FRONTIERS IN DIGESTIVE DISEASES

SYMPOSIUM

MARCH 9 | 2024

"Frontiers in GI Cell Biology: Crosstalk and Communication"

Saturday, 3/9/24

8:00 am - 3:00 pm

Baylor St. Luke's
7200 Cambridge
Houston Tx, 77030

Baylor College of Medicine

Provided by Baylor College of Medicine

ACCREDITATION/CREDIT DESIGNATION STATEMENTS

Baylor College of Medicine is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to provide continuing medical education for physicians.

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DDC
Texas Medical Center Digestive Diseases Center

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About the Texas Medical Center Digestive Disease Center (DDC)

The Texas Medical Center Digestive Diseases Center facilitates cutting-edge digestive diseases research, promotes translational collaborative research between basic and clinical areas, develops new projects, nurtures new investigators, and provides GI educational activities.

The DDC is a federally funded center (NIH P30DK056338) designed to serve basic and clinical scientists at institutions within the Texas Medical Center, including Baylor College of Medicine, The University of Texas Health Science Center at Houston, and the MD Anderson Cancer Center.

The DDC supports three basic science cores: Tissue Analysis and Molecular Imaging, Functional Genomics and Microbiome, Gastrointestinal Experimental Module Systems; and one clinical core: Study Design and Clinical Research.

Current DDC membership includes 118 members, with 78 full members and 40 associate members. DDC members have approximately $48 million in digestive diseases-related research funding as of 2023. The overall theme of the DDC is “Molecular mechanisms and outcomes of injury, infection, or metabolic dysfunction of the digestive system.”

Visit the DDC website to learn more about upcoming enrichment activities, funding opportunities, and other valuable resources.

Visit the DDC online!

https://www.bcm.edu/research/research-centers/texas-medical-center-digestive-

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"Frontiers in GI Cell Biology: Crosstalk and Communication"

AGENDA

FRONTIERS IN DIGESTIVE DISEASES SYMPOSIUM
MARCH 9 | 2024

7:30 AM - 8:10 AM **BREAKFAST**

8:10 AM - 8:25 AM **WELCOME REMARKS**

Hashem B. El-Serag, M.D., M.P.H., DDC Co-Director
Jason C. Mills, M.D., Ph.D., DDC Co-Director

SESSION I

8:25 AM - 9:05 AM **"Niche Regulation of Gastrointestinal Stem Cell Dynamics"**

Linda C. Samuelson, Ph.D.
John A. Williams Professor of Gastrointestinal Physiology
University of Michigan, Ann Arbor, MI

9:05 AM - 9:45 AM **"A nervous bowel is a happy bowel . . . Making sense of Hirschsprung disease"**

Robert O. Heuckeroth, M.D., Ph.D.
Research Director, Suzi and Scott Lustgarten Center for GI Motility
Children's Hospital of Philadelphia, Philadelphia, PA

9:45 AM - 10:05 AM **"Building a functional intestine for children with intestinal failure: how to innervate and innovate"**

Allison L. Speer, M.D., FACS, FAAP (2017 Pilot Feasibility Awardee)
Associate Professor, Pediatric Surgery
The University of Texas Health Science Center, Houston, TX

10:05 AM - 10:45 AM **"Engineering gastrointestinal organoids for digestive disease research"**

James M. Wells, Ph.D.
Director for Research, Division of Endocrinology
Cincinnati Children's Hospital, Cincinnati, OH

10:45 AM - 11:05 AM **COFFEE BREAK**

SESSION II

11:05 AM - 11:45 AM **"Gut epithelial cells: interpreters of gut microbiome/immune interactions"**

Rodney D. Newberry, M.D.
Dr. Nicholas V. Costrini Professor of Gastroenterology and Inflammatory Bowel Disease
Washington University School of Medicine, St. Louis, MO

11:45 AM - 12:05 PM **"Microbial regulation of intestinal epithelial hormones"**

Sara Di Rienzi, Ph.D. (2017 Pilot Feasibility Awardee)
Assistant Professor, Molecular Virology and Microbiology
Baylor College of Medicine, Houston, TX

12:05 PM - 12:45 PM **"Unraveling mucus microbe interactions in the intestine"**

Mindy Engevik Ph.D.
Assistant Professor, Regenerative Medicine and Cell Biology
Medical University of South Carolina, Charleston, SC

12:45 PM - 2:15 PM **POSTER SESSION (BOXED LUNCHES PROVIDED)**

2:15 PM - 2:45 PM **BREAKOUT SESSIONS WITH SELECT SPEAKERS**

#1. Linda Samuelson, Ph.D.
#2. Rodney Newberry, M.D.
#3. James Wells, Ph.D.
#4. Robert O. Heuckeroth, M.D., Ph.D.
#5. Mindy Engevik, Ph.D.

2:45 PM - 3:00 PM **POSTER AWARDS / CLOSING REMARKS**

Geoffrey Preidis, M.D., Ph.D., DDC Enrichment Program Director

Provided by Baylor College of Medicine

Texas Medical Center Digestive Diseases Center
15th Annual Frontiers in Digestive Diseases Symposium
"Frontiers in GI Cell Biology: Crosstalk and Communication"

Saturday, March 9, 202 | 8 am – 3 pm | Baylor St. Luke's Medical Center | 7200 Cambridge

The symposium will offer a balance of research information and clinical applications regarding cell biology crosstalk and communication in GI injury, infection, and metabolism and provide insight into new research developments and current clinical best practices. This program is presented by the Texas Medical Center Digestive Disease Center.

TARGET AUDIENCE: Gastroenterologists, Hepatologists, Digestive Diseases Researchers, Scientists, Medical Students and Trainees, and other healthcare professionals with an interest in digestive diseases research.

LEARNING OBJECTIVES: At the conclusion of the conference, participants should be able to:

- Define cell biology crosstalk and communication in GI tract, its history and future applications in treating and preventing digestive diseases.
- Interpret current research developments regarding cell biology crosstalk and communication in the GI tract.
- Apply cell biology crosstalk and communication in GI tract to the detection and treatment of digestive diseases.

EDUCATIONAL METHODS: Lecture, Small Group Breakout Session, Poster Session and Panel Discussion

ACTIVITY EVALUATION: Evaluation by questionnaire will address program content, presentation, and possible bias.

ACCREDITATION/CREDIT DESIGNATION STATEMENT: Baylor College of Medicine is accredited by the Accreditation Council for Continuing Medical Education to provide continuing medical education for physicians. Baylor College of Medicine designates this live activity for a maximum of *4.50 AMA PRA Category 1 Credits™.* Physicians should claim only the credit commensurate with the extent of their participation in the activity.

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Weill Cornell Medicine

Gyongyi Szabo, M.D., Ph.D.
Harvard Medical School

Gary D. Wu, M.D.
University of Pennsylvania

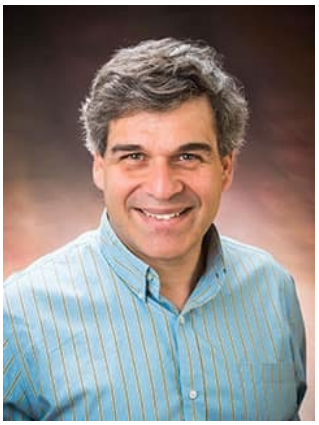
Keynote Speaker

Linda Samuelson, Ph.D.
University of Michigan

Dr. Linda Samuelson is the John A Williams Collegiate Professor of Gastrointestinal Physiology at the University of Michigan. She obtained her BS degree from Michigan State University and her PhD from the University of Chicago, followed by postdoctoral training at the University of Michigan, where she subsequently joined the faculty in the Physiology Department. She is currently Professor of Molecular & Integrative Physiology, and Professor of Internal Medicine in the Division of Gastroenterology & Hepatology at the University of Michigan. She also serves as Director of the Center for Cell Plasticity and Organ Design, an interdisciplinary center that colalesses strengths in developmental and stem cell biology with tissue engineering, to solve problems impacting organ development, homeostasis, and regeneration.

Dr. Samuelson's research program seeks to uncover basic mechanisms regulating gastrointestinal stem cell function to maintain tissue homeostasis and orchestrate repair after injury. Her ongoing research focuses on defining the cells and signals that constitute the stem cell niche in stomach and intestine. These studies have unveiled the essential role of the Notch signaling pathway for stem cell self-renewal and cell fate determination. Her findings have made important contributions to our understanding of the cellular and molecular basis of gastrointestinal tissue health.

Symposium Speakers



Robert Heuckeroth, M.D., Ph.D.
Children's Hospital of Philadelphia

Dr. Heuckeroth is a pediatric gastroenterologist in the Division of Gastroenterology, Hepatology and Nutrition at Children's Hospital of Philadelphia where he runs a basic research lab, cares for children with serious bowel motility disorders, and serves as Director of the Suzi and Scott Lustgarten Center for GI Motility at CHOP. Dr. Heuckeroth is a professor of Pediatrics and the Irma and Norman Braman Endowed Chair for Research in GI Motility Disorders. He investigates mechanisms controlling bowel motility to find new ways to treat, diagnose, and prevent intestinal motility disorders. His research focuses on the enteric nervous system and smooth muscle biology as he works to find new ways to improve the lives of children with bowel motility disorders.

Allison Speer, M.D.
The University of Texas Health Science Center

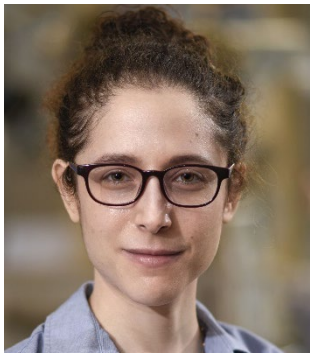
Dr. Speer is an Associate Professor of Pediatric Surgery at the McGovern Medical School at the University of Texas Health Science Center (UTHealth Houston). She has received several research awards including the American College of Surgeons Surgical Forum Excellence in Research Award and grant funding from the Ethicon-Society of University Surgeons Surgical Research Fellowship Award. Dr. Speer's clinical interests include intestinal failure and rehabilitation, advanced minimally invasive surgery, and thoracic/foregut surgery. She is the surgical director of the Short bowel syndrome Therapy and Rehabilitation program. She is also a basic-translational scientist focused on developing novel regenerative medicine strategies for intestinal failure such as tissue-engineered intestine and cell-based therapies.

James Wells, Ph.D.
Cincinnati Children's Hospital

Dr. Wells is Director for Research in the Division of Endocrinology and Chief Scientific Officer for the Center for Stem Cell and Organoid Medicine at Cincinnati Children's Hospital Medical Center. His basic research examines the molecular mechanisms involved in the embryonic development of endocrine cells including pancreatic beta cells and tissues of the intestinal tract. Translational projects have focused on identifying new approaches to improve child health in several ways: 1. to identify and use embryonic pathways to generate complex, three-dimensional organ tissues from pluripotent stem cells, 2. use these tissues to develop new in vitro human models for diabetes and digestive disease research, and 3. develop long-term, therapeutic strategies for cell and tissue-replacement therapies.

Rodney Newberry, M.D.
Washington University at St. Louis

Dr. Newberry is the Dr. Nicholas V. Costrini Professor of Gastroenterology and Inflammatory Bowel Disease at Washington University School of Medicine in St. Louis. His lab investigates how the intestinal immune system maintains balance between tolerance to innocuous substances while maintaining the ability to mount inflammatory responses to potential infectious organisms. Using state of the art imaging techniques, he discovered a novel mechanism delivering innocuous substances across the epithelium to immune cells in the intestine. Further studies are underway to define how this delivery mechanism is regulated and its role in tolerance to substances in the diet. He is also investigating how diet and other environmental cues shape the intestinal immune system.



Sara Di Rienzi, Ph.D.
Baylor College of Medicine

Dr. Di Rienzi is Assistant Professor in Molecular Virology and Microbiology at Baylor College of Medicine investigating dietary and microbial modulation of gut hormones. She is a 2021 DDC Pilot Feasibility Awardee. Her current research is characterizing novel hormone secreting cells in the gut and how gut microbes regulate the release of these novel gut hormones. The ultimate goals of Dr. Rienzi's research are to improve treatment of diseases through effective and informed use of diet and beneficial microbes.

Mindy Engevik, Ph.D.
Medical University of South Carolina

Dr. Engevik is Assistant Professor of Medicine in the Department of Regenerative Medicine and Cell Biology at the Medical University of South Carolina. She is a 2018 DDC Pilot Feasibility Awardee. Dr. Engevik's research continues to focus on microbial-host crosstalk, now with an emphasis on interactions between microbes and the intestinal mucus layer. This focus encompasses the two-sided nature of these interactions: the beneficial effects on host health mediated by commensal microbe modulation of the mucus layer, and the subversion of mucus function during infection by colonizing pathogens. These efforts utilize both animal and human organoid model systems through the DDRCC and CDLD cores.

Poster Session

"Metabolic Regulator ERRγ is Essential and Sufficient for Acid-Pumping Cell Differentiation"

Sumimasa Arimura, Ph.D.
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Poster #1
See abstract on page 14

"Menopause partially explains the risk of advanced MASLD fibrosis as women age"

Maya Balakrishnan, MD MPH
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Abstract Only
See abstract on page 15

"Delineating cellular differences between human intestinal enteroids from infants and adults using single cell RNA-seq analysis"

Sohini Banerjee, Ph.D.
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Poster #2
See abstract on page 16

"Effects of early-life malnutrition and timed maternal separation on gastric emptying and gastrointestinal pathophysiology in neonatal mice"

Donovan Berens, MD
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Abstract Only
See abstract on page 17

"Gut Microbiota Dynamics in Neonatal Hypoxic-Ischemic Encephalopathy: a Potential Target for Modulating Long-Term Neurodevelopmental Outcomes"

Viola Caretti, M.D., Ph.D.
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Poster #3
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"Characteristics and incidence of gastric cancers among patients with autoimmune and non-autoimmune atrophic gastritis in a U.S. population"

Kadon Caskey, M.D.
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Poster #4
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"Smooth muscle contractile responses to bile acids in mouse ileum require TGR5 but not ASBT"

Diana Chang
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Abstract Only
See abstract on page 20

"4-Methylumbelliferone modulates gut microbiota to inhibit liver fibrogenesis"

Xi Chen, M.D., Ph.D.
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Poster #5
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"Crucial Role of Secretory Organelle Dynamics in Pancreatic Acinar Cell Response to Injury, Metaplasia, and Tumorigenesis"

Charles Cho, M.D., Ph.D.
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Poster #6
See abstract on page 22

"Inequalities in the risk of developing cirrhosis complications over time: a cohort study."

George Cholankeril, M.D.
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Poster #7
See abstract on page 23

Poster Session

"Stromal antigen 1 as a modulator of program death ligand-1 expression: implications for colorectal cancer immunotherapy"

Mart Angelo Dela Cruz, BS
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Poster #8
See abstract on page 24

"Agreement between stomach biopsy culture and stool next-generation sequencing for determining Helicobacter Pylori antibiotic susceptibility profiles in a screening predominantly Hispanic U.S. population"

Samanthika Devalaraju, M.D.
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Poster #9
See abstract on page 25

"Uncovering the mechanism regulating ESRRG function by SIRT1 in gastric parietal cell differentiation"

Margarita Divenko, MS
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Poster #10
See abstract on page 26

"Essential role of p53 in intestinal epithelium homeostasis and stem cell differentiation"

Amit Dubey, Ph.D.
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Poster #11
See abstract on page 27

"Mitigating cholestasis in TPN-fed piglets via enteral agonism of farnesoid X receptor-fibroblast growth factor 19 axis"

Sarah Elefson, Ph.D.
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Poster #12
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"Phase 3 Biomarker Study for HCC Surveillance Using a novel HES v2.0 Algorithm. A Prospective Collection with Retrospective Blinded Evaluation"

Hashem El-Serag, M.D., M.P.H.
Baylor College of Medicine
Poster #13
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"Serum Metabolic Biomarker Signature Is Predictive of the Risk of Hepatocellular Cancer in Patients with Cirrhosis"

Hashem El-Serag, M.D., M.P.H.
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Abstract Only
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"Purinergic Signaling Drives Multiple Aspects of Rotavirus Pathophysiology"

Kristen Engevik, Ph.D.
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Poster #15
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"P2Y1 receptor signaling influences epithelial responses to intestinal inflammation"

Kristen Engevik, Ph.D.
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Poster #16
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Poster Session

"Probiotic Limosilactobacillus reuteri DSM 17938 alters gut microbiota and reduces circulating monocytes in immunocompromised RAG1KO mice"

Ana Fadhel Alvarez, M.D.
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Poster #17
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"Do Gastrointestinal Symptoms Correlate with GI Inflammation or Gut Permeability in Children with Autism Spectrum Disorder?"

Vaishali Harne, M.D.
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Poster #18
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"Evaluating potential norovirus antivirals in human intestinal enteroids"

Nicole Hayes, MSc
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Poster #19
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"The ephrin receptor A7 (EPHA7) gene may regulate enteric nervous system development in human intestinal organoids"

Madushani Herath, Ph.D.
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Poster #20
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"Coagulation Factor Genes Identified as Potential Candidates Driving Alcohol-Associated Acute Pancreatitis in Aging Mice via Spatial Transcriptomic Profiling"

Isabella Hernandez, M.D.
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See abstract on page 37

"Doo dots: a dried fecal spot (DFS)-based bioanalytical platform for bile acid quantitation"

Thomas Horvath, Ph.D.
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Poster #22
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"Longitudinal dynamics of fecal bile acids in pediatric patients with primary sclerosing cholangitis-ulcerative colitis (PSC-UC) undergoing oral vancomycin therapy (OVT)"

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Poster #23
See abstract on page 39

"Fibrosis of ganglionic intestine may contribute to bowel dysfunction in human Hirschsprung disease"

Britney Hsu
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Poster #24
See abstract on page 40

"Characterization of epigenetic changes during the metaplastic process by single-cell RNA sequencing"

Yang-Zhe Huang
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"Development of an Algorithm to Automate the Detection of Emergency Presentations Among Pancreatic Cancer Patients"

Natalia Khalaf, M.D., M.P.H.
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Poster #26
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Poster Session

"Eradication rates of empiric Helicobacter pylori treatment regimens in a U.S. safety-net population"

Min Kim, BS
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See abstract on page 43

"Somatic Mutations of Esophageal Adenocarcinoma: A Comparison Between Black and White Patients"

Hyeyeun Lim, Ph.D.
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See abstract on page 44

"Comparative DNA Methylation Profiling of Cirrhosis With And Without Hepatocellular Carcinoma"

Hyeyeun Lim, Ph.D.
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Poster #29
See abstract on page 45

"Ghrelin: a crucial hormone for epithelial integrity and function in the mouse colon"

Zeyu Liu, MS
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Poster #30
See abstract on page 46

"Microbially-derived branched chain fatty acids regulate intestinal permeability in malnutrition"

Lauren Lynch, BA
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Abstract Only
See abstract on page 47

"Investigating the interplay between diet and the gut microbiome in necrotizing enterocolitis (NEC)"

Valeria Melendez Hebib, MS
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Poster #31
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"Deciphering the role of IL-4 in colon repair after acute colitis"

Nicolas Moreno, BS
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Poster #32
See abstract on page 49

"Glucagon-like peptide 1 (GLP-1) agonists semaglutide and tirzepatide and colorectal cancer risk: a cell culture study"

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Poster #33
See abstract on page 50

"Liver steatosis in malnourished mice is altered by the gut microbiome"

Larissa Neves, BS
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Poster #34
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"Investigating the role of the PERK-integrated stress response pathway during paligenosis and metaplasia"

Thanh Nguyen
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See abstract on page 52

Poster Session

"Real-world patterns of objective inflammation assessment in a large, multicenter cohort: potential gaps and barriers to treat to target"

Peter Nguyen
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Poster #36
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"Ghrelin signaling in macrophages: an intricate link of gut-brain axis in aging"

Jiyeon Noh
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See abstract on page 54

"Evaluating Mechanisms of Human Norovirus Gastroenteritis Using Human Intestinal Enteroids"

Hephzibah Nwanosike, BSc
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Poster #38
See abstract on page 55

"Chronotherapy Will Play a Key Role in Future Personalized Anti-HCC Treatment"

Noha Osman, Ph.D.
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Poster #39
See abstract on page 56

"De-N-glycosylation at the fulcrum of intestinal innate immune response and barrier function"

Ashutosh Pandey, Ph.D.
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Poster #40
See abstract on page 57

"Evaluation of human milk oligosaccharides as therapeutics for noroviruses"

Ketki Patil, MS
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See abstract on page 58

"Smooth muscle specific loss MBNL as a model of DM1 causes muscle hypercontraction and delayed gastrointestinal motility"

Janel Peterson, BS
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Poster #42
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"Bmpr2 And Grem1 Knockouts In Fibroblasts Do Not Alter Severity Of Alcohol-Associated Chronic Pancreatitis In Mice"

Amy Qin, M.D.
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Poster #43
See abstract on page 60

"Chronic arsenic exposure induces thymidylate synthase (TYMS) in colorectal cancer (CRC) cells: implications for 5-Fluorouracil Chemoresistance"

Priya Roy, MS
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Poster #44
See abstract on page 61

Poster Session

"Probiotic limosilactobacillus reuteri dsm 17938 ameliorates maternal separation stress in newborn mice and alters adolescent behavior"

Zeina Saleh, M.D.
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Poster #45
See abstract on page 62

"Acinar to ductal metaplasia is an evolutionary dead end and does not actively contribute to pancreas regeneration after damage"

Rutvi Shah, MS
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Poster #46
See abstract on page 63

"New patterns of cell plasticity in longitudinal study of human tissue: Implications for healing after radiofrequency ablation in Barrett's Esophagus"

Sarah To, Ph.D.
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Poster #47
See abstract on page 64

"Early-life malnutrition suppresses hepatic Cyp7b1 transcription by inducing Srebp1"

Xiaoyang Wan, Ph.D.
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Poster #48
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"Decoding human norovirus tropism via single-cell RNA analysis"

Luqiong Wang, MS
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Poster #49
See abstract on page 66

"Limosilactobacillus reuteri-engineered growth factors to mitigate radiation damage in human intestinal organoids"

Katherine Wozniak, Ph.D.
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Poster #14
See abstract on page 67

"Eosinophil protects against colitis in a novel cyclooxygenase (COX)-2-IL-22 dependent manner"

Yang Yang, Ph.D.
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Poster #50
See abstract on page 68

"YAP1 facilitates the re-entry of differentiated cells into the cell cycle during paligenosis through phospho-dependent, non-canonical Hippo pathway regulation"

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Poster #51
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"HES1 loss and counteracting ATOH1 up-regulation promotes intestinal stem cell regeneration of ulcerative colitis"

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Poster #52
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"DDC Tissue Analysis & Molecular Imaging Core"

Michael Mancini, Ph.D.
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Poster #53
Poster Only

Poster Session

"DDC Tissue Analysis & Molecular Imaging Core"

Deborah Schady, M.D.
Texas Children's Hospital
Poster #54
Poster Only

"DDC Functional Genomics & Microbiome Core"

James Versalovic, M.D., Ph.D.
Baylor College of Medicine
Poster #55
Poster Only

"Gastrointestinal Experimental Model Systems Core: Organoids"

Sarah Blutt, Ph.D.
Baylor College of Medicine
Poster #56
Poster Only

"Gastrointestinal Experimental Model Systems Core: Gnotobiotic Facility"

Margaret Conner, Ph.D.
Baylor College of Medicine
Poster #57
Poster Only

"Study Design & Clinical Research Core"

Fasiha Kanwal, M.D., MSHS
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Poster #58
Poster Only

Metabolic regulator ERRγ is essential and sufficient for acid-pumping cell differentiation

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Acid-producing gastric parietal cells (PCs) are highly metabolically active, digesting food and killing pathogens. Because loss of PCs is common in a variety of diseases, from atrophic gastritis to gastric cancer, treatments that reverse their loss would be valuable. However, the mechanism of PC differentiation has remained largely unclear. Here, we show that the earliest progenitors of PCs in the mouse stomach preferentially express the cell metabolism regulator and orphan nuclear receptor estrogen-related receptor gamma (*Esrrg*, encoding ERRγ) as they arise from gastric (isthmus) stem cells. Lineage tracing of *Esrrg* in vivo showed that expression of *Esrrg* largely committed progenitors to differentiate into mature PCs. Expression of *Esrrg* allowed stepwise tracing of the molecular and cellular stages of PC differentiation. Furthermore, mice with *Esrrg* deletion in whole gastric epithelium or during PC differentiation showed a complete absence of PCs. Conversely, overexpression of *Esrrg* or treatment with a pan-ERR agonist promoted PC differentiation in gastric corpus organoid cultures, and the use of the agonist also increased the PC population in wild-type mice. Collectively, our results demonstrate that the metabolism-regulating transcription factor ERRγ is essential and sufficient for PC differentiation and suggest that ERRγ may be a therapeutic target for diseases associated with PC loss.

Menopause partially explains the risk of advanced MASLD fibrosis as women age

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Background: Aging is associated with a higher risk of advanced fibrosis in women with metabolic dysfunction associated steatotic liver disease (MASLD) than men with MASLD. Among study populations with MASLD and average age ≥ 50 years, women have a 56% higher risk of advanced fibrosis compared to men (versus 17% increased risk among women compared to men in study populations with MASLD and average age < 50 years). Animal studies have shown that that loss of circulating estrogen, as occurs during menopause, is associated with rapid fibrogenesis among female rodents with hepatic steatosis. Understanding the effect of menopause on fibrosis has potential therapeutic implications vis a vis estrogen replacement therapy. Therefore, we undertook this study to examine whether menopause mediates the effect of age on advanced fibrosis in women.

Table 1. Study population characteristics (n=194)	
Age (mean, SD)	**48.3**
Body mass index , mean (SD)	35.2 (6.5)
Obesity categories, N (%)	
Overweight or lower (BMI: <30)	41 (21.1)
Class1(BMI:30-35)	67 (34.5)
Class2(BMI:35-40)	40 (20.6)
Class3(BMI:>=40+)	46 (23.7)
Diabetes status	
No diabetes / prediabetes	**77 (39.69)**
Type 2 Diabetes	117 (60.3)
Hypertension, N(%)	
No	99 (51.0)
Yes	95 (48.97)
Menopause , N (%)	
No	91 (46.9)
Yes	103 (53.09)
Menopause age , mean (SD)	45.17(6.86)
ALT, mean (SD)	87.69(60.99)
HbA1c , mean (SD)	7.08(1.7)
NAFLD Activity Score (NAS) , mean (SD)	4.08(1.3)
Steatosis Severity , N (%)	
Mild steatosis	79 (40.7)
Moderate steatosis	80 (41.2)
Severe steatosis	35 (18.0)
NASH, N (%)	
No	79 (40.7)
Yes	115 (59.28)
Advanced Fibrosis	
No (Metavir Stage 0/1/2)	138 (71.1)
Yes (Metavir Stage 3/4)	56 (28.87)

Methods: This cross-sectional study included 194 women with biopsy-proven MASLD recruited prospectively as part of the Harris County MASLD Cohort (Houston, Texas). We defined advanced fibrosis as the presence of bridging fibrosis or cirrhosis on liver biopsy. Menopausal status was determined using a validated baseline reproductive questionnaire. Logistic regression models examined associations between age (continuous variable) and advanced fibrosis. Causal mediation analysis examined whether menopause mediated the association between age and MASLD fibrosis. All analyses were adjusted for obesity classification, T2DM, and PNPLA3 genotype.

Results: The mean age of the study population was 48.3 years and 56 (28.8%) had advanced fibrosis. The mediation analysis results are presented in the Figure. The total effect of age on advanced fibrosis estimated by OR was 1.107 (95% CI, 1.048-1.166) mediated through the menopause pathway. About 37.8% (p 0.044) of the total effect was mediated by menopause.

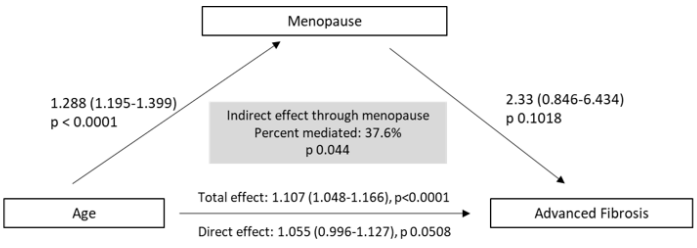

Figure 1. Causal mediation analysis of the relationship between age and advanced fibrosis mediated by the menopause pathway. The numbers represent odds ratios and their 95% confidence intervals. All analyses were adjusted for obesity, type 2 diabetes, and PNPLA3 genotype.

Conclusions: Menopause partially mediates the effect of age on fibrosis risk among women with MASLD. Although menopause appears to mediate a substantial proportion of the effect of age on fibrosis risk, additional behavioral and clinical mediating factors need to be examined.

Delineating cellular differences between human intestinal enteroids from infants and adults using single cell RNA-seq analysis

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The cellular landscape and the physiological characteristics of the human gastrointestinal (GI) tract is dynamic during early infancy, changing in response to development, functional requirements and environmental exposures. Human intestinal enteroids (HIE) are physiologically relevant models of the human intestine. In previous work, we performed transcriptional analysis of jejunal HIEs using total RNA sequencing from infants and adults and identified transcriptional differences in pathways associated with RNA processing, ribonucleoprotein complex biogenesis, cell-cell adhesion, and immune responses. Functional validation studies also showed significant differences in cell differentiation and proliferation, barrier function and innate immune response to a poliovirus vaccine. Total RNA sequencing analysis, however, does not allow elucidation of which intestinal cell types contribute to observed differences between infant and adult HIEs. The goal of the present study is to use single-cell RNA sequencing to explore the diversity of individual cell types in HIEs derived from children and adults, to quantitatively analyze and infer intercellular communication networks, and to obtain deeper insights into the differential responses of each cell type in differentiated HIEs.

Single cell transcriptional profiles were obtained from HIE lines established using surgical samples from one infant (J1005) and one adult (J11). UMAP clustering identified distinct clusters of cells for the infant and adult HIEs. Single cell transcriptomics confirmed previous observations of greater number of enteroendocrine cells (EECs) in infants HIEs also in addition to highlighting the diversity of EEC subtypes in these cultures. Differential transcriptional profiles were observed for the infant and adult HIEs. Further analysis of the gene expression, including validation of differences using single cell datasets from additional adult HIEs is being performed. These studies will help predict signaling interactions between cellular populations in infant and adult lines. The present study will advance our fundamental knowledge of developmental differences between adult and infant HIEs and provide deeper understanding of intestinal maturation, disease susceptibility, and potential for development of therapeutics.

Effects of early-life malnutrition and timed maternal separation on gastric emptying and gastrointestinal pathophysiology in neonatal mice

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Background: The etiology of pediatric gastroparesis (GP) has not been fully elucidated. Malnutrition-associated changes in gastrointestinal motility have been previously described in patients with anorexia nervosa and small-for-gestational age infants. We have previously reported a mouse model of early-life GP using neonatal timed maternal separation (TmSep). Mouse pups undergoing TmSep are nutritionally stunted and are exposed to the stress of maternal separation. To determine the differential effects of early postnatal malnutrition and stress, we examined the effects of TmSep stress and malnutrition – alone or in combination – on pathophysiology characterized by gastric emptying, bowel length, and body weight.

Methods: C57BL/6 mouse pups were randomized on day of life (DOL) 5 until conclusion of the experiment on DOL 14. Mouse pups were allocated to the following groups: control, long interval (LI) TmSep for 12 hours daily, short interval (SI) TmSep for 3 hours daily, crowded litters without maternal separation, and crowded litters with short interval (SI) maternal separation. We assessed gastric emptying using fluorescein isothiocyanate (FITC)-dextran gavage by quantifying the amount of FITC-dextran in the stomach and small bowel after 15 minutes. We also assessed body weight, antral-cecal length, and whole bowel length.

Results: Pups in the control group had significantly higher mean body weight than those in the (LI) TmSep, crowded, and (SI) crowded groups which trended together. Mean body weight was similar between pups in the control and (SI) TmSep groups. Gastric emptying of FITC-dextran was similar between pups in the control, (SI) TmSep, and (SI) crowded groups. Pups in the crowded and (LI) TmSep groups had significantly reduced gastric emptying of FITC-dextran. Pups in the control group had significantly longer antral-cecal length and whole bowel length when compared to those in the (LI) TmSep, crowded, and (SI) crowded groups which trended together. Mean antral-cecal length and whole bowel length were similar between pups in the control and (SI) TmSep groups.

Conclusions: Exposure to short interval timed maternal separation alone is insufficient to replicate our previously established model of early-life GP. Malnutrition induced by long interval TmSep and crowded litters conferred a lower mean body weight, shorter antral-cecal length, shorter whole bowel length, and decreased gastric emptying of FITC-dextran relative to controls. GP pathophysiology observed in TmSep may be more influenced by malnutrition than by stress from maternal separation.

Gut Microbiota Dynamics in Neonatal Hypoxic-Ischemic Encephalopathy: a Potential Target for Modulating Long-Term Neurodevelopmental Outcomes

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Background: Neonatal hypoxic-ischemic encephalopathy (nHIE) is a leading cause of mortality and chronic neurological disabilities, including attention deficit hyperactive disorder. The gut microbiota plays a crucial role in brain development, and its perturbation is associated with neurobehavioral changes in pre-clinical models. However, it is unknown whether neonatal brain injury affects the gut microbiota into adulthood and if changes in its homeostatic development impact neurodevelopmental outcomes. We **hypothesize** that nHIE pathologically alters the gut microbiota throughout development and that therapeutic modulation of the gut microbiome to mimic healthy gut post-nHIE ameliorates neuro-behavioral outcomes in adulthood. **Methods:** C57BL/6 pups at post-natal day 9 (corresponding to term in neonates) underwent the modified Rice-Vannucci surgery (mRV) to induce nHIE or sham surgery followed by hypoxia (controls). Fecal samples were collected 1 day, 2 months, and 3 months after surgery for 16S rRNA sequencing (16S). All mice underwent baseline behavioral tests at 2 months. Thereafter, both nHIE and sham mice received fecal microbiota transfers (FMT) from age-matched naïve donors, and behavioral deficits were assessed by re-administering tests 1 and 3 months post- FMT. To probe the influence of HIE-derived microbiota on behavior, naïve mice were also transplanted with FMT from nHIE or sham mice. Stool, gut, and brain were collected for 16S sequencing and histopathological analysis. **Results:** Principal-coordinate analysis (PCoA) of weighted UniFrac distances from the averaged 16S sequencing revealed statistically significant different microbiota clusters between the nHIE and the sham group 1 day (acute) and 3 months (chronic) after surgery ($p = 0.022$ & 0.037, $R2 = 0.5$ & 0.1, respectively), indicating a persistent shift in β- (between samples) diversity in the microbiota of nHIE-pups vs controls. nHIE pups had significantly lower mean relative abundance of Lactobacillus 1 day after injury and both of *Lactobacillus* and *Bifidobacterium* genera 3 months after injury ($p< 0.05$, n=5-6 per sex/group). This confirms that specific beneficial bacteria important for neonatal brain development are lost after nHIE. Additionally, opportunistic pathogens or pathobionts were significantly enriched in the nHIE group vs controls. Behavioral analysis revealed an increased mean velocity, distance moved, and cumulative time in the border area with decreased time in the center, indicating a hyperactive phenotype in nHIE mice ($p=0.0001$, $p=0.0168$, $p=0.025$). At 5 months (corresponding to early thirties in humans), nHIE mice with a naïve FMT had normalization of their hyperactive phenotype, as shown by a significant reduction in mean velocity and distance moved ($p=0.028$ and $p=0.0322$; n=3-5 per sex/group), while sham mice had no change. Naïve mice given an FMT from nHIE donors became hypoactive with reduced mean velocity and distance moved ($p=0.0004$ and $p=0.0004$; n=3-5 per sex/group), while naïve mice with sham FMT had no change. **Conclusions:** nHIE causes unhealthy alternation in gut microbiota at both acute and chronic time points. The transplantation of HIE-derived biome into naïve mice results in behavioral deficits, supporting a causative role of the gut microbiome in neuro impairments after nHIE. Conversely, the normalization of hyperactive behaviors in HIE mice following naïve FMT supports gut microbiota as a potential therapeutic target. As a next step, we will determine if motor, cognitive, and social activity impairments can also be normalized in nHIE pups after FMT. These results underscore the potential of gut microbiota modulation as a long-term treatment for neurodevelopmental disabilities after neonatal hypoxic brain injury.

Characteristics and incidence of gastric cancers among patients with autoimmune and non-autoimmune atrophic gastritis in a U.S. population

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Background: Rates of gastric neuroendocrine tumors (NETs) are increasing in the U.S. Autoimmune atrophic gastritis (AAG) is characterized by immune-mediated destruction of oxyntic glands resulting in gastric atrophy and is the primary risk factor for gastric NETs. We aimed to determine the demographic and clinical risk factors for AAG and the incidence of gastric cancers among patients with AAG and non-autoimmune atrophic gastritis (NAAG). **Methods:** For this retrospective cohort study, AAG cases and NAAG controls were identified using the histopathology database of patients who underwent gastric biopsy from Ben Taub Hospital (Houston, Texas) from March 2006-July 2021. Cases were defined by the presence of AAG or atrophic gastritis with enterochromaffin-cell hyperplasia on histopathology. All histopathology reports of suspected cases were reviewed for the study by a GI pathologist, and select slides were re-examined if needed. NAAG controls were defined by the presence of gastric intestinal metaplasia or atrophic gastritis without autoimmune features on gastric biopsy histopathology. Demographic, clinical, and biomarker risk factors for AAG were assessed using chi-2 and t-test. Cases and controls were reviewed to determine age-adjusted incidence rates of gastric cancers (adenocarcinoma, NET, lymphoma, other) up to June 1, 2023. Predictors of cancer were calculated using hazard ratios (HR) and 95% confidence intervals (CI). **Results:** A total 172 AAG cases and 455 NAAG controls were included. AAG cases were more likely to be female (78.5% vs. 50.8%, p<0.01), younger (mean age 54.5 vs. 58.0 years, p<0.01), and Hispanic (71.5% vs. 47.9, p<0.01). Cases were less likely to have *H. pylori* (29.7% vs. 55.6%, p<0.01) but more like to have anti-parietal cell antibody (84.4% vs. 37.5%, p<0.01) and gastrin levels >200 pg/mL (89.6% vs. 31.3%, p <0.01) than controls (Figure 1). AAG cases had 26 incident cancers (88% NET) and 27 incident dysplasia in 3874 person-year follow-up time compared to 31 incident cancers (71% adenocarcinoma) and 36 incident dysplasia in NAAG controls over 6393 person-year follow-up time. The age-adjusted incidence rate of gastric cancers was 6.7 per 1000 person-years in AAG cases and 4.8 per 1000 person-years in NAAG controls (incidence rate ratio 1.15, 95% CI 0.90-2.63 (Figure 2)). The presence of AAG was associated with increased gastric cancer risk (HR 4.73, 95% CI 1.14-19.59) compared to NAAG after adjusting for sex, age, race/ethnicity, and *H. pylori*. **Conclusions:** AAG cases were more likely to be female, younger, Hispanic and have elevated anti-parietal cell antibody and gastrin levels compared to NAAG controls. Cases with AAG had a 1.15-fold higher gastric cancer risk than NAAG controls and were more likely to develop incident NETs. Given the heightened cancer risk with AAG, future studies should focus on strategies for early cancer detection.

	Non-autoimmune Atrophic Gastritis Controls N=455	Autoimmune Atrophic Gastritis Cases N=172	p-value
Sex			<0.01
Male	224 (49.2)	37 (21.5)	
Female	231 (50.8)	135 (78.5)	
Age, mean (SD)	58.0 (11.3)	54.5 (11.1)	<0.01
Race			<0.01
Non-Hispanic White	42 (9.2)	8 (4.7)	
Black	122 (26.8)	36 (20.9)	
Hispanic	218 (47.9)	123 (71.5)	
Asian	30 (6.6)	5 (2.9)	
Other	43 (9.5)	0 (0.0)	
H. pylori			<0.01
Positive	253 (55.6)	51 (29.7)	
Negative	202 (44.4)	121 (70.4)	
Anti-Parietal Cell antibody			<0.01
Positive	3 (37.5)	76 (84.4)	
Negative	5 (62.5)	14 (15.6)	
Unknown	447	82	
Anti-intrinsic factor antibody			0.12
Positive	5 (27.8)	54 (47.4)	
Negative	13 (72.2)	60 (52.6)	
Unknown	437	58	
Gastrin level (pg/mL)			<0.01
>=200	5 (31.3)	43 (89.6)	
<200	11 (68.8)	5 (10.4)	
Unknown	439	124	

Figure 1: Demographic, clinical, and biomarker characteristics of autoimmune atrophic gastritis cases compared to non-autoimmune atrophic gastritis controls.

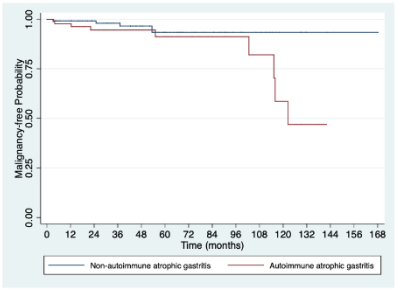

Figure 2: Kaplan-Meier curves of malignancy-free probability stratified by autoimmune atrophic gastritis vs. non-autoimmune atrophic gastritis status.

Smooth muscle contractile responses to bile acids in mouse ileum require TGR5 but not ASBT

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Background: Many disorders of gut-brain interaction (DGBIs) are more prevalent in women than men and feature alterations in gastrointestinal motility and bile acid homeostasis. Mechanisms by which bile acids regulate gastrointestinal motility are poorly characterized. We recently validated an adapted tissue bath technique using everted mouse ileum, which revealed differential contractile responses to ursodeoxycholic acid (UDCA) and deoxycholic acid (DCA). Here, we aimed to determine whether these responses are dependent on host sex, the plasma membrane bile acid receptor TGR5, or the apical sodium-dependent bile acid transporter ASBT.

Methods: Ileal segments from male and female mice were everted and suspended in tissue baths. Contractile responses to physiologic concentrations of UDCA and DCA were quantified with or without TGR5 or ASBT inhibitors. Phosphorylation of extracellular signal-regulated kinase (ERK) and myosin light chain (MLC), markers of TGR5 activation and smooth muscle contraction, respectively, were assessed with western blot.

Results: There were no sex differences in the dose-dependent contractile responses to bile acids. At 100 µmol/L, UDCA but not DCA increased MLC phosphorylation and increased contractility. TGR5 inhibition decreased ERK phosphorylation and led to decreases in contractility, phosphorylated MLC, and surprisingly, total MLC. ASBT inhibition did not affect contractile responses.

Conclusion: Differential effects of UDCA and DCA on ileal smooth muscle contractility are not dependent on host sex or ASBT-mediated transport. Bile acids signal through mucosal TGR5, which regulates smooth muscle contractility by complex mechanisms. Understanding how bile acids differentially regulate gastrointestinal motility could facilitate new therapeutic options for specific DGBIs.

4-Methylumbelliferone modulates gut microbiota to inhibit liver fibrogenesis

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4-Methylumbelliferone (4-MU) is the active component of the Hymecromone, a choleretic and antispasmodic treatment approved in Europe and China, with great safety profile. It is also widely used to inhibit hyaluronan (HA) synthesis in research. Given a positive correlation between circulating HA levels and the severity of liver fibrosis, 4-MU has been tested in several animal models of liver fibrosis. In most of the experimental settings, 4-MU showed great efficacy in reducing liver fibrosis severity, regardless of the etiology of the disease, igniting the interest to repurpose the compound for treating liver fibrosis. In our report, we primarily used a colin-deficient, amino acids defined, high-fat diet (CDAHFD) treatment regimen to establish liver fibrosis in mice, which captures many characteristics of liver fibrosis in nonalcoholic steatohepatitis (NASH). We showed 4-MU effectively inhibits liver fibrosis in mice treated with CDAHFD. To test whether 4-MU works through suppress HA production, several transgenic mouse models with either enhanced HA production or digestion in different liver cell populations were exposed to CDAHFD, and all showed reduced liver fibrogenesis, highlighting a complicated role of HA in liver fibrogenesis. Even though 4-MU is a choleretic agent in humans, it did not modulate liver, gut, or stool bile acids (BAs) content, nor affect intestinal FXR, which is activated by BAs. In contrast, 4-MU reshapes gut microbiota and gut microbiota ablation significantly blunts 4-MU's anti-fibrosis property. In summary, elevated HA accompanies development of liver fibrosis, but its role in fibrogenesis is complicated, and 4-MU ameliorates liver fibrosis via an FXR-independent, gut microbiota-dependent mechanism.

Highlights:

- 4-MU inhibit NASH fibrosis
- Has2 overexpression prevents HSC activation but promotes myofibroblast proliferation and ECM buildup after HSC is activated.
- Intestinal epithelial cell FXR is not needed for 4-MU to reduce liver fibrosis.
- Hepatocyte FXR is not needed for 4-MU to reduce liver fibrosis.
- The gut microbiota is partially responsible for 4-MU's anti-fibrotic effect in the liver.

Crucial Role of Secretory Organelle Dynamics in Pancreatic Acinar Cell Response to Injury, Metaplasia, and Tumorigenesis

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In the pancreas, repeated acute pancreatitis and chronic pancreatitis cause an increased risk of progression to pancreatic intraepithelial neoplasia (PanIN) and pancreatic ductal adenocarcinoma (PDAC) via a metaplastic event in the secretory acinar cell known as acinar-to-ductal metaplasia (ADM). ADM proceeds through an evolutionarily conserved cell program called paligenosis, a three-step process (autophagy, metaplastic gene expression, and proliferation) that terminally differentiated cells use to re-enter the cell cycle.
Little is known about the precise changes that occur in this crucial secretory translation axis during paligenosis and how this process is involved in tumorigenesis in pancreatic acinar cells. Our work here will show that one of the most obvious changes that occur during paligenosis is in the organelles required for secretion, in particular the ribosomes associated with the rough endoplasmic reticulum (rER).

We observed that during the initial catabolic phase of paligenosis, an almost simultaneous reduction in the amount of ribosomal small and large subunits and the amount of ER was noted, which occurs through both autophagy and the ubiquitin-proteasome system. We also found that the functional and structural remodeling of ribosomes and the ER occurred through two injury-responsive, ribosome-related proteins, IFRD1 and ZFP36L1. An 80S-assembled ribosome-binding protein, IFRD1, also known as TIS7, increased and specifically inhibited the translation of the ribosome it was bound to, thereby serving as a ‘brake’ on ER translation. Next, ZFP36L1, also known as TIS11B, accumulated near the ER, serving as a haven to protect ribosomes and mRNAs, in particular AU-rich cytosolic transcripts, required for anabolic growth.
Next, we showed that those ribosome salvaging mechanisms collaborate with ribosome biogenesis in the later, anabolic stage of paligenosis. Loss of *Nat10*, which we show is required for ribosome biogenesis, blocked both progression through the cell cycle and post-injury restoration of secretory cell architecture. The *Nat10*Δ/Δ phenotype was ameliorated when we also deleted the tumor suppressor p53, indicating ribosome biogenesis is a cell proliferation licensing step for paligenosis.

Finally, we showed that loss of paligenotic regulation of the dynamic ribosome changes we have observed blocks not just paligenosis but also prevents the progression of cancer at the pre-cancerous stages: deletion of *Nat10* blocks development of PanIN lesions in the *Kras G12D* mutant mouse model of PDAC tumorigenesis.

These findings provide important answers and further understanding of how paligenosis, specifically dynamic regulation of ribosomes, works within acinar cells to fuel PanIN and PDAC, suggesting drugs that target ribosomes might eventually add to our anti-cancer arsenal.

Inequalities in the risk of developing cirrhosis complications over time: a cohort study.

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Background: Current understanding of the rates and trajectories of progression to cirrhosis complications is derived from outdated data. We examined the progression patterns of cirrhosis and their implications, focusing on the different outcomes of prolonged illness *vs*. early mortality using contemporary data. **Methods**: We conducted a retrospective cohort study of adult patients with compensated cirrhosis (CC) from 130 Veterans Affairs hospitals diagnosed between 10/1/2010 and 08/30/2015, with follow-up through 08/31/2023. Utilizing an unadjusted semi-Markov multistate model, we examined transitions from CC to intermediate disease events (ascites, hepatic encephalopathy [HE], esophageal variceal bleeding [EVB], hepatocellular carcinoma [HCC], any two complications, or three or more complications) and, ultimately, to death. We also examined the effects of age and etiology (HCV, alcohol, MASH). **Results**: Of 24,728 patients with CC (median age 62 years, 18% HCV, 25% alcohol, 26% HCV and alcohol, 30% MASH), 30% developed ascites, 10% HE, 9% HCC, and 1% developed EVB as the first decompensation-related event. Most patients with CC progressed to a single complication (50%), 3% experienced ≥2 complications at the onset of decompensation, and 21% died before any complications. In total, 26% remained as CC during a median follow up of 6.2 years. The risk of progressing from 1st complication to a subsequent complication was much higher than that from CC to 1st complication (Figure 1). The risk of developing a 2nd complication was higher in patients with EVB, whereas patients with ascites, HE, or HCC had a higher risk of progressing directly to death (Figure 1). The progression intensity (instantaneous hazard) was the highest from 3 complications to death, followed by from 2 complications to death (Figure 2). Younger patients had an accelerated progression intensity across most transitions. Risk of progression to HCC was higher for patients with HCV than for alcohol related cirrhosis (HR = 2.13, 95% CI: 1.85 – 2.45). More patients with alcohol-related cirrhosis progressed to non-HCC complications and at a faster pace than HCV cirrhosis (HR from CC to ascites for HCV- vs. alcohol-related cirrhosis = 0.53, 95% CI=0.49- 0.57). Progression rates were similar for patients with alcohol and MASH across most transitions. **Conclusions**: In a cohort of patients with compensated cirrhosis, half progressed while others remain compensated, and a large proportion died. The progression between states varied by age/etiology. Transitions accelerated as patients progressed in their clinical course, underscoring the need for future trials to focus on the high yield period before patients develop first complication. These data have implications for counseling patients and planning interventions.

Figure 1. Progression risk at 12 months from entry into a disease state in patients with cirrhosis.

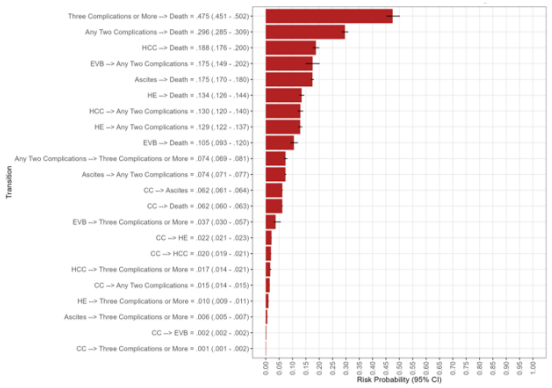
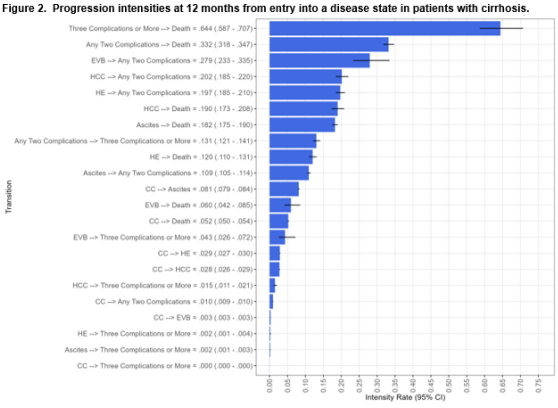


Figure 2. Progression intensities at 12 months from entry into a disease state in patients with cirrhosis.



Stromal antigen 1 as a modulator of program death ligand-1 expression: Implications for colorectal cancer immunotherapy

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Introduction: Immune checkpoint inhibition targeting program death receptor 1 (PD-L1)/program death ligand 1 (PD1) have revolutionized treatment of several malignancies. In colorectal cancer (CRC), immunotherapy has been FDA approved for the subset of cancers that are microsatellite unstable (~20%). These therapeutic antibodies target PD1-PD-L1 interaction prevention, countering the mechanism of immune quiescence elaborated by the tumor. However, the determinants of PD1/PD-L1 expression are incompletely understood as to targeting these receptors/ligands to the cell surface and where it may be activate. However, the determinants of PD1/PD-L1 expression, including targeting of these receptors/ligands to the cell surface and location of activation, remain incompletely understood.

We have shown stromal antigen 1 (SA-1), a high order chromatin remodeler (*Can Res Prev* 2016), is lost in early CRC carcinogenesis as a modality of transcriptional activation. Microarray data indicated that SA-1 loss led to increased PD-L1, and SA-1 overexpression suppressed PD-L1 levels. Furthermore, in silico work showed there may be a physical interaction between SA-1 and PD-L1. We therefore wanted to assess the ability of SA-1 to impact PD1 therapeutics.

Methods: To study the role of SA-1 on PD-L1 expression, we devised an SA-1 shRNA construct to suppress SA-1 in the human CRC cell line HT29. RNA was processed for qPCR of SA-1 and PD-L1. For functional assessment of PD1 working, we devised a co-culture with HT29 shSA-1-construct and natural killer cell line NK 92. NK cells were stained for identification with CD2 and CD69 for activity while HT29 cells were stained for exogenous PD-L1 for flow cytometry. Protein from HT29 cells were harvested for PD-L1 ELISA and immunoblot to assess caspase 8 and cleaved caspase 3. For SA-1-PDL1 linkage, immunoprecipitation (IP) of SA-1 was performed.

Results: SA1 construct showed a 52% reduction in SA1 mRNA ($p<0.006$) while PD-L1 was increased by 99% . ELISA revealed a 30% increase of PD-L1 with SA1 shRNA ($p<0.04$). Flow cytometry revealed the SA-1 construct induced PD-L1 expression by 61% ($p<0.001$).

HT29 SA-1 shRNA construct co-culture with NK92 resulted in an upregulated CD69 in NK92 (20%, $p<0.003$). Immunoblot of co-cultured HT29 cells+SA-1 shRNA suggested less extrinsic apoptosis (Table 1). IP for SA-1 in HT29 cells saw banding for PD-L1.

Conclusions: For the first time we show SA-1 modulates PD-L1 and thus targeted immunotherapy. Specifically, SA-1 loss in colon carcinogenesis leads to increased PD-L1. Moreover, given our demonstration of the physical interaction with SA-1 and PD-1, this implicates SA-1 downregulation allows PD-L1 to escape nuclear/cytoplasmic sequestration and be activated at the plasma membrane. Our work provides novel biological insights and may serve as another biomarker for patients who may benefit from immunotherapy.

Agreement between stomach biopsy culture and stool next-generation sequencing for determining *Helicobacter Pylori* antibiotic susceptibility profiles in a screening predominantly Hispanic U.S. population

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Background: Tailored *Helicobacter pylori* therapy based on antibiotic susceptibility profiling is recommended over empiric therapies. We compared antibiotic susceptibility profiles and eradication success rates among *H. pylori* infected subjects using gastric biopsy culture and next-generation sequencing (NGS) of stool. **Methods**: In this cross-sectional screening study, we prospectively recruited asymptomatic subjects 30-65 years old from Harris Health primary care clinics (Houston, Texas) between April 2021-October 2023. Those with prior *H. pylori* testing or treatment, gastric cancer, or gastric surgery were excluded. Screening for *H. pylori* was done using IgG serology. *H. pylori* positive subjects who consented underwent endoscopy with gastric biopsies (2 antral, 2 corpus pooled) sent for culture. After August 2022, all were also offered single stool NGS *H. pylori* susceptibility testing. All subjects received antibiotic regimens tailored to susceptibility profiles. We determined rates of antibiotic resistance based on biopsy culture or stool NGS along with agreement between the two tests using Cohen's kappa. Successful eradication was determined using stool antigen and compared among treatment regimens. **Results**: Among 210 *H. pylori* infected subjects, 30.5% were men, 1.4% white, 6.2% black, 90.0% Hispanic, and 2.4% Asian. Overall, 79 subjects received both tests, 70 only biopsy cultures, and 61 only stool NGS. Ten subjects had unsuccessful susceptibility profiling using culture, 5 using stool NGS, and 2 using both tests. Resistance rates by culture were metronidazole 57.0%, levofloxacin 23.0%, clarithromycin 8.8%, amoxicillin 2.2%, and tetracycline 0% (Table 1). Resistance rates by stool NGS were metronidazole 27.3%, levofloxacin 33.6%, clarithromycin 12.3%, amoxicillin 0%, tetracycline 1.6%, and rifabutin 0%. The agreement between the two tests was low for clarithromycin (k=0.392) and metronidazole (k=0.191) among 69 subjects tested using both methods. A total 206 subjects received tailored antibiotic therapy, 36.4% of whom received eradication testing. Subjects treated with clarithromycin-triple (n=38) or levofloxacin-triple therapies (n=3) based on culture results had higher eradication rates (94-100%) than based on stool NGS (clarithromycin-triple 83%, n=10; levofloxacin-triple 0%, n=1) (Table 2). Metronidazole-triple therapy had lower eradication rates using culture (33-50%, n=7) than using stool NGS (100%, n=3). **Conclusion**: Overall eradication rates were higher with biopsy culture (92%) than with single stool NGS (83%) except for metronidazole-triple therapy. The level of agreement in *H. pylori* resistance between biopsy culture and stool NGS was substantial for levofloxacin and low for clarithromycin and metronidazole. Studies are ongoing comparing the two tests to better optimize stool NGS for convenient susceptibility profiling.

Conflicts of Interest: Dr. Graham is a consultant for RedHill Biopharma and Phathom Pharmaceuticals regarding novel *H. pylori* therapies and has received research support for culture of *Helicobacter pylori.* He is also a consultant Janssen Research & Development regarding potential gastrointestinal effects of drugs under development and has collaborated on research projects with American Molecular regarding molecular diagnostics for *H. pylori*. The other authors have no conflicts of interest.

Uncovering the mechanism regulating ESRRG function by SIRT1 in gastric parietal cell differentiation

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Background: The differentiation of gastric parietal cells (PCs) from stem cells is poorly understood despite the importance of PCs for gastric health and acid secretion. We have previously shown the nuclear receptor Estrogen-related receptor gamma (ESRRG) is essential for PC specification from isthmal progenitors, however the mechanisms regulating ESRRG transcriptional activity during differentiation remain unknown. As a nuclear receptor, ESRRG function is primarily modulated by interactions with co-regulatory proteins. We hypothesized that Sirtuin 1 (SIRT1) acts as an ESRRG co-activator in early progenitor cells to induce ESRRG transactivation and promote transcription of ESRRG-regulated genes essential for PC differentiation.

Methods: To evaluate the role of SIRT1 in modulating ESRRG activity, we utilized *in vivo* PCs ablation and stomach injury models along with *in vitro* gastric organoid culture. Techniques included single-cell RNA sequencing (scRNA-seq), western blotting (WB), quantitative PCR (qRT-PCR), co-immunoprecipitation (Co-IP), and immunofluorescence (IF) and hematoxylin and eosin staining (H&E) to assess SIRT1-ESRRG binding and the effects of SIRT1 activation on PC differentiation. The SIRT1 agonist resveratrol was used to pharmacologically activate SIRT1 *in vitro* and *in vivo*.

Results: We show for the first time that ESRRG interacts with SIRT1 in a gastric cancer cell line, suggesting SIRT1 may co-activate ESRRG in the stomach. Resveratrol treatment enhanced PC differentiation in gastric organoids and increased PC counts during homeostasis and injury recovery in mouse models. Resveratrol facilitated SIRT1 nuclear localization in PCs, likely enabling SIRT1-ESRRG interaction.

Conclusion: Our findings suggest activating SIRT1-ESRRG co-regulation induces PC differentiation, representing a novel therapeutic approach for treating gastritis by promoting PC regeneration using FDA-approved compounds like resveratrol. Additional studies are needed to further elucidate the molecular mechanisms underlying SIRT1-ESRRG signalling in gastric progenitor cells, as well as to establish the therapeutic potential of pharmacological activation of this pathway for improving PC differentiation and gastric health.

Essential role of p53 in intestinal epithelium homeostasis and stem cell differentiation

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INTRODUCTION: Tumor suppressor *p53* regulates self-renewal and differentiation in some adult stem cells but its role in intestinal stem cells remains to be fully described. Intestinal epithelium homeostasis is maintained by two main pools of stem cells: *Lgr5+* and reserve stem cells. We recently reported that *Krt15+* cells also harbor self-renewal, multipotent and regenerative capacities. Therefore, we hypothesize that *Trp53* loss specifically in *Krt15+* stem cells will perturb epithelial homeostasis and differentiation.

METHODS: We generated *Krt15-CrePR1;Trp53*$^{fl/fl}$ (*Krt15*$^{\triangle}$*Trp53*) mice and induced Cre recombination by injecting RU486 (PR agonist). Mice were euthanized at different time points after Cre recombination. Organoid cultures were established from the intestine.

RESULTS: Two months following Cre recombination, intestinal morphology wasn't severely affected. However, we observed significant changes in organoid cultures especially following Wnt hyperactivation. Indeed, contrarily to control organoids which formed cystic structures following GSK3 inhibition, *Trp53*-depleted organoids remained mainly budded structures suggesting that differentiation is still occurring. These results correlated with increased expression of Paneth cell-specific genes and decreased expression of EphB2 receptor and its target genes, a pathway important in cell fate. Twelve months following Cre recombination, *Trp53* loss specifically in *Krt15+* cells led to several morphological alterations in the small intestine. Increased crypt length and villi width was observed in *Krt15*$^{\triangle}$*Trp53* mice but cell proliferation wasn't affected. However, number of Paneth cells was increased, and aberrant presence of goblet cells was noted in *Krt15*$^{\triangle}$*Trp53* mice. We also observed increased number of Tuft and goblet cells in the villi of experimental mice. Surprisingly, we observed some crypt cells that expressed goblet and Paneth cell markers and decreased Notch pathway activation, suggesting that *Trp53* loss leads to a dysregulation of secretory cell lineages. Brief, our results suggest that loss of *Trp53* in *Krt15+* stem cells affects intestinal morphology and cell fate decision potentially through Ephrin signaling.

Mitigating cholestasis in TPN-fed piglets via enteral agonism of farnesoid X receptor-fibroblast growth factor 19 axis

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Infants who cannot tolerate enteral feedings are administered total parenteral nutrition (TPN) to ensure the delivery of necessary nutrients. Long-term use of TPN can result in cholestasis. Enterally fed bile acids have been used as a previous mitigation strategy during cholestasis to support normal bile acid metabolism, but new, more effective, non-bile acid therapeutic agents are being developed. The study objective was to determine if treatment with the bile acid ursodeoxycholic acid (UDCA), a non-bile acid Tropifexor (TPX), or fibroblast growth factor 19 (FGF19) will activate the farnesoid X receptor (FXR)-FGF19 axis and prevent or mitigate cholestasis. For three weeks, piglets received TPN and were treated enterally with either UDCA (25 mg/kg*day) or TPX (7.5 µg/kg*day) in minimal milk-based formula (12 mL/kg*day), or with daily intravenous FGF19 (0.25 mg/d) injection. Control and FGF19 pigs also received minimal milk-based formula (12 mL/kg*day). After three weeks, plasma markers for cholestasis including total bile acids, bilirubin, and gamma-glutamyl transferase were lower in pigs that received TPX compared to the control treatment. TPX increased ileal mRNA abundance of FGF19, ileal lipid binding protein, and organic solute transporter α compared to control pigs. All treatments reduced the mRNA abundance of apical sodium-dependent bile acid transporter compared to the control treatment. TPX also increased mRNA abundance of hepatic small heterodimer protein and bile salt export pump. Total bile acid concentrations were lower in the plasma and higher in the colon of TPX treated pigs compared to controls. The majority of all bile acids were glycine conjugated, and UDCA treated pigs had the highest conjugated UDCA in the plasma, liver, and distal ileum compared to the control; the latter suggesting enterohepatic recirculation of conjugated UDCA. In control and FGF19-treated pigs, the dominant bile acid in the liver is conjugated hyocholic (HCA) while conjugated CDCA and conjugated UCDA are dominant in TPX and UDCA pigs, respectively. In the distal ileum, conjugated HCA is the dominant bile acid in all treatment groups. Overall, UDCA and FGF19 had minimal effects on the FXR-FGF19 axis, while TPX prevents cholestasis in TPN-fed piglets.

Phase 3 Biomarker Study for HCC Surveillance Using a novel HES v2.0 Algorithm. A Prospective Collection with Retrospective Blinded Evaluation

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Background: Several blood-biomarker panels are promising surveillance tests for early HCC detection in patients with cirrhosis. However, missed cancers (low sensitivity) and false positive tests challenge the use of currently available blood-biomarker panels, including alpha-fetoprotein, triple biomarkers (AFP, lens culinaris agglutinin-reactive AFP [AFP-L3], and des-gamma-carboxy prothrombin [DCP]) and GALAD (a panel of triple markers, age, gender). We previously developed and validated *HCC Early Detection Screening (HES) algorithm v1.0,* which contains AFP, alanine aminotransferase, platelet count and age. Here, we developed *HES v2.0.* with additional biomarkers and evaluated the comparative performance of HES v2.0 with GALAD for HCC detection.

Methods: We used harmonized data from 2 prospective cohort studies from seven centers in the U.S. We enrolled patients with cirrhosis and no past or present HCC followed in a surveillance program consisting of liver imaging (mostly ultrasound) combined with AFP. Blood samples were prospectively collected every 6 months and underwent retrospective blinded assays for AFP, AFP L-3 and DCP. We developed HES v2.0, which incorporates AFP-L3% and DCP in addition to the change in AFP level, AFP-L3 and DCP level in the prior year, in combination with age, ALT, platelets and etiology (HCV, alcoholic liver disease, neither). Patients were followed until development of HCC, transplantation, death, or 07/2022. We calculated sensitivity at a fixed 10% false positive rate (FPR) for detecting HCC for AFP, AFP-L3DCP, and GALAD. For HES v2.0, we used 10-fold cross validation to estimate its sensitivity at 10% FPR. Sensitivity is the probability of at least one positive screen in HCC cases within 6, 12 or 24 months before HCC diagnosis. The FPR is the probability of a positive screen in all the screenings conducted in control patients.

Results. A total of 2437 patients contributed to a total of 6811 surveillance episodes, of whom 149 patients developed HCC. We limited the analysis to 118 HCC cases and 2221 controls with complete information on biomarkers. The mean age of the cohort was 59.8 years (SD 10.2) and 36.6% were women. The cirrhosis etiology was HCV in 36.7%, alcohol 17.0%, and NAFLD in 33.1%. At a fixed 10% FPR, patient-level sensitivity of HES v2.0 was 52.7, 48.1 and 46.7% within 6, 12 and 24 months prior to HCC diagnosis, which was higher than corresponding values for GALAD (46.7, 41.4 and 36.3%, respectively), triple biomarkers, or any of the individual biomarkers (Table 1).

Conclusions: In North American patients with cirrhosis, HES v2.0 results in a considerable improvement in sensitivity for HCC detection without any increase in false positive results compared with GALAD. External validation of HES v2.0 in is required.

Table 1. Sensitivity of biomarkers and biomarker panels at a fixed FPR of 10% for HCC detection

	6 months prior to HCC diagnosis	12 months prior to HCC diagnosis	24 months prior to HCC diagnosis
AFP	35.6	35.7	30.4
AFP-L3%	37.8	41.4	38.2
DCP	33.3	31.4	31.4
GALAD	46.7	41.4	36.3
HES V.2	52.7	48.1	46.7

FPR: False Positive Rate

Serum Metabolic Biomarker Signature Is Predictive of the Risk of Hepatocellular Cancer in Patients with Cirrhosis

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Background. While cirrhosis is the precursor for hepatocellular carcinoma (HCC), the annual risk of HCC in cirrhosis is only 1-3%. Risk stratification of HCC among patients with cirrhosis can allow for more precise prognostication, targeted prevention and early detection. We previously developed a HCC risk stratification model, which contains demographic (race, ethnicity, and sex), clinical (liver disease etiology) and lifestyle (alcohol, smoking, BMI) features. Several inflammatory and metabolic biomarkers including insulin resistance, adipocytokines, proinflammatory and anti-inflammatory cytokines have been associated with HCC risk. We sought to develop a robust metabolic biomarker panel predictive of HCC, and test its incremental value to our base predictive model. **Methods.** We used data from a prospective cohort of adult patients with cirrhosis recruited at 8 liver clinics in 4 cities between 12/2016 and 8/2021 and followed until development of HCC, transplantation, death, or 12/31/2021. We reviewed published literature and identified potential metabolic biomarkers reported to be associated with HCC risk. Subsequently, we utilized custom designed FirePlex immunoassays to measure serum levels of these 39 promising biomarkers of metabolic dysfunction and inflammation. Next, we used stepwise regression and the least absolute shrinkage and selection operator (Lasso) to identify optimal panels of biomarkers that were predictive of HCC. Using the Fine-Gray competing risk model, we estimated the association of the metabolic panels with HCC risk. C-index and AUROC was used to evaluate model discriminatory performance. The incremental predictive value of metabolite panel was evaluated by comparing its predictive performance to our validated base model. **Results.** We analyzed data from 2,446 patients with cirrhosis, of whom 112 developed HCC. The mean age was 57.5 years, 37.6% were women, 51.2% non-Hispanic White, 30.6% Hispanic, and 16.0% were African American. HCV was present in 34.3% (20.6% cured), NAFLD in 34.3%, alcohol-related liver disease in 28.1%, diabetes in 46.9% and overweight/obese in over 50%e. We identified a 9 biomarker panel (P9) (Table 1). Adding P9 to the base model (with race, ethnicity, sex, liver disease etiology, alcohol, smoking, BMI) increased the C-index for overall predictive discrimination from 0.72 to 0.74 and 0.75, respectively (p=0.03), and also improved the AUROC at 1 and 2 years by 12% and 14%, respectively (p < 0.0001) (Table 2). **Conclusions.** We identified a biomarker panel of 9 circulating serum biomarkers that is significantly associated with HCC risk among patients with cirrhosis. This biomarker panel improves the predictive ability of a risk stratification model that contains clinical and lifestyle factors. Further validation of these findings is required.

Table 1. The components of the biomarker panels
IGF_1
IL_10
Latent_TGF_beta_1
Adipsin
Fetuin_A
IL_1_beta
Macrophage Stimulating Protein alpha chain (MSPa)
Serum Amyloid A (SAA)
TNF_alpha

Table 2. Performance characteristics of HCC risk stratification models with and without metabolic biomarker panel.

	C statistic (95% CI)	1-year AUC (95% CI)	2-year AUC (95% CI)
Base model (race, ethnicity, sex, liver disease etiology, alcohol, smoking, BMI)	0.719 (0.660, 0.778)	0.719 (0.623, 0.815)	0.725 (0.647, 0.803)
Base Model +P9	0.753 (0.698, 0.808)	0.790 (0.702, 0.878)	0.769 (0.696, 0.842)

Purinergic Signaling Drives Multiple Aspects of Rotavirus Pathophysiology

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Background: Rotavirus causes life-threatening diarrhea in children, resulting in ~200,000 deaths each year. Rotavirus infects a limited number of cells at the tips of the villi in the small intestine; however, rotavirus is known to dysregulate cells distant from the site of infection through paracrine signaling. We recently identified that rotavirus infected cells release the purinergic signaling molecule ADP, which binds to the P2Y1 receptor on nearby uninfected cells; causing a signaling cascade that affects distant uninfected cells. *In vitro* P2Y1 was found to contribute to chloride and serotonin secretion; suggesting P2Y1 may activate pathways responsible for diarrhea. **Hypothesis:** We hypothesize that P2Y1 orchestrates cell-cell communication involved in diarrhea and infection severity. **Methods:** To elucidate the role of purinergic signaling via P2Y1 receptors during rotavirus infection, we used the mouse-like rotavirus (rD6/2) to investigate the effects of purinergic signaling in the context of homologous murine rotavirus infection *in vivo*. C57Black6 mouse pups were orally gavaged rD6/2 rotavirus at day 4-6 of age and assessed over the course of 5 days. Beginning at day 1 post infection, infected pups were treated daily by oral gavage with saline or 4mg/kg MRS2500, a P2Y1-selective, competitive antagonist. Stool was collected and scored for diarrhea daily prior to each treatment. Pups were euthanized and small intestine tissue was collected at 3- and 5-days post infection for immunostaining, qRT-PCR and luminal contents. Intestinal organoids derived from Balb/C pup jejunum were inoculated with rD6/2 rotavirus, followed by supplementation with vehicle or MRS2500 to assess fluid secretion by organoid swelling over time. **Results:** Rotavirus-infected pups exhibited significant diarrhea incidence and high diarrhea scores on days 2, 3, and 4 post infection. Treatment of rotavirus-infected mouse pups with MRS2500 resulted in decreased severity and incidence of diarrhea compared to vehicle treated controls. This was consistent with i*n vitro,* rD6/2-infected organoids exhibiting a significant increase in organoid swelling compared to uninfected organoids, which was significantly prevented by inhibition of P2Y1 signaling. Viral stool shedding from MRS2500 treated pups was significantly lower than saline-treated pups starting at day 4 post infection, which suggests P2Y1 signaling may amplify rotavirus replication *in vivo*. Finally, transmission experiments in litters with infected (but not treated) and pretreated MRS2500 uninfected pups show a decreased incidence in diarrhea suggesting that P2Y1 inhibition has a potential protective role in reducing the effects of transmission. **Conclusion**: Collectively these findings establish a conserved role of purinergic signaling in the pathophysiology of rotavirus infection. Additionally, our data indicates P2Y1 as a new candidate for host-targeted therapeutics that could have both antiviral and antidiarrheal effects against rotavirus pathophysiology.

P2Y1 receptor signaling influences epithelial responses to intestinal inflammation

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Background: Purines are among the most influential and ancient biochemical molecules in evolutionary history. In recent years, several studies have identified that nucleotides are released from cells during infection and tissue injury and serve as paracrine signaling molecules that help coordinate host defense and repair responses. These important extracellular signaling molecules activate purinergic receptors, of which there are 19 different purinergic receptor subtypes, which are classified into P1 and P2 receptors. Several studies have revealed crucial roles for P2 purinergic receptors during inflammatory and infectious diseases; however, these studies have largely examined purinergic signaling in immune cells and the enteric nervous system. Yet, while epithelial cells serve as the first barrier against infection and inflammation, the role of purinergic signaling within the gastrointestinal tract remains largely unknown. Our group recently discovered a novel and prominent role for P2Y1 in mediating host responses during rotavirus infection. To extend these studies, we sought to elucidate the P2 purinergic receptor repertoire expressed within gut epithelium and assess their functional role during insult. **Methods & Results:** Using the Human Protein Atlas, we queried single-cell RNA sequencing data for P2 purinergic receptor expression in small intestine, colon, and rectum. *In silico* analysis revealed high expression of P2Y1, P2Y2, P2Y11 and P2X4 among the purinergic receptors throughout the gastrointestinal tract. Interestingly, P2Y1 showed highest expression in intestinal enterocytes and goblet cells. Further, we compared P2 receptor expressions between commonly used, cancer-derived intestinal cell lines (T84, HT29 and Caco2) and crypt-derived human intestinal organoids (HIOs). Both HIOs and T84 and Caco2 cell lines had similar mRNA expressions of P2Y1, P2Y2, P2Y11 and P2X4. However, live calcium imaging studies showed robust responses to P2Y receptor-specific agonists in HIOs, particularly P2Y1 and P2Y2 agonists, but the cancer-derived cell lines failed to respond to these agonists, suggesting these cell lines have defects in P2Y signaling. To assess alterations in P2 receptor expression in intestinal inflammation, we turned to RNAsequencing data from ulcerative colitis (UC) patients (GSE38713 and GSE75214) and observed that P2Y1 expression was significantly reduced in inflamed tissue. *In vitro* functionally, blocking P2Y1, but not P2Y2, significantly delayed wound healing in epithelial scratch assays, indicating a potential role in epithelial injury repair. To delineate the role of epithelial P2Y1 *in vivo*, we generated $Villin^{Cre}$ $P2Y1^{flex/flox}$ mice. At baseline, the knockout animals did not exhibit overt intestinal defects; however, in the DSS-induced colitis model, the epithelial P2Y1 KO mice had significantly greater weight loss and epithelial damage than their wildtype littermates. **Conclusions**: These findings confirm that the gastrointestinal tract express P2Y1, P2Y2, P2Y11 and P2X4 purinergic receptors and P2Y1 signaling plays important functional roles in both gastrointestinal epithelial homeostasis and repair of injury.

Probiotic *Limosilactobacillus reuteri* DSM 17938 alters gut microbiota and reduces circulating monocytes in immunocompromised RAG1KO mice

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Background: Autoimmune conditions involve both innate and adaptive immunity. Probiotic DSM 17938 has been shown to modulate adaptive immunity including inflammatory and regulatory T-cells in mouse models of necrotizing enterocolitis, IPEX syndrome, and multiple sclerosis. However, it is unclear how this specific strain affects innate immunity. Therefore, RAG1KO mice (T- and B-cell deficient) were used to test our hypothesis that oral administration of DSM 17938 by gavage would result in a more diverse microbiota and improved immune responses.

Objective: To evaluate the effect of DSM 17938 on gut microbiota and innate immunity in RAG1KO mice.

Methods: Mice (WT and RAG1KO, 8-9 weeks old) were fed DSM 17938 10^7 CFU/mouse/day or controlled media (MRS) by gavage three times a week for 28 days. Cecum to rectum content was collected for 16S rRNA sequencing for microbiome analysis. The blood immune cells including T-cells, B-cells, natural killer cells, monocyte/macrophages and dendritic cells were analyzed by flow cytometry using immune cell specific markers. Tissues including liver, small intestine, and colon were evaluated histologically.

Results: Probiotic treatment changed gut microbiota, with a significant increase in alpha diversity evaluated as Faith PD ($p=0.001$) and Observed Species ($p=0.01$) compared to control treatment. Bacterial clusters in the probiotic-treated group were significantly shifted from the controls ($p<0.0001$). Microbial composition included phyla Firmicutes (45.6-66.2%) and Bacteroidota (32.5-47.5%). *Anaeroplasma* and *Clostridia* have been shown to be positively correlated with the severity of multiple sclerosis, and we previously showed they were reduced by oral administration of DSM 17938. *Alistipes*, considered a beneficial microbe belonging to the *Rikenellaceae* family, are typically depleted in autoimmune diseases. The relative abundance of *Alistipes* was significantly reduced in RAG1KO mice compared to WT control ($p=0.0072$). However, we found a reduction in relative abundance of genera *Anaeroplasma* and *Clostridia*, and increased abundance of *Alistipes* in probiotic fed mice compared to RAG1KO mice without probiotic. Blood $CD11b^+$ monocytes were increased in RAG1KO mice compared to WT ($p=0.0149$). Amongst these cells, $Ly6C^+$ ($p=0.0103$) and $F4/80^+$ ($p=0.0124$) were reduced by DSM 17938 treatment. No histological inflammation in tissues including liver, small intestine and colon was noted in RAG1KO mice-fed probiotic DSM 17938.

Conclusions: Probiotic DSM 17938 modulates innate immunity and gut microbiota. Specifically, the $Ly6C^+$ monocyte subset is reduced, which is known to precede influx of pro-inflammatory macrophages in the gut. DSM 17938's anti-inflammatory effects could aid in treatment of immunodeficiencies such as severe combined immunodeficiency (SCID) and other inborn errors in innate immunity.

DO Gastrointestinal Symptoms Correlate with GI Inflammation or Gut Permeability in Children with Autism Spectrum Disorder?

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Background: Autism spectrum disorder (ASD) is becoming increasingly common in children with 1 in 36 children affected. Gastrointestinal (GI) symptoms including selective eating, constipation, diarrhea, bloating, and gastroesophageal reflux are present in 23-70% of children with ASD. The hypotheses for GI symptoms in ASD include alterations in the gut microbiome, 'leaky gut,' and 'brain–gut' axis. We hypothesized that children with severe symptoms of ASD have worse GI symptoms, alteration in gut permeability, and inflammation. Zonulin, a peptide that regulates intercellular tight junctions is considered a marker of intestinal permeability. Fecal calprotectin is a marker for intestinal inflammation. S100A9, a component of the inflammatory cascade, is upregulated in inflamed tissues. S100B is a glial cell marker of brain inflammation.

Objective: To assess the association between GI symptoms, intestinal inflammation, and permeability with ASD severity in children.

Methods: Children (n=315) with suspected ASD with no other preexisting neurological or GI disorder were screened and diagnosed using Social Communication Questionnaire (SCQ), Autism Diagnostic Interview–Revised (ADI-R), and Social Responsiveness Scale, 2nd Ed. (SRS-2, T-score, and raw score). GI symptoms were assessed using the GI severity index (GSI). Plasma zonulin, S100A9, fecal calprotectin, and S100B levels were measured using ELISA. Statistical analysis was performed using the univariable General Linear Model (GLM).

Results: Sixty-two children (76% males, 24% females) with median age 8.6 years and normal BMI, CRP, WBC, ESR, and alanine aminotransferase levels were included. 24% of patients were nonverbal. Mean GSI score in ASD patients was 7.52±2.39 of total 14. Non-verbal children and boys showed a statistically significant increase in SRS Raw scores ($p < 0.01$ and < 0.05 respectively). Nonverbal boys had a trend toward higher scores on GSI (8.7±3.1) and SCQ (27.2±4.4) compared to verbal boys. Plasma zonulin (5.99±3.06 ng/mL) approached a significant positive correlation with SCQ score ($p = 0.06$). SRS T score and Raw score showed a significant correlation with GSI ($p=0.03$ and $p=0.06$ respectively), indicating worse GI symptoms in children with more severe ASD. GSI did not correlate significantly with zonulin, S100A9, or fecal calprotectin ($P=0.21$, 0.52 and 0.33 respectively). S100B levels were uniformly low.

Conclusions: Children on the autism spectrum with severe ASD, especially nonverbal males, have worse GI symptoms which leads to significant morbidity. In our single center sample, there was no suggestion of gastrointestinal or systemic inflammation in children with ASD. However, there was a marginally significant correlation between ASD severity and zonulin levels, suggesting altered gut permeability in a small subset of children with ASD.

Evaluating potential norovirus antivirals in human intestinal enteroids

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Human noroviruses (HuNoVs) are the leading cause of foodborne gastroenteritis across all age groups. Despite the substantial global disease burden, there are currently no targeted antivirals approved for the treatment or prevention of HuNoV gastroenteritis. We utilized a standardized pipeline to screen potential HuNoV antivirals that were identified through structure-based studies, biochemical analyses, and medicinal chemistry-based approaches.

We first determined the half maximal tissue culture infectious dose ($TCID_{50}$) for different HuNoV strains in jejunal human intestinal enteroid (HIE) lines. We then tested the effect of potential antivirals on the replication of 100 $TCID_{50}$s of each virus by RT-qPCR. For drugs identified through structure-based studies and biochemical analyses, we tested three concentrations of potential inhibitors and carried out lactate-dehydrogenase assays in tandem to quantify the cytotoxicity of each inhibitor. For a high throughput screen of compounds synthesized through medicinal chemistry studies, we assessed the effect of 10 μM compound on virus (100 $TCID_{50}$s) replication.

A peptidomimetic antiviral drug rupintrivir that inhibited the prototype GI.1 and globally-dominant GII.4 proteases in a fluorescence resonance energy transfer (FRET) based assay did not inhibit the replication of either strain when tested in HIEs. However, when evaluated in combination with ritonavir, GI.1 HuNoV replication was inhibited. The combination of rupintrivir and ritonavir was not cytotoxic in HIEs at any of the tested concentrations. The inhibitory effect seen when rupintrivir and ritonavir are used together suggests a need for further exploring the combination approach to testing HuNoV antivirals. SYC-2626, a newly discovered quinazolinone compound with activity against Zika and dengue viruses significantly inhibited the replication of GII.4 and GII.3 HuNoV strains by >90% at 40 μM and above. Screening ~100 analogs of SYC-2626 in HIEs is on-going. Additional studies include half maximal inhibitory concentration (IC_{50}) analyses and cytotoxicity assays for the newly identified antivirals.

The development of a standard pipeline for antiviral testing in HIEs allows for screening several classes of compounds as inhibitors of HuNoV replication. Prioritized compounds can then be tested in multiple HIE lines to assess differences in potency between donors. Together, these studies can lead to the identification of novel therapeutics for HuNoVs.

The ephrin receptor A7 (*EPHA7*) gene may regulate enteric nervous system development in human intestinal organoids

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Introduction: Intestinal failure is a life-threatening condition that currently lacks a definitive treatment strategy. Human intestinal organoid (HIO)-derived tissue-engineered intestine is a promising cure; however, substantial challenges preclude clinical application. One of these challenges is optimizing the development of the enteric nervous system (ENS) within HIOs. Ephrin receptor A7 (EPHA7) belongs to of the largest known subfamily of receptor tyrosine kinases, the Eph receptors, and their corresponding family of ligands called ephrins. The ephrins and Eph receptors have been implicated in developmental events, particularly to guide the spatial patterning of axons, cells, and connections within the nervous system. In this study, we aimed to understand if *EPHA7* gene and EPHA7 protein expression in transplanted (tHIOs) impacted ENS development.

Methods: HIOs and enteric neural crest cells (ENCCs) were generated from human embryonic stem cells and co-cultured *in vitro* for 28-40 days. HIOs with or without ENCCs were transplanted into adult immunodeficient mice and harvested between 6-20 weeks. Epithelial and ENS developmental maturity were determined by grading the epithelium with H&E as previously described and the ENS with immunohistochemistry (IHC) for neuronal marker TUJ1 on a scale of 0-4, with 0 being absent and 4 recapitulating human small intestine controls. RNA was isolated and bulk RNAseq was performed and analyzed. IHC was performed for EPHA7 and the epithelial expression was quantified utilizing ImageJ.

Results: Bulk RNAseq demonstrated differential upregulation of *EPHA7* expression between tHIOs without ENS (ENS Grade 0) and tHIOs with a more developmentally mature ENS (ENS Grade 3). IHC demonstrated EPHA7 epithelial expression in the majority of tHIOs and human small intestine controls, and the level of expression was similar between tHIOs with ENS Grades 0-3.

Conclusion: While the upregulated *EPHA7* expression in tHIOs with a more developmentally mature ENS versus tHIOs without ENS suggests a potential regulatory impact of *EPHA7* expression on ENS development and maturity, EPHA7 epithelial expression did not appear to have the same effect. It remains unclear how *EPHA7* may influence ENS development in tHIOs. Further studies are needed to better understand the role of ephrins and Eph receptors during ENS development within tHIOs.

Coagulation Factor Genes Identified as Potential Candidates Driving Alcohol-Associated Acute Pancreatitis in Aging Mice via Spatial Transcriptomic Profiling

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Background: The rapidly aging population is consuming more alcohol, resulting in increased alcohol-associated acute pancreatitis (AAP) with high mortality. However, the underlying mechanisms are poorly understood, and no specific therapies are available. This study aimed to establish an AAP mouse model mirroring aging individuals and used Visium spatial transcriptomics to analyze pancreatic tissue for spatial gene expression differences between aging and young mice with AAP. The goal was to identify key AAP pathophysiology genes in aging for potential therapeutic targeting. **Methods**: AAP was induced in aging (18 mo) and young (3 mo) C57BL/6 male mice via alcohol diet and cerulein injection. Pancreata were collected for acute pancreatitis (AP) assessment (n=4 mice/group) and Visium spatial transcriptomics (n=2 mice/group). Bioinformatics identified distinct tissue clusters and spatially distributed differentially expressed genes (DEGs). **Results**: Aging mice showed significantly severe AAP compared to young mice (5.0-fold increase in AP scores, $p<0.05$). Visium analysis revealed eight distinct tissue clusters: five acinar, two stromal, and one islet. These clusters were then merged into three main clusters, acinar, stromal, and islet, for comparative analysis between aging and young AAP mice. Over 1,300 DEGs were identified, with top hits including Mmp8, Ppbp, Serpina3m, Cxcl13, Hamp, Cd177, Fga, Mmp25, Tarm1, and Acod1. Notably, Fga, encoding fibrinogen α chain, showed significant upregulation in acinar cluster (log2FC = 3.2), with concurrent upregulation of genes encoding fibrinogen β and γ chains. Fibrinogen, a key factor in coagulation, contributes to hypercoagulation, potentially exacerbating outcomes in aging AAP. Increased fibrinogen levels are associated with worsened outcomes in elderly patients with AP, suggesting its detrimental role in aging AAP pathogenesis. **Conclusions**: This study uncovers spatial heterogeneity of inflammatory processes in aging AAP mice, highlighting potential molecular drivers such as Fga. These findings offer insights into the dysregulation of the coagulation cascade which can be potentially regulated for therapeutic development.

Doo dots: a dried fecal spot (DFS)-based bioanalytical platform for bile acid quantitation

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Introduction: Dried blood spot (DBS) analysis has existed since the 1960s with Guthrie's implementation of neonatal screening for phenylketonuria (PKU) disease. Since then, whole blood has been the dominant matrix analyzed by the "Guthrie card" platform. Recent adaptations have allowed for oncard plasma processing followed by subsequent bioanalysis. Our team is focusing development effort on creating a dried fecal spot (DFS)-based bioanalytical sampling platform that can be used for analytical microbiology and microbiome applications. To address questions regarding the utility of the DFS platform for the measurement of fecal bile acids, we collected feces from five subjects for each of the following cohorts: 1) healthy individuals, 2) individuals with diarrhea, and 3) Clostridioides difficile-infected patients, and each sample was homogenized and extracted according to our published methods. **Methods:** A 25 µL volume of each homogenized fecal sample extract was loaded onto both sample channels integrated into each Capitainer quantitative DBS (qDBS) device (1 device per study subject), and all DFS samples were allowed to dry overnight. The DFS loaded qDBS devices were placed inside proprietary shipping envelopes in preparation for ambient temperature shipping across country in the standard mail. The residual liquid volume for all fecal sample extracts was immediately frozen at -80°C, and then were shipped under dry ice conditions from Charleston, SC to Houston, TX for bioanalysis. Upon arrival at the Texas Children's Microbiome Center bioanalytical lab, the frozen methanolic sample extracts were stored frozen at -80°C while the DFS samples were allowed to age at ambient temperature for a period of 4-months on a benchtop. Prior to analysis, DFS spots were removed from the device and homogenized by bead-beating in a 200-µL volume of an internal standard (IS) solution that contained 16 deuterated-bile acid analogs at a concentration of 250 nM for each. Additionally, the frozen fecal sample extracts were thawed, and diluted at an IS volume consistent with the DFS preparations so that direct comparisons could be made. All of the samples were analyzed in a single analytical batch using our published liquid-chromatography-tandem mass spectrometry (LC-MS/MS)-based quantitative bioanalytical method using precisely prepared calibration curves that span a linear dynamic range of 2.44-2,500 nM. **Results/Conclusions:** When we plotted the non-conjugated and conjugated primary and secondary bile acid concentration data for all three subject cohorts, we found that the concentration patterns were nearly superimposable between the DFS sample and the traditional methanol sample extract. Furthermore, a two-sided Mann-Whitney U Test showed that there were no statistical differences observed for any of the non-conjugated and conjugated primary and secondary bile acid comparisons made across all three subject cohorts. We concluded there were no stability issues observed for the bile acid content of the DFS loaded onto the qDBS device during ambient shipping and storage (for 4 months). *The convenience of ambient-temperature shipment and storage of DFS samples cannot be overstated* – this would especially be true in instances of at-home metabolite or therapeutic biomonitoring, or when performing analytical microbiology/microbiome assessments of fecal samples sets collected from remote locations across the globe.

Longitudinal dynamics of fecal bile acids in pediatric patients with primary sclerosing cholangitis-ulcerative colitis (PSC-UC) undergoing oral vancomycin therapy (OVT)

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Background: Primary sclerosing cholangitis (PSC) is a rare cholestatic liver disease affecting ~1,500 pediatric patients in the US, with most also suffering from ulcerative colitis (PSC-UC), a subtype of chronic inflammatory bowel disease (IBD). Within ten years of diagnosis, many will develop clinical complications (50%) that may require liver transplantation (30%), and these patients have the highest lifetime risk of developing colorectal cancer amongst all IBD. There is currently no effective medical therapy to delay the progression of liver disease or the onset of clinical complications in PSC. Although the etiology of PSC is poorly understood, dysbiosis of the gut microbiome and bacterial-derived metabolites (i.e., bile acids (BAs) have been implicated in PSC. Given the potential for gut dysbiosis in PSC-UC pathogenesis, an empiric treatment with oral vancomycin therapy (OVT) has evolved and has shown some benefit in individual cases and small-uncontrolled case series. It remains unclear, however, how OVT affects the gut microbiome in this clinical setting. We have developed a targeted metabolomics method to study the dynamic response of fecal bile acid concentrations contained in stool specimens collected from pediatric patients with PSC-UC (pPSC-UC) in response to OVT. **Longitudinal Study:** An IRB-approved prospective longitudinal study (BCM-IRB# H-43759) was conducted at BCM/TCH to examine the fecal and salivary microbiome dynamics in pPSC-UC. The study included the following four groups: i) healthy controls (HC, n=18); ii) UC patients without PSC (UC, n=17); iii) pPSC-UC patients receiving OVT (VPUC, n=10); and, iv) pPSC-UC patients without OVT, i.e., "OVT-free controls" (PUC, n=5). Stool and salivary samples were collected from all subjects at study initiation, and for most (depending on retention in the study) at months 3, 6, 9, and 12; additional collections were made on days 2 and 7 for the VPUC study subjects after starting oral vancomycin therapy (250mg bid, or 125mg qid). **Materials & Results:** 500 mg of stool was processed for microbiome analysis using the OMNIgene® GUT stool collection kit, and the bile acids were extracted from the resultant fecal slurry using an organic solvent system. The bile acid (e.g., CA, β-MCA, CDCA, DCA, UDCA, LCA, GCA, GCDCA, GDCA, GUDCA, GLCA, TLCA, TCA, TCDCA, TDCA, TUDCA) content of the extracted fecal sample extracts were quantified using a reverse-phase chromatography-tandem mass spectrometry (LC-MS/MS) method. In response to OVT therapy, we observed increasing fecal levels of several non-conjugated primary bile acids (CA, CDCA) and decreased levels in several secondary BAs (DCA & LCA) in the post-OVT fecal sample extracts. **Conclusion:** Our unprecedented cohort of longitudinal stool samples collected from pediatric patients with PSC-UC offers a unique opportunity to study the effects of OVT on the dynamics of a targeted set of BAs, which are bioactive metabolic products likely of significant functional relevance.

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Fibrosis of ganglionic intestine may contribute to bowel dysfunction in human Hirschsprung disease

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Introduction: Hirschsprung disease (HSCR) is characterized by a deficit of enteric neurons in the distal bowel. Although the proximal ganglionic bowel is considered normal, many patients continue to experience constipation, enterocolitis, and bowel dysfunction after pull through surgery. Previously, we showed that mouse ganglionic proximal HSCR colon exhibited increased collagen content, dysregulated ECM-related gene expression, and decreased mechanoreceptor expression when compared to the equivalent segment of WT colon. We now aim to characterize the differences in collagen content of human proximal ganglionic intestine of HSCR patients compared to that of age-matched controls.

Methods: Proximal ganglionic intestine segments were collected from patients with HSCR (n=6) and equivalent intestine segments were collected from age-matched controls (n=3). Controls were patients undergoing stoma closure after trauma. Collagen content across the submucosa and muscularis propria were calculated from trichrome staining and compared statistically using a paired t-test after normalization to age-matched controls. To determine the effects of distension alone on collagen content, proximal and distal intestine segments were collected from patients with atresia (n=3), and a similar analysis was performed.

Results: The interstitial collagen content of human proximal ganglionic HSCR intestine was approximately 1.4 times greater than that of proximal age-matched control intestine. This was statistically significant in the submucosa but did not reach a level of statistical significance in the muscularis propria. In comparison, the collagen content of proximal dilated intestine in atresia patients was not significantly different than that of the distal intestine.

Conclusions: Human ganglionic proximal HSCR intestine demonstrated increased collagen content compared to that of age-matched controls. Dilated intestine in patients without HSCR did not demonstrate an increase in collagen content, suggesting that this aberration is a function of HSCR and not distention. This mechanism may underlie the abnormal function of the ganglionic intestine in HSCR.

Characterization of epigenetic changes during the metaplastic process by single-cell RNA sequencing

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Adenocarcinomas, the most common cancers in adults, typically arise from precursor lesions that are induced by chronic injury or inflammation. As normal cells first become metaplastic and then progress to dysplasia and neoplasia, they dramatically change phenotype. Cellular identity and lineage determination throughout development are in large part regulated by epigenomic changes. Given that tumorigenesis is a type of abnormal development, it follows that similar epigenetic changes occur during metaplasia; however, research on this remains relatively scant. Here, we investigate epigenetic changes in mature, digestive-enzyme-secreting gastric chief cells as they reprogram to progenitor-like, mucinous Spasmolytic Polypeptide Expressing Metaplasia (SPEM) cells. The reprogramming process occurs via a stereotypical, evolutionarily conserved sequence of cellular and molecular events known as *paligenosis*. We can induce chief cell paligenosis across the whole body of the stomach by intraperitoneal injections of high-dose tamoxifen (HDT), which kills acid-pumping parietal cells and causes SPEM within 48 to 72 hours. During paligenosis, cells undergo massive ultrastructural remodeling including recycling of the vast majority of the endoplasmic reticulum (ER) that they used for secreting zymogenic enzymes, and they induce expression of a number of mucins as well as progenitor-associated genes like *Sox9*. Thereafter, they re-enter the cell cycle to help regenerate injured tissue. To begin to identify epigenetic factors regulating the large changes in cell structure and gene expression, we are contrasting single-cell RNA-sequencing analysis of HDT-treated mouse gastric corpus vs mice in which parietal cells are directly ablated by a genetic diphtheria toxin model that does not cause SPEM. The sc-RNA-seq confirms loss of parietal cells in both models and SPEM predominantly in HDT. We observe induction of multiple chromatin-modifying genes during paligenosis with few such epigenetic genes expressed in mature chief cells at homeostasis, suggesting epigenetic processes might be more inert within the fully differentiated chief cells than during the dynamic paligenosis process as cells are actively rearranging phenotype. Complementing sc-RNA-seq with proteomic analysis by mass spectrometry, we have identified the DNA methyltransferase DNMT1 as a key epigenetic factor upregulated during paligenosis. DNMT1 has been previously shown to be associated with metaplasia in the stomach of patients infected with *H. pylori,* where both metaplasia and infection are major risk factors for gastric cancer. We confirm with immunostaining that DNMT1 is absent in chief cells at homeostasis but undergoes dramatic increase after HDT treatment. In summary, using single-cell transcriptomic methods, we are demonstrating active epigenetic-related gene expression changes during paligenosis and are following up on one DNA-modifying gene in particular, the DNA methyltransferase, DNMT1, that emerges during this process and has been previously correlated with tumorigenesis of gastric cancer.

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Development of an Algorithm to Automate the Detection of Emergency Presentations Among Pancreatic Cancer Patients

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Introduction: Cancer diagnosis made via emergency presentation (EP), defined as a new cancer diagnosis made following an emergency care episode or unplanned inpatient admission, is associated with worse patient outcomes and greater organizational stress on healthcare systems. Pancreatic cancer has among the highest rates of EPs of all cancer types in European studies, however, remains understudied among U.S. populations. In this study, we developed an automated algorithm to detect pancreatic cancer EPs in the national Veterans Affairs (VA) healthcare system for large-scale study of EP rates and associated outcomes. **Methods:** We performed manual record reviews on a retrospective cohort of pancreatic cancer patients (N=243) diagnosed between 2007-2019 at a tertiary care VA medical center. We classified each patient by EP status (yes/no). An EP was defined as a new cancer diagnosis made within 30 days of a cancer-related emergency department visit. Using EP status determined by record review as the gold standard, we developed, tested, and iteratively refined an automated algorithm to detect pancreatic cancer EPs. We then applied this algorithm to the national VA database (N=11,525 pancreatic cancer cases across 146 VA facilities) between 2007-2019. We used logistic regression analysis to study sociodemographic and clinical characteristics associated with EPs and Cox proportional hazards models to study survival by EP status. **Results:** The developed algorithm had a positive predictive value of 89.5%, negative predictive value of 80.5%, sensitivity of 90.6%, and specificity of 78.5% for identifying EPs among pancreatic cancer patients in the local VA dataset (N=243, 66.7% EP rate). In national VA data, the algorithm identified EPs in 49.9% (5,751/11,525) of newly diagnosed pancreatic cancer cases. In multivariable logistic regression, EPs were associated with more advanced stage disease compared to non-emergency presenters (Table 1). One-year mortality among patients with EPs was 77.3% compared to 59.4% for non-emergency presenters after adjusting for age, race, sex, rurality, and cancer stage (hazards ratio 1.58; 95% confidence interval 1.51-1.66). **Conclusion:** We developed an automated algorithm to identify pancreatic cancer EPs in a national U.S. healthcare system with strong performance characteristics. We found that EPs among pancreatic cancer were common (49.9%), particularly among those with more advanced stage disease, and independently associated with decreased survival. This is a critical first step towards facilitating further large-scale on the topic within national U.S. healthcare systems. Improved understanding of process breakdowns including patient-, provider- and system-related factors that contribute to EPs can help identify important care gaps and inform future quality improvement efforts.

Table 1. Multivariate associations between patient characteristics and outcome (diagnosis of pancreatic cancer via emergency presentation).

Patient Characteristic	Adjusted Odds Ratio (95% Confidence Interval)	P-Value
Age	0.99 (0.99-0.99)	<0.01
Sex: Male	*-reference-*	
Sex: Female	1.06 (0.83-1.35)	0.65
Race/Ethnicity: Non-Hispanic White	*-reference-*	
Race/Ethnicity: Non-Hispanic Black	1.16 (1.04-1.29)	0.01
Race/Ethnicity: Hispanic	1.16 (0.96-1.39)	0.12
Race/Ethnicity: Asian	1.1 (0.51-2.36)	0.81
Race/Ethnicity: Pacific Islander	0.97 (0.59-1.6)	0.9
Race/Ethnicity: American Indian/Alaskan Native	1.02 (0.64-1.64)	0.93
Race/Ethnicity: Other/Unknown	0.88 (0.76-1.02)	0.1
Elixhauser Comorbidity Index	1.02 (1.01-1.02)	<0.01
Geographic Designation: Metropolitan	*-reference-*	
Geographic Designation: Micropolitan	0.81 (0.71-0.92)	<0.01
Geographic Designation: Small Town	0.75 (0.64-0.88)	<0.01
Geographic Designation: Rural	0.81 (0.67-0.97)	0.03
Cancer Stage: 1	*-reference-*	
Cancer Stage: 2	1.42 (1.25-1.61)	<0.01
Cancer Stage: 3	1.44 (1.23-1.67)	<0.01
Cancer Stage: 4	1.85 (1.65-2.06)	<0.01

Eradication rates of empiric *Helicobacter pylori* treatment regimens in a U.S. safety-net population

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Background: Although tailored treatment of *Helicobacter pylori* is recommended, susceptibility testing in the community is limited. Determining eradication rates of treatment regimens may serve as a surrogate of antibiotic resistance. Our aim was to compare the eradication rates of empiric treatment regimens among *H. pylori* infected patients in a U.S. safety-net hospital system.

Methods: In this retrospective cross-sectional study, we included adult patients positive for *H. pylori* from July 2021-April 2022 in the Harris Health System (Houston, Texas). Using EPIC Slicer Dicer (electronic medical record-based data mining tool), we identified patients with a positive *H. pylori* test (IgG serology, stool antigen, urea breath test [UBT]) followed by a treatment regimen within one year followed by an eradication test (stool antigen, UBT). We confirmed treatment regimen on manual chart review and extracted demographic, laboratory, and endoscopy data. We compared rates of eradication among commonly used empiric treatment regimens. We additionally examined predictors of successful eradication based on demographic (age, sex, race/ethnicity, birthplace, BMI), medication (treatment regimen, duration, concurrent proton pump inhibitor [PPI] prescription), and system factors (prescriber specialty, care by gastroenterologist, receipt of endoscopy) reported as odds ratios (OR) and 95% confidence intervals (CI).

Results: Among 1106 *H. pylori* positive patients, 28.3% were male, 2.5% white, 10.7% black, 80.6% Hispanic, and 4.4% Asian with mean age 51.3 years (standard deviation 12.6 years). Bismuth-tetracycline-metronidazole was the most commonly prescribed regimen (57.4%) followed by clarithromycin-amoxicillin-metronidazole (20.9%) and clarithromycin-amoxicillin (15.7%). Most patients were prescribed concurrent PPIs (98.3%) and 44.0% were prescribed 14-day therapy. Eradication rates were highest for bismuth-tetracycline-metronidazole therapy (83.9%) followed by clarithromycin-amoxicillin-metronidazole therapy (80.1%) and clarithromycin-amoxicillin therapy (79.3%) (Table 1). Compared to bismuth-tetracycline-metronidazole regimen, clarithromycin-metronidazole (adjOR 0.29, 95% CI 0.12-0.66) and levofloxacin-amoxicillin (adjOR 0.11, 95% CI 0.03-0.49) regimens were associated with unsuccessful eradication while clarithromycin-amoxicillin-metronidazole and clarithromycin-amoxicillin regimens were not significantly different in predicting eradication success (Table 2).

Conclusion: Overall, all empiric regimens had eradication success rates of <85%. Bismuth-tetracycline-metronidazole therapy had the highest eradication rate, while metronidazole and levofloxacin triple therapies were predictors of unsuccessful eradication. This study supports moving towards tailored therapies based on proven susceptibility to achieve eradication rates of >90%.

Somatic Mutations of Esophageal Adenocarcinoma: A Comparison Between Black and White Patients

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INTRODUCTION: Esophageal cancer is one of the more common cancers and was responsible for 5.5% of all deaths from cancer worldwide in 2020. There are two main histological subtypes of esophageal cancer, esophageal adenocarcinoma (EAC) and esophageal squamous cell carcinoma (ESCC). In Western countries, EAC is the predominant subtype, representing 40-70% of all esophageal cancers. In the United States, EAC incidence has increased substantially over the last four decades, and absolute rates of EAC have remained highest among non-Hispanic White males. While there have been improvements in therapies and surgery, fewer than 20% of EAC patients survive for five years post-diagnosis. Although Black patients have a lower incidence, the average onset age of advanced cases is younger, and survival rates are lower than White patients. Gastroesophageal reflux disease [GERD] symptoms, smoking, obesity, and low socio-economic status are well-known risk factors of EAC but are unlikely to explain fully the differences in incidence and outcomes between Black and White patients.

METHODS: In our current study, we sought to assess the somatic mutation profiles derived from formalin-fixed paraffin-embedded (FFPE) tumor samples from male patients with EAC by conducting whole-exome sequencing (WES). We performed somatic mutational signature analysis to compare the pattern of single nucleotide variants (SNVs) in EAC samples to pre-defined esophageal cancer (ESCA)-specific signatures from the SIGNAL database. MutSigCV 2.0 was used to identify significant EAC-related genes (*q-value* < 0.1).

Results: We identified three molecular EAC subgroups. Group 1 is associated with age and *NTHL1* deficiency-related signatures. Group 2 occurs primarily in Black patients and is associated with signatures related to DNA damage from oxidative stress and *NTHL1* deficiency-related signatures. Group 3 is associated with defective homologous recombination-based DNA often caused by *BRCA* mutation in White patients. We observed significantly mutated race-related genes (*LCE2B* in Black*, SDR39U1* in White) (q-value < 0.1).

Conclusions: our present study is the first comprehensive exome sequencing analysis to compare the difference in mutations between Black and White EAC patients using mutational signature analysis. Our findings underscore the possibility of distinct molecular mutation patterns in EAC among different races. Further comprehensive epidemiologic and epigenetics studies are necessary to validate our findings in larger sample sizes and understand the underlying mutational mechanisms of EAC.

Comparative DNA Methylation Profiling of Cirrhosis With and Without Hepatocellular Carcinoma

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Background: Hepatocellular carcinoma (HCC) is a leading cause of death worldwide. In the United States, HCC incidence and mortality have increased rapidly over the past two decades with a <20% 5-year survival rate. Despite advancements in treatment, cure remains only possible for fewer than 10% of HCC patients, and most HCC patients are diagnosed with advanced disease. Most cases of HCC arise in the background of cirrhosis. Understanding of the mechanism leading to the transition from cirrhosis to HCC, and identifying key biomarkers are crucial to the development of effective screening strategies and to improved survival. DNA methylation has been shown to be associated with gene expression modulation and plays an important role in both physiological and pathological processes. Aberrant DNA methylation occurs in an early phase of carcinogenesis and can be detected in peripheral blood mononuclear cells (PBMCs), which are thought to detect the epigenetic reprogramming of the host immune system during the development of malignant tumors. However, the difference of DNA methylation patterns in PBMC between cirrhosis and HCC is still unclear.

Methods: Twenty-two patients with cirrhosis who later developed HCC (cases) were gender/age-matched with 22 patients with cirrhosis who remained cancer-free during an average 4-year follow-up (controls). PBMC was isolated from whole-blood samples collected at pre-diagnostic baseline from all cases and controls. All samples were analyzed by Infinium MethylationEPIC microarrays covering ~ 850K CpG sites. We used the Champ (Chip Analysis Methylation Pipeline) package to analyze the methylation comparing PBMC from cases and controls to identify differentially methylated CpGs sites and genes and their representation in specific biological pathways.

Results: In total, 508 differentially methylated probes (DMPs) were identified by comparing HCC patients and cirrhosis patients, with 161 hypomethylated and 347 hypermethylated (*p*-value < 0.05). Gene set enrichment analysis (GSEA) analysis revealed that the hypermethylated probes were mainly associated with functions such as regulation of nucleobase-containing compound metabolic process and heterocycle biosynthetic process. In contrast, the hypomethylated probes were mainly enriched in the organ system process. Protein-protein interaction analysis identified *RB1* and *HIST1HIE* with key pathways like the p53 pathway (PPI score=0.99, 0.99, respectively). Twenty-six CpG sites were located on promoter regions with *p*-value < 0.05 and | ▵ Beta value| > 0.1. The top 3 significantly hypomethylated CpG sites on promoter regions were cg04531182, cg10596483, and cg19263494 and corresponded to the *KLRC4-KLRK1*, *JRK,* and *PMF1* genes, respectively. The top 3 significantly hypermethylated CpG sites on promoter regions were cg25460273, cg08425810, and cg22706186 and corresponded to the *PTPRE*, *AGAP2*, and *FAM19A1* genes, respectively.

Conclusions: We identified the methylation landscape of cirrhosis and HCC and provided potentially new methylation markers of progression risk. Comprehensive epidemiologic and epigenetics studies are necessary to validate our findings in larger sample sizes.

Ghrelin: a crucial hormone for epithelial integrity and function in the mouse colon

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Ghrelin, commonly referred to as the "hunger hormone," is primarily produced in the gastrointestinal (GI) tract and is renown for its role in appetite stimulation. However, its effect extends far beyond appetite regulation, it plays a critical role in a range of physiological functions such energy homeostasis and metabolism.

The digestive tract, involved in nutrient absorption and processing, has central to overall health. A key component of this complex system is the intestinal crypts, the ones in the colon are as colon crypts. These structures are essential for understanding the gut's dynamics and its crucial role in health. The colon crypts contain various epithelial cells including stem cells, enterocytes, goblet cells, paneth cells, tuft cells, and enteroendocrine cells, working in concert to preserve the colon's barrier function.

Our study of ghrelin-null mice reveals that the absence of ghrelin leads to a reduction in colon length and crypt depth, as well as showing increased goblet cell population—a pattern akin to the early stages of colitis development. Furthermore, ghrelin deficiency significantly upregulates the expression of pro-inflammatory genes such as Tnfa, Il1β, and iNOS in the colon crypts. To corroborate these findings, we generated colonoids from both wild-type and ghrelin-null mice. The colonoids lacking ghrelin exhibited notably slower growth and compromised barrier function compared to their wild-type counterparts. Gene expression analyses of epithelial cell markers revealed that ghrelin deletion alters the expression profile of these cells in both crypts and organoids, evidenced by a decrease of Lgr5 (stem cell) and Lyz1 (paneth cell) expression, and a marked increase in Muc2 (goblet cell) expression. Intriguingly, the absence of ghrelin also affected the gene expression of enteroendocrine cell subpopulations, increasing ChgA, Zcchc12, and Gip, but decreasing Gcg expression.

In conclusion, our research has uncovered novel functions of ghrelin in the colon in maintaining crypt depth, modulating the expression of inflammation-related genes, and remodeling epithelial cell populations. Our findings demonstrate that ghrelin plays a critical role in regulation of the epithelial cell population and maintenance of gut barrier integrity, and that ghrelin may have potential to serve as a potential therapeutic target for colon-related diseases such as colitis.

Microbially-derived branched chain fatty acids regulate intestinal permeability in malnutrition.

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Background: Malnutrition claims over 5 million lives of children under 5 years annually. Malnourished populations suffer from increased intestinal permeability and gut microbial alterations. Bacterial translocation from the gut to distant organs can cause sepsis, a major cause of mortality in malnutrition. The gut microbiome and its metabolites, including branched chain fatty acids (BCFAs) that are derived from microbial fermentation of protein, help regulate intestinal barrier integrity. Additionally, increased gut barrier permeability can be caused by a defective intestinal mucus layer. We hypothesized that increased permeability in the malnourished colon is caused by decreased microbial BCFA production and an altered colonic mucus layer. **Methods:** Specific pathogen free (SPF) and germ free C57BL/6 mice were weaned to a low-protein low-fat diet to induce malnutrition or to control chow. Stool was analyzed by metagenomic sequencing and mass spectrometry targeting BCFAs. Permeability was assessed *ex vivo* in Ussing chambers by quantifying the flux of 4 kDA fluorescein isothiocyanate across the colonic epithelium. To measure bacterial translocation, livers and spleen were sterilely harvested and homogenized, then cultured aerobically and anaerobically on Brain Heart Infusion agar plates at 37°C. Gene expression was analyzed in colon homogenates by qPCR. *In vitro* permeability was assessed by measuring transepithelial electrical resistance (TEER) across human-derived organoid monolayers seeded on semi-permeable transwell supports. BCFAs were applied at doses spanning the physiologic range (1 mM – 10 mM) for 24 hours. **Results:** Malnutrition tripled *ex vivo* colonic permeability ($P < 0.0001$) and dramatically increased the number of culturable anaerobic bacteria in spleen and liver tissue from SPF mice ($P < 0.05$). Strikingly, germ-free mice were protected from malnutrition-induced increased gut permeability. Malnutrition resulted in decreased microbial community richness (33.7 vs. 38.7 OTUs, $P = 0.07$) and evenness (2.3 vs 2.7 Fisher diversity index, $P < 0.05$). Compared to controls, malnourished mice had dramatically decreased quantities of the BCFAs 2-methylbutyrate, isobutyrate, and isovalerate ($P < 0.01$). These changes were associated with a 40% reduction of mucin gene transcription (*Muc2* and *Muc4*, $P < 0.05$) and a 40% decrease in transcription of the BCFA receptor *Ffar3* ($P < 0.05$). Relative abundance of the mucus degrading bacterium *Akkermansia munciniphila* was increased in malnourished colonic microbial communities ($P < 0.05$). The BCFA isovaleric acid increased TEER *in vitro* in human-derived colonic organoid monolayers in a dose-dependent manner (58% increase in TEER, 0 vs. 10mM isovaleric acid, $P < 0.01$). **Conclusion:** The gut microbiome plays a causal role in malnutrition-induced gut barrier dysfunction, and the microbial-derived BCFA isovaleric acid supports intestinal barrier function *in vitro.* Ongoing studies will examine whether isovalerate supplementation might rescue malnutrition-associated intestinal barrier defects and reduce the risk of sepsis. This knowledge could translate into novel microbiota-targeting therapies to restore the gut barrier in malnutrition.

Investigating the interplay between diet and the gut microbiome in necrotizing enterocolitis (NEC)

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Necrotizing enterocolitis (NEC) is the leading cause of death from gastrointestinal disease in premature infants. Although its pathogenesis is incompletely understood, prematurity, diet, and gut microbiome dysbiosis have been identified as risk factors for this disease. Breastmilk feeding significantly decreases the incidence of NEC in preterm infants compared to infant formula, however, the mechanisms through which these diets affect the pathogenesis of NEC remain elusive. This project aims to elucidate how the interplay between diet and the gut microbiome affects susceptibility to NEC.

Feeding studies using a preterm piglet model of NEC have demonstrated that feeding a commercially available infant formula results in higher ((100% vs 0%, $P<0.01$) incidence of NEC compared to donor human milk (DHM), respectively. Whole genome sequence analysis of the gut microbiome composition revealed that colonization by *Clostridium perfringens* (*C. perfringens*) was higher ($P<0.05$) in formula-fed and diseased piglets (30-50%) than in healthy and DHM-fed piglets (0-10%). Moreover, the abundance of *C. perfringens* positively correlated ($R= 0.8$, $P<0.01$) with disease severity suggesting *C. perfringens* colonization is associated with the pathogenesis and progression of NEC.

To elucidate how diet composition affects abundance and virulence of *C. perfringens*, we isolated this bacterium from piglets afflicted by NEC and infant stool samples. Our results show that *C. perfringens* isolates from piglets with NEC exhibit a trend of higher growth ($9.93x10^9$ CFU/mL difference on average) and higher (+50%, $P<0.001$) levels of toxin perfringolysin O, when cultured in formula compared to DHM. Importantly, we found that both piglet and infant isolates grow to higher densities in maltodextrin, the most abundant carbohydrate in preterm infant formula, compared to lactose, the most abundant carbohydrate in breast milk. These findings suggest that the carbohydrate present in formula, but not human milk, may differentially select for gut microbes that increase the risk for NEC. Future experiments will focus on the effect of dietary components on the cytotoxicity of *C. perfringens* with the goal of further understanding the relationship between this species, diet, and the pathogenesis of NEC.

Deciphering the role of IL-4 in colon repair after acute colitis

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Introduction: Therapies that promote epithelial repair and homeostasis would be major advancements in the treatment of inflammatory bowel disease (IBD), encompassing Crohn's disease (CD) and ulcerative colitis (UC). IBD is characterized by relapsing and remitting inflammation and dysbiosis which is often exacerbated by incomplete mucosal healing. Existing therapies have high failure and non-response rates, failing to control relapse effectively. Thus, there is a critical need to explore mechanisms that restore mucosal homeostasis after IBD flares. This project aims to characterize the interactions of Interleukin-4 (IL4) with immune cells and the microbiome and their contribution to colon homeostasis after colitis. **Methods:** Genetically modified mice including IL-4 and IL4Rα deleted mice, neutrophil specific IL4Rα deleted mice, Il4/eGFP-enhanced transcript (4get) mice, and appropriate controls were treated with 3% dextran sulfate sodium (DSS) for 5 days to induce colon injury. Mice were observed during DSS treatment and until day 21, recording daily weight changes and qualitative assessment of colitis symptoms. Flow cytometry was used to assess cellular dynamics during and after colitis. Bone marrow macrophages were derived by culture of bone marrow mononuclear cells with L929 media for 7 days, and in vitro stimulation was performed using 20ng/mL of recombinant IL-4. Transcript-level changes were measured by qPCR. **Results and Discussion:** After treatment of 4get mice with DSS, flow cytometry revealed an influx of IL-4-producing eosinophils into the colon on days 8 and 14. These GFP^+ eosinophils accounted for >90% of IL-4-producing cells on day 8 and >75% on day 14, suggesting that eosinophils enter the colon during acute colitis, and produce IL-4 during acute injury and recovery. After DSS treatment, IL-4 deficient mice experienced more severe weight loss from day 6-21 when compared with control mice treated in separate cages. On day 21, IL-4 deficient mice had significantly shorter colon length compared to controls. However, when co-housed IL-4 deficient and littermate control mice were treated with DSS, there were no phenotypic differences, suggesting a likely role for the microbiome in regulating colon inflammation after DSS. After DSS treatment, neutrophil-specific IL4Rα deleted mice demonstrated premature mortality compared with littermate controls, suggesting that IL4 signaling through neutrophils may be important for controlling inflammation during acute colitis. Interestingly, IL-4 stimulation of bone-marrow-derived macrophages in vitro revealed 20-fold increases in transcription of HB-EGF, an EGFR ligand that has demonstrated protective effects during TNFα and DSS-induced acute colitis in mice. IL-4 stimulation of bone marrow macrophages generated from IL4Rα deletion mice did not upregulate HB-EGF. **Future direction:** To analyze the impact of IL-4 deficiency on the microbiome, 16S microbiome sequencing of naïve and DSS-treated IL4 deficient and control mice will be performed. Then, we will perform microbiota normalization by antibiotic treatment and reciprocal fecal material transfer between the IL-4 deficient and C57BL/6J mice, followed by DSS treatment to understand if the protective role of IL-4 during colitis can be shared by the microbiome. To analyze the impact of neutrophil-specific IL4Rα deletion on neutrophil activation and migration during colitis, we will perform DSS treatment followed by immunofluorescence staining to identify neutrophil presence and location using whole-body and neutrophil-specific IL4Rα deletion mice. To account for the direct action of IL-4 and macrophage-derived HB-EGF on the colon epithelium, we will culture colon organoids and treat them with DSS in-vitro followed by the introduction of IL-4 and/or IL-4 stimulated macrophages and assess cellular proliferation and migration kinetics.

Glucagon-like peptide 1 (GLP-1) agonists semaglutide and tirzepatide and colorectal cancer risk: a cell culture study

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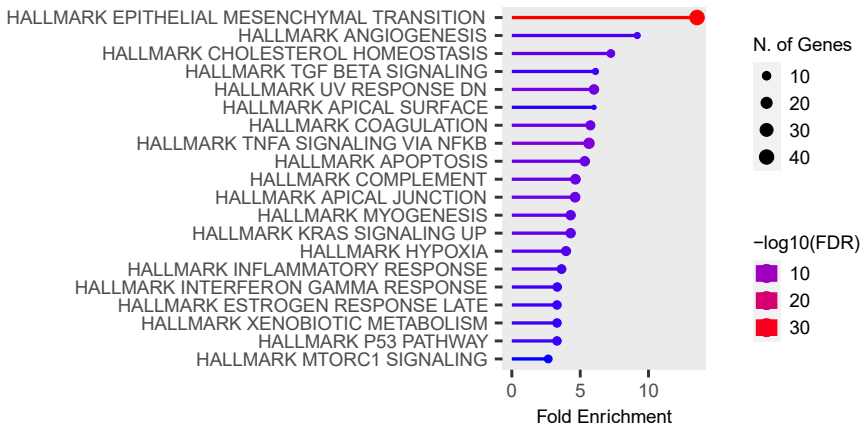
Background: GLP-1 agonists are increasingly utilized for both diabetes and obesity, two powerful CRC risk factors. GLP-1 receptors are found on CRCs. Most of the epidemiological data does not suggest an increase in CRC risk (there is a definitive increase in thyroid cancer); however, these reports are hampered by limited follow-up. We therefore wanted to assess the impact of two agents, semaglutide (GLP-1) and tirzepatide (a dual GLP-1 agonists) that are in widespread clinical use on cell proliferation as a marker of potential pro-neoplastic effects. **Methods:** Cell Culture: Using standard techniques, we grew human immortalized (nontransformed) colonocyte cell line HCEC-1CT. Cellular Assays: Cell number was determined via cell count via DAPI nuclear stain and corroborated with WST-1. RNA seq: RNA was isolated with RNeasy mini kits, and sequencing was performed using the Illumina® NovaSeqTM platform. Molecular Assays: RT-PCR was performed with TaqMan using GAPDH via comparative CT assay. Western blot analysis was performed, and GAPDH was used as a loading control. **Results:** As noted in Table 1, treatment of both semaglutide and trizepatide increased cell number without clear dose-responsive. We did a pilot PCNA assessment by Western blot showing congruence (e.g., HCT116 treated with 500nM tirzepatide had a 253% increase ($p<0.01$). To better evaluate biological plausibility, we performed RNA sequencing. Figure 1 shows the results of the "Hallmark analysis," revealing several dysregulated hallmarks associated with colorectal carcinogenesis, including Epithelial-Mesenchymal Transition, Angiogenesis, and Apoptosis, as well as TGF-beta, K-ras, and p53 signaling. **Conclusions:** Our data suggests that semaglutide and tirzepatide increase cell proliferation and thus may be potentially pro-neoplastic in both non-transformed colon and CRC cell lines. RNAseq shows numerous pathways associated with colon carcinogenesis, supporting plausibility. Given the intrinsic limitations of cell culture, this data needs to be interpreted with caution. Moreover, this system cannot account for the counterbalancing antineoplastic impact of improved obesity and diabetes. This work underscores the need for rigorous epidemiological and animal model studies.

HCEC-1CT (Cell Count)				
Semaglutide	500 nm	1 uM	5 uM	10 uM
Percent Change	62.4	60.1	57.8	35.6
p-value	4.00E-06	0.000016	0.000026	0.0035
Tirzepatide	50 nm	250 nm	500 nm	1 uM
Percent Change	17.9	15.1	20.2	21.1
p-value	0.00086	0.01	0.0024	0.00084
HCT-116 (WST)				
Semaglutide	500 nM	1 uM	5 uM	10 uM
Percent Change	10.7	9.3	27.6	24.9
p-value	0.061	0.037	0.0019	0.0021
Tirzepatide	50 nM	250 nM	500 nM	1 uM
Percent Change	12.0	37.0	23.8	19.5
p-value	0.034	1.08E-05	0.0059	0.014

Table 1: DAPI Stain of HCEC-1CT exposed to semaglutide and tirzpatide compared to untreated HCEC-1CT. WST assay of HCT-116 of exposed to semaglutide and tirzpatide compared to untreated HCT-116.



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Liver steatosis in malnourished mice is altered by the gut microbiome

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Each year, 45% of childhood deaths are attributed to malnutrition. Chronic malnutrition increases gut barrier permeability, causes dyslipidemia and metabolic dysfunction, and dramatically alters the gut microbiome. In severely malnourished children, accumulation of triglycerides in hepatocytes results in hepatic steatosis. The microbiome influences host metabolism through microbial-derived metabolites and an altered microbiome has been implicated in the progression of liver disease. Our laboratory previously reported that malnourished mice that develop liver steatosis have dramatic alterations of both the microbiome and microbial metabolites, including branched-chain fatty acids, which are fermentation products of dietary protein. Therefore, we hypothesize that in malnutrition, the altered gut microbiome, and the reduction of branched-chain fatty acids, contributes to the development of steatosis. To test this hypothesis, we modeled early postnatal malnutrition by feeding mice a low-protein, low-fat diet (LPLFD) that is isocaloric to a control chow diet; both diets were triply-irradiated to facilitate germ-free (GF) studies. At 8 weeks of life, we examined hepatic lipid accumulation with oil red O staining, hepatic triglyceride and cholesterol levels, and hepatic protein expression with western blot. SPF malnourished mice had profound steatosis that was significantly attenuated in GF malnourished mice. In addition, total cholesterol and cholesterol ester were significantly increased in SPF malnourished mice compared to GF malnourished mice. Together, these results are the first to implicate a potentially causal role of the gut microbiome in malnutrition-induced hepatic steatosis. Given the dramatic increase of lipid droplets and cholesterol esters found in SPF malnourished mice, we explored whether lipid droplet associated perilipin proteins were altered in malnourished mice. We found that Perilipin2 is increased in both SPF and GF malnourished mice. However, Perilipin5 is slightly reduced in SPF malnourished mice and increased in GF malnourished mice. These changes may impact the function and localization of Perilipin5. Phosphorylation of Perilipin5, which is Protein Kinase A-dependent, triggers its mobilization into the nucleus where it stimulates the expression of fatty acid oxidation genes. Moreover, Protein Kinase A is activated in the presence of isovalerate, a branched-chain fatty acid that is reduced in SPF malnourished mice. Thus, these data suggest a potentially novel therapeutic link between microbially-derived branched chain fatty acids, Perilipin5 phosphorylation, increased fatty acid oxidation, and attenuation of malnutrition-induced liver steatosis.

Investigating the role of the PERK-integrated stress response pathway during paligenosis and metaplasia

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Cellular reprogramming is essential for the regeneration of adult organs lacking dedicated stem cells post-injury or inflammation. Because such cell plasticity is likely to be executed by a conserved cellular program, investigating the cellular-molecular features underlying this process is crucial. Our lab introduced the concept of paligenosis, where mature cells undergo a 3-stage sequential regenerative process of organelle autodegradation, activation of a progenitor-gene network, and cell cycle re-entry, in which mutations of key regulators within any stage can potentially lead to metaplasia and pre-cancerous lesions. This project focuses on identifying the upstream triggers of paligenosis. Using a high-dose tamoxifen injury model in murine stomach corpus chief cells, ultrastructural changes in the rough endoplasmic reticulum (rER) during paligenosis progression were observed, suggesting dynamic ER changes as an upstream event in paligenosis. The integrated stress response (ISR) is a well-studied pathway that monitors ER functioning, with ER-resident kinase PERK being a key ER stress sensor that phosphorylates the eukaryotic translation initiation factor 2 subunit 1 (eIF2α), causing halt of global translation while upregulating a specific set of genes to restore homeostasis. Data indicates that HDT injury activated the PERK-ISR in paligenotic cells and caused global attenuation of protein synthesis. This project aims to further elucidate the necessity and sufficiency of the PERK-ISR pathway in initiating paligenosis, utilizing PERK-ISR activators and inhibitors, as well as *Perk*$^{\Delta/\Delta}$ mice crossed with chief cell-specific promotor mice. In summary, this study aims to provide new insights into the regulation of the rough ER and its role during paligenosis, contributing to our understanding of metaplasia formation. The outcome of the project could potentially identify new therapeutic targets for blocking precancerous lesions in settings where paligenosis of mature, differentiated cells occurs.

Real-world patterns of objective inflammation assessment in a large, multicenter cohort: potential gaps and barriers to treat to target

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BACKGROUND: A Treat-to-Target (T2T) strategy for inflammatory bowel disease (IBD) improves clinical outcomes. The cornerstone of T2T is the routine monitoring of disease control using objective markers of inflammation. Despite this, objective testing is often delayed or not performed. This study aims to describe patterns and associated factors of intended inflammation assessment for patients with IBD within the multicenter quality improvement collaborative, IBD Qorus.

METHODS: Patient and provider survey data from IBD Qorus were prospectively collected from 6/2021 to10/2022 across 35 academic and community practices during routine clinical care visits. Patients with unknown status of mucosal healing were excluded. The primary outcome was "timely assessment" as defined by provider-reported intent to obtain fecal calprotectin (FC), imaging, or endoscopy within 12 months. Factors associated with the primary outcome were assessed using univariate and multivariate logistic regression, adjusting for age, gender, IBD type, and clinical factors.

RESULTS: 4,831 encounter-level surveys from the study period were included for analysis. An intent to assess inflammation within the next 6 months was reported in 73.9% of encounters. 77.1% planned to use FC and57.7% imaging or endoscopy. An intent to assess inflammation within 12 months was reported in 89.3% of encounters. Patients declined FC in 29.8% of encounters and declined imaging or endoscopy in 8.3%. In the primary univariate analysis, intent for timely assessment was associated with ulcerative colitis (UC), recent emergency department visit or hospitalization, lack of clinical remission, mild Provider Global Assessment, current steroid use, lack of immunomodulator use, lack of steroid-free mucosal healing, care at an academic site, and intent to change therapy. On multivariate analyses, UC (OR 1.38, 95% CI 1.11-1.71), steroid use (OR2.1, 95% CI 1.1-4.6), and plans to change therapy (OR 1.84, 95% CI 1.2-2.9) remained significantly associated with higher odds of timely assessment. In contrast, steroid-free mucosal healing (OR 0.52, 95% CI 0.41-0.66) and care in community practice (OR 0.63, 95% CI 0.51-0.77) remained significantly associated with lower odds of timely assessment.

CONCLUSION: In IBD Qorus, there is a strong intent to assess objective markers of inflammation. However, lower odds of intended assessment were found among patients with Crohn’s, patients in steroid-free mucosal healing, and those receiving care in community practice, revealing potential care gaps. Interestingly, providers frequently reported that patients declined FC testing, and declined this at higher rates than that for endoscopy.

Ghrelin signaling in macrophages: an intricate link of gut-brain axis in aging

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The prevalence of inflammatory bowel disease (IBD) among the elderly is growing globally, and the severity and comorbidity in the elderly is worse than younger patients. Epidemiological studies indicate there is a strong correlation between IBD and cognitive dysfunctions. However, the intricate complication between gut and brain during aging are not well understood. The nutrient-sensing hormone ghrelin is primarily produced in the gastrointestinal tract, functioning through its receptor, growth hormone secretagogue receptor (GHSR). While the expression of the receptor is well-established in the brain, we found GHSR is also highly expressed in intestinal macrophages and its expression in the colon is dose-responsively correlated with colitis inducing reagent, dextran sulfate sodium (DSS), suggesting that ghrelin signaling may play an important role in the inflammation in IBD.

We hypothesize that GHSR reprograms intestinal macrophage function, thereby regulating gut permeability, the severity of experimental colitis, and further contributing to cognitive dysfunction and neuroinflammation in aging. We used myeloid-specific GHSR knockout mice (*MΦ-KO*) to conduct FITC-dextran assay to assess gut permeability, novel object recognition and Y maze spatial memory test to assess cognitive functions. To investigate the role of macrophage GHSR in colitis and its associated neuroinflammation in aging, we induced experimental colitis in young (3-month-old) and old (20-month-old), *MΦ-KO* mice using 3.5% DSS in drinking water for 5 days followed by 3 days of water for recovery. The severity of colitis was assessed by Disease Activity Index (DAI) daily. The DAI is scored based on body weight decrease, rectal bleeding, and fecal consistency. At termination, the colon and brain were collected for flow cytometry analyses.

We demonstrated that old *MΦ-KO* mice showed improved gut permeability and cognitive memory functions. In experimental colitis, *MΦ-KO* mice showed better DAI compared to controls, and remarkably, the protective effect was more pronounced in old mice. The old *MΦ-KO* mice showed attenuated intestinal inflammation exhibiting reduced pro-inflammatory colonic macrophages and decreased pro-inflammatory microglia in the brain. These findings offer a new mechanistic insight for gut-brain axis in aging, suggesting macrophage GHSR is a critical pathogenic link between intestinal injury and neuroinflammation. Our study suggests that targeting GHSR in macrophages could serve as a novel immunotherapy for rejuvenating gut and brain fitness in aging.

Evaluating Mechanisms of Human Norovirus Gastroenteritis Using Human Intestinal Enteroids.

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Human noroviruses (HuNoVs) are the leading cause of nonbacterial gastroenteritis worldwide. They are associated with 19-21 million cases of gastroenteritis in the US and about 219,000 deaths globally each year. HuNoVs infections result in acute, self-limiting illness in immunocompetent individuals and chronic infections in some immunocompromised patients. However, the mechanisms by which HuNoVs cause diarrhea and vomiting are poorly defined. HuNoVs primarily infect enterocytes in the small intestine. However, a previous study using jejunal biopsies from a 9-month-old transplant patient showed the presence of HuNoV RNA in neurohormonal cells in the gut called enteroendocrine cells (EECs). Upon stimulation, these cells signal to the enteric nervous system and activate brain centers involved in nausea and vomiting. We hypothesized that secretion of neuroactive hormones such as serotonin from infected EECs is a mechanism associated with HuNoV induced vomiting. To test this hypothesis, we used human intestinal enteroids (HIEs), a physiologically relevant model to study HuNoVs. Specifically, we used an HIE line that has been genetically modified to overexpress the neurogenin 3 (NGN3) transcript, which promotes differentiation of EECs. We quantified the replication of three GII.4 HuNoV isolates using RT-qPCR and observed significantly lower genome equivalents of HuNoV RNA in the induced NGN3 HIEs. Low numbers of GII.4 infected cells were also observed. We also did not observe a significant difference in the secretion of serotonin, after infection with a HuNoV GII.4 strain. This may be due to the low infectivity of the NGN3 HIE line. As there are at least 15 different types of EECs categorized based on morphology, regional distribution, and hormone production, it is possible that specific EEC subpopulations in NGN3 HIEs are not susceptible to HuNoV infection. In on-going studies, we are examining whether infection of enterocytes induces EECs to secrete other hormones associated with vomiting.

Chronotherapy Will Play a Key Role in Future Personalized Anti-HCC Treatment

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Hepatocellular carcinoma (HCC) has displayed the fastest rate of increase in both incidence and cancer-related death in the U.S in recent years. HCC is usually diagnosed at an advanced stage without curative options and displays strong resistance to systemic and immunotherapies. The most known “HCC-driver genes” identified in human metastatic HCCs do not offer treatment options. The lack of preclinical relevant animal models that develop HCC following the same molecular pathogenesis pathway as that observed in humans significantly has impaired the efforts of improving the current anti-HCC therapy. We have established a chronic jet lagged wild-type (WT) mouse model previously and a humanized HCC model recently. Both spontaneously develop non-alcoholic fatty liver disease (NAFLD)-related HCCs following the same mechanism as that described for human NAFLD-related HCCs. The whole genome-sequencing analysis of premalignant dysplasia nodules (PMDN) and HCCs from jet-lagged WT mice revealed that NAFLD-induced hepatocarcinogenesis is independent of the known human HCC-drivers, highly heterogeneous in mutational landscapes, and with strikingly high mutational frequencies. However, stable circadian entrainment of humanized mice efficiently coupled the transcriptomes in different HCCs to that in benign liver tissues despite the heterogeneity in individual HCC mutational landscapes. The circadian coupling of HCC transcriptome was associated with significantly reduced risk of tumor progression and metastasis and prolonged host survival. Chronic circadian disruption drastically shifted the HCC transcriptome to induce the activation of all hallmarks of cancer, changes in both tumor and host immunity, and disruption of peripheral prognostic biomarkers, which were associated with accelerated tumor progression and metastasis and early mortality of the hosts. Together, our studies demonstrate that chronic circadian dysfunction is an independent liver carcinogen and that personalized anti-HCC chronotherapy may significantly improve anti-HCC therapeutic index.

De-*N*-glycosylation at the fulcrum of intestinal innate immune response and barrier function

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The first known congenital disorder of deglycosylation (CDDG) is caused by recessive pathogenic variants in the *NGLY1* gene, which encodes for the enzyme *N*-glycanase 1. NGLY1 removes *N*-linked glycans from misfolded glycoproteins and is thought to function in the endoplasmic reticulum-associated degradation (ERAD) pathway. Patients with NGLY1 deficiency show a broad spectrum of symptoms including global developmental delay, hypotonia, abnormal involuntary movements, alacrima, and chronic constipation. However, there is no cure yet and the biologically relevant functions of NGLY1 are not fully understood. We used the *Drosophila* model to identify the *N*-glycoproteins and signaling pathways regulated by NGLY1. Loss of *Pngl* (Drosophila NGLY1) results in developmental delay and lethality. We have previously reported key roles for *Pngl* in the visceral mesoderm in the regulation of BMP and AMPKα signaling. Here, we show that loss of *Pngl* in a specific secretory cell type in the gut leads to gut barrier defects, which cause starvation and increased JNK activity. These defects combined with additional cell-autonomous abnormalities in the gut epithelium and fat body (which is analogous to mammalian liver) result in Foxo overactivation, which induces a hyperactive innate immune response and enhanced lipid catabolism, thereby contributing to lethality associated with loss of *Pngl*. Gut bacterial dysbiosis is associated with the host innate immune response. In agreement with innate immunity hyperactivation in *Pngl* mutant guts, germ-free rearing of these larvae significantly improved their developmental delay. However, it did not rescue lethality. In contrast, raising *Pngl*-mutant larvae on isocaloric, fat-rich diets improved animal survival in a dosage-dependent manner. Our data indicate that Pngl functions in *Drosophila* larvae to establish the gut barrier and to maintain innate immune and metabolic homeostasis during larval development and that the immune and metabolic consequences of loss of Pngl are primarily mediated through non-bacterial insults.

Evaluation of human milk oligosaccharides as therapeutics for noroviruses

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Human noroviruses (HuNoVs) are the leading cause of acute gastroenteritis worldwide. Currently, there are no approved vaccines or targeted antivirals. Histo-blood group antigens (HBGAs) are cellular attachment factors for HuNoVs on the intestinal epithelium. X-ray crystallography and binding studies show that HuNoV virus-like particles (VLPs) bind to structurally analogous compounds present in breast milk called human milk oligosaccharides (HMOs). These data lead to the hypothesis that HMOs can act as decoy receptors to prevent the attachment of HuNoV to HBGAs.

We aimed to evaluate the effect of an α(1,2)- fucosylated HMO (2' fucoslyllactose, 2'FL) in the inhibition of HuNoV replication using human intestinal enteroids (HIEs). We used a standard antiviral testing pipeline developed for HuNoVs to test the effect of 2'FL on the replication of a globally dominant GII.4 HuNoV strain. The half maximal tissue culture infectious dose ($TCID_{50}$) for the globally dominant GII.4 HuNoV strain was determined in several small intestinal HIE lines from adults and children. We then assessed the effect of 2'FL on the replication of 100 TCID50 of HuNoV by RT-qPCR. 2'FL significantly reduced GII.4 replication in duodenal and jejunal lines from adults, although the magnitude of inhibition was higher in duodenal HIEs compared to jejunal lines. The duodenal and jejunal HIEs were derived from distinct donors; therefore, to assess if there are segment-specific differences in the absence of genetic differences between persons, 2'FL was tested in HIEs from all segments of the small intestine from a single adult donor. Significant reduction of GII.4 replication was seen in all the segments, suggesting the potential for donor-specific differences in 2'FL activity. However, when studies were carried out in duodenal and jejunal HIEs from children, significant inhibition of replication was seen in pediatric duodenal HIEs but not in infant jejunal lines. Additional studies are being carried out to determine the mechanisms contributing to the differential effect on adult and infant jejunal HIEs.

These data suggest 2'FL has the potential to be developed as a treatment for HuNoV gastroenteritis, especially for adults. This work will aid in future studies on the development of HMOs as therapeutics for HuNoVs.

Smooth muscle specific loss MBNL as a model of DM1 causes muscle hypercontraction and delayed gastrointestinal motility

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Myotonic dystrophy type 1 (DM1) is the most common adult-onset muscular dystrophy, causing highly variable multisystemic symptoms that predominantly affect the brain, heart, and skeletal muscle. Gastrointestinal (GI) disturbances are highly prevalent and reported by 80% of affected individuals as affecting daily life and well-being. The cause of DM1 GI dysfunction is currently unknown but evidence supports a role for smooth muscle dysfunction. The goal of this project is to elucidate the role of muscleblind-like (MBNL) loss of activity, a primary mechanism of DM1 pathology, in GI smooth muscle function.

We used mice expressing a smooth muscle specific, tamoxifen inducible *Myh11-Cre*ER^{T2} driver and floxed *Mbnl1* and *Mbnl2* alleles to induce conditional double knockout of MBNL (denoted as cdKO) and assessed upper and lower GI motility. Small bowel transit was quantified by gavaging mice with a cell impermeable FITC dye conjugated to a large dextran molecule that allowed for the quantification of bolus movement after 25 minutes. The progression of the mean geometric center of FITC-dextran was significantly delayed in cdKO small bowel compared to controls by at least 1.22 segments ($p \leq 0.01$), indicating delays in small intestine motility. Colonic motility was measured by bead expulsion assay, where bead latency was significantly increased in cdKO mice by at least 168 seconds ($p \leq 0.006$) compared to controls, indicating impaired large bowel motility. Gross anatomical measurements showed a reduction in cdKO small intestine length ($p \leq 0.0001$) with increased jejunal and colonic muscularis thickness in cdKO mice ($p \leq 0.005$). *Ex vivo* force transduction assays were performed to measure jejunal and proximal colon longitudinal muscle contractility and cdKO tissues showed decreased contractile activity at baseline and in response to increasing concentrations of cholinergic stimulus ($p \leq 0.04$) while contraction frequency was maintained. Finally, RNA isolated from human and mouse jejunum and colon smooth muscle was used to identify DM1-associated splicing changes by RT-PCR. CRE-only and CRE-lacking controls were used for all assays.

Conserved, misregulated splicing of *Mypt1* exon 24 was identified in both mouse and human jejunal and colonic tissues and was found to affect Mypt1 leucine zipper domain inclusion by western blot. Given the potential for Mypt1 to alter overall myosin light chain phosphatase (MLCP) activity, the predominate regulator for smooth muscle relaxation, we evaluated phosphorylation sites of myosin light chain 2 (MLC20) to determine smooth muscle contraction levels. Increased ser19 Mlc20 phosphorylation ($p \leq 0.005$) indicated increased contraction of cdKO smooth muscle, which could be explained by decreased MLCP activity resulting from increased Mypt1 phosphorylation at the inhibitory thr696 site ($p \leq 0.01$). *Ex vivo* force transduction experiments will be repeated using a Rho kinase (RhoK) inhibitor to test whether tissue contractile activity can be rescued by increasing MLCP activity through decreased RhoK-dependent phosphorylation. Treated tissues will also be used to evaluate rescue of pMlc20 and pMypt1 levels in cdKO mice by western blot.

In conclusion, we have successfully generated the first mouse model of DM1 GI dysfunction and shown that smooth muscle specific knockout of MBNL causes delayed GI motility and decreased contractile activity. We suspect that dysregulated splicing of *Mypt1* transcripts interferes with smooth muscle relaxation during peristalsis that may be rescued by exogenously increasing MLCP activity.

Bmpr2 And Grem1 Knockouts In Fibroblasts Do Not Alter Severity Of Alcohol-Associated Chronic Pancreatitis In Mice

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Background: Chronic pancreatitis (CP), a debilitating disease characterized by progressive pancreatic fibrosis, is commonly caused by alcohol consumption. The underlying mechanisms behind alcohol effects on CP progression remain unclear. Pancreatic fibroblasts have been shown to play a key role in fibrosis in CP, and express both bone morphogenetic protein type 2 receptor (Bmpr2) and Gremlin 1 (Grem1), an endogenous BMP antagonist. Previously, we demonstrated that global KO of Bmpr2 exacerbates CP-induced pancreatic fibrosis, while KO of Grem1 attenuates CP-induced pancreatic fibrosis, suggesting an anti-fibrogenic role of BMP signaling in CP. Utilizing alcohol-associated CP mouse models, we observed increased Bmpr2 and Grem1 expression. In this study, we developed fibroblast-specific KOs and investigated the role of Bmpr2 and Grem1 in alcohol-associated CP. **Methods:** Inducible $Bmpr2^{fibroblast(fb)KO}$ and $Grem1^{fbKO}$ mice were generated by crossing $Col1a2^{CreER}$ with $Bmpr2^{flox/flox(f/f)}$ and $Grem1^{f/f}$ mice (from Regeneron Pharm), respectively. $Bmpr2^{f/f}$ and $Grem1^{f/f}$ mice were used as respective controls. Male and female adult mice were used. To assess KO effects, pancreatic fibroblasts were isolated from mice after tamoxifen administration and qPCR was performed. CP was induced in mice fed with Lieber-DeCarli liquid diet supplemented with 4% alcohol and received cerulein (50µg/kg, ip, 5 injections/day, 3 days/week for 4 weeks). Tamoxifen was injected after the first week of CP induction for ablation of CP-induced Bmpr2 or Grem1 gene expression. The mice were euthanized after CP induction (n=9-15/group). The pancreata were harvested for histopathological evaluation and Sirius red staining for fibrosis quantification. **Results:** Isolated pancreatic fibroblasts showed 68% reduction of Bmpr2 and 50% reduction of Grem1 mRNA levels in $Bmpr2^{fbKO}$ mice and $Grem1^{fbKO}$ mice, respectively, compared to the controls ($p<0.05$). Upon CP induction, $Bmpr2^{fbKO}$ and $Grem1^{fbKO}$ showed similar CP scores and fibrosis compared to the controls ($p>0.05$); $Bmpr2^{fbKO}$ mice exhibited a trend of more severe cases with CP score>20 (5/11 in $Bmpr2^{fbKO}$ vs 2/9 in controls). **Conclusions:** Specific gene KOs of Bmpr2 or Grem1 in pancreatic fibroblasts did not alter alcohol-associated CP severity or fibrosis compared to the controls. Taken together with our previous studies using the global KOs, fibroblast-specific KOs of Bmpr2 and Grem1 may not be sufficient to alter CP severity. Other cell types may be involved in the development of alcohol-associated CP.

Chronic arsenic exposure induces thymidylate synthase (TYMS) in colorectal cancer (CRC) cells: implications for 5-Fluorouracil Chemoresistance

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Background: Arsenic (As) is an established carcinogen for bladder and skin cancer. Emerging evidence implicates CRC with a ~10-60% increase in mortality (Kasmi et al *Environ Health* 2023), although the increase in CRC incidence is less definitive. This leads us to speculate that As may potentially lead to more aggressive/chemotherapy-resistant CRCs. 5-fluorouracil (5-FU) is the stalwart for CRC treatment. Therefore, we wanted to assess As's ability to resist 5-FU. **Methods:** Cell Culture: We chronically treated human CRC lines HT29 and HCT116 (6-10 weeks) with low doses (0.1, 0.25 ug). Molecular Assays: RNA seq: RNA seq was Illumina® NovaSeqTM platform. TaqMan based RT-PCR and Western blot analysis used GAPDH was used as loading control. Cellular Assays: Cell number was determined via tetrazolium-based assays (WST-1) and cell count via DAPI nuclear stain. **Results:** We evaluated our RNAseq data from HT29 chronically 0.25 uM As-treated with for chemoresistance genes and noted that TYMS overexpressed TYMS by 134% ($p<0.01$) and we corroborated with individual RT-PCR. Lower dose (As 0.1 uM) in HT29 showed a 45%. To show robustness, we performed in a different cell line (HCT-116, a microsatellite unstable human line) and results mirrored the previous data (180% and 45% in 0.25 uM and 0.1 uM, respectively). With regards to efficacy of 25uM 5-FU (table 1), in HT29 cells 0.1 and 0.25 uM blunted cell killing of 5-FU by 15% and 28% respectively. This was confirmed in HCT116 with the 0.25 uM arsenic suppressing 5-FU efficacy by 17% (no change with the 0.1 uM). Finally, we assessed 5-FU induced apoptosis by measuring activated (cleaved) caspase 3 on Western blot and noted that 0.1 uM and 0.25 uM arsenic mitigated 5-FU induced apoptosis by 66% and 33% respectively (table 2). **Conclusion:** For the first time, we demonstrated that chronic As exposure leads to blunting of the 5-FU efficacy via induction of TYMS. This provides insights into the increased mortality of CRC by As. With the increasing amounts of As in drinking water caused by climate change, the potential clinical ramifications of these findings may increase. We believe that factoring in the role of As and other toxicants may be important in providing precision medicine to CRC therapy.

HT-29	No As with 5-FU	0.1 uM As with 5-FU	0.25 uM As with 5-FU
Percent Fold Change Compared to control As without 5-FU	-68.4	-58.2	-49.1
P-Value	1.30E-13	3.90E-12	1.40E-10
HCT-116	No As with 5-FU	0.1 uM As with 5-FU	0.25 uM As with 5-FU
Percent Fold Change Compared to control As without 5-FU	-69.5	-71.9	-57.6
P-Value	8.30E-13	1.50E-10	3.2E-09

Table 1: DAPI staining of cells treated with As HCT-116 and HT-29 exposed to 5-FU. Fold change compared to As alone.

	0.1uM As	0.25uM As	5-FU	0.1uM As with 5-FU	0.25uM As with 5FU
Percent Fold Change in cleaved PARP	36.8	53.9	1219.0	409.1	819.1

Table 2: Western Blot density analysis data of cleaved PARP in HCT-116 percent fold change is compared to the control unexposed As and 5-FU HCT-116

Probiotic *limosilactobacillus reuteri* dsm 17938 ameliorates maternal separation stress in newborn mice and alters adolescent behavior

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Background: Gut microbial dysbiosis plays a key role in early life stress and stress-related adult neuropsychiatric disorders. Resetting gut microbiota by early-life microbial manipulation may produce long-term benefits. Probiotic DSM 17938 modulates gut microbiota, alters systemic metabolites, and facilitates immune regulation. In mice, maternal separation (MS) results in disorganized maternal care, behavioral deficits, and dysbiosis that persist into adulthood. Cholecystokinin (CCK) generated by both gut and brain, and corticosterone are associated with the stress response. Glial fibrillary acidic protein (GFAP) produced by brain glial cells may be involved in stress-associated gliosis.

Objective: To determine whether DSM 17938 can reduce stress-associated changes in newborn mice and affect subsequent adult behavior by modulating gut microbiota.

Methods: Mouse pups were exposed to unpredictable MS (MSU) from d7 to d20 of life, daily, 3 hours/day with daily supplemental DSM 17938 (10^7 CFU/mouse/day) or phosphate buffered saline (PBS), given by gavage. They were weighed weekly. CCK, GFAP, and corticosterone in brain tissue were measured by ELISA, and stool microbiota was analyzed by 16S rRNA sequencing on d21. Behavioral evaluation of 10-week-old mice included Y-maze Test (YMT) for cognitive function and Tail Suspension Test (TST) for depression-like behavior.

Results: MSU resulted in a significant decrease in growth at d14 and d21 of life ($p= 0.035$ and <0.0001, respectively). Orally feeding DSM 17938 these mice significantly improved weight gain compared to stressed mice fed with PBS. Protein levels of CCK in brain tissue were reduced following MSU, while DSM 17938 significantly increased CCK in those subjected to MSU ($p=0.04$). Interestingly, GFAP levels showed an opposite trend to CCK levels, indicating that the decrease in CCK was likely secondary to a detrimental effect on neuronal health. PBS-fed newborn mice with MSU significantly increased corticosterone level, which was reduced by DSM 17938. On behavioral testing of the older mice, adult mice experiencing early MSU demonstrated reduced % of correct cycles on YMT and mobility time on TST. DSM 17938 treated offsprings demonstrated better cognitive function and less depressive-like behavior compared to PBS treated controls. Stress resulted in a significantly reduced fecal Shannon diversity and distinctly shifted clusters compared to no stress, which could be corrected by oral administration of DSM 17938.

Conclusions: DSM 17938 beneficially affects stress-related physical and biochemical changes caused by MS in neonates. Additionally, we found that early life intervention of gut microbiota by DSM 17938 had a beneficial effect on subsequent adult behavior.

Acinar to ductal metaplasia is an evolutionary dead end and does not actively contribute to pancreas regeneration after damage

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One of the major risk factors strongly associated with PDAC is pancreatitis and meta-analysis studies have revealed that inflammation of the pancreas increases the relative risk of developing pancreatic cancer by up to 13-fold. During pancreatitis, acinar cells transiently undergo metaplasia known as acinar to ductal metaplasia (ADM). It is well known that in the presence of activated oncogenes, for example mutated *Kras*, this metaplastic state can progress into PDAC with ADM being the first step towards tumorigenesis. To understand the pathophysiological role of ADM during pancreatitis and tumorigenesis, we utilized different mouse models to study the function and fate of ADM cells. We found that although ADM can effectively mitigate pancreatitis-induced tissue damage, it is not a regenerative process. Instead, cells that underwent ADM were eventually cleared out from the tissue. Immunophenotyping studies further demonstrated a rise in the abundance of phagocytic cells in the tissue during later stages of recovery from pancreatitis that might suggest cell clearance. To better capture the dynamic process distinct sub-populations of epithelial cells undergo during ADM induction and resolution, we are leveraging spatial transcriptomics of the entire pancreatic tissue at different timepoints after damage induction. We are also actively studying how oncogenic *Kras* activation reverts this process and contributes to tumorigenesis. This study will shed light on initiation of pancreatic cancer and how it can be prevented in the context of evolution from pancreatitis.

New patterns of cell plasticity in longitudinal study of human tissue: Implications for healing after radiofrequency ablation in Barrett's Esophagus

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Barrett's Esophagus (BE), a precursor of esophageal adenocarcinoma (EAC), develops from chronic epithelial damage due to gastrointestinal reflux, replacing normal squamous epithelium with columnar intestinal metaplasia. Radiofrequency Ablation (RFA) is performed in high-risk patients to reduce the risk of BE progressing to cancer. RFA induces a wound across three epithelial cell types (stratified squamous, Barrett's metaplasia, and gastric epithelium), typically healing completely with squamous epithelium. However, the mechanisms underlying the repair post-RFA and the origin of the neosquamous epithelium remain unclear. Recent studies suggest re-epithelialization by squamous cells at the proximal ablated wound edge and by patches of squamous cells emerging as islands derived from ducts of the underlying esophageal submucosal glands throughout the wound. Notably, buried intestinal metaplasia foci beneath the neosquamous mucosa were also observed during the healing process, potentially serving as a source for BE recurrence.

To understand the neo-epithelialization process and the origins of neosquamous epithelium, we characterized the histological changes in endoscopic biopsies from BE patients at 1, 2, and 4 weeks post-RFA. Immunohistochemistry revealed the emergence of glands expressing both CDX2 (intestinal) and p63 (squamous) at 1 week post-RFA, a ductal structure expressing a mixture of p63, SOX9 (intestinal progenitor), and CK7 (metaplastic) but negative for CDX2 at 2 weeks post-RFA, and Barrett's glands expressing CK7, CDX2, SOX2 (gastric) but negative for p63 at 4 weeks post-RFA. These findings uncover an unexpected previously unidentified highly plastic transitional cells with potential as neosquamous mucosa progenitors during RFA-induced healing in BE patients.

In parallel, we also explored the heterogeneity of endoscopic biopsies from BE patients before and after RFA using Spatial Transcriptomics (ST). This endeavour marks the first of its kind to analyse tissue architecture and local tissue interactions during the post-RFA healing process. Biopsies collected from BE patients before RFA and at 1, 2, and 4 weeks post-RFA were subjected to ST analysis. The transcriptomic data underwent cell type identification, differential gene expression analysis, and pathway analysis. We identified six distinct subpopulations within the biopsies and reveal the spatial distribution of these subsets within the tissue, uncovering potential interactions among them. We observed a unique subpopulation exhibiting an epidermal development signature at 1 week post-RFA, followed by a mixture of epidermal cell and keratinocyte differentiation profiles with distinct transcriptional programs at 2 and 4 weeks post-RFA, indicating the progression of squamous cell development during the healing process. Our ongoing work aims to identify, for the first time, novel molecular markers associated with neo-epithelization of the esophageal epithelium following RFA.

Early-life malnutrition suppresses hepatic Cyp7b1 transcription by inducing Srebp1

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Background: Severe acute malnutrition decreases hepatic bile acid synthesis, and this results in impaired intestinal absorption of dietary fats and fat-soluble vitamins, overgrowth of intestinal bacteria, and intolerance to therapeutic refeeding. Our mouse model of malnutrition exhibits a decreased bile acid pool and profoundly decreased hepatic expression of one of the three rate-limiting enzymes in bile acid synthesis: oxysterol 7-α hydroxylase (Cyp7b1). Expression of this key enzyme is tightly controlled by transcriptional regulators including the sterol regulatory element-binding protein-1 (Srebp1), which is known to repress Cyp7b1 transcription. The overall aim of this project is to determine whether targeted inhibition of Srebp1 can restore bile acid synthesis and improve growth in malnourished mice.

Methods: Lactating dams were randomized to receive a low-protein, low-fat diet (LPLFD; 5% fat, 7% protein, and 88% carbohydrate) or isocaloric control diet (15% fat, 20% protein, and 65% carbohydrate). On day-of-life 21, pups were weaned to their respective dam's diet and continued on LPLFD or control diet for the remainder of the experiment. At 8 weeks of age, livers were harvested to assess mRNA and protein expression of Cyp7b1 and Srebp1 by qPCR and western blot, and chromatin immunoprecipitation (ChIP) was used to assess Srebp1 binding to the Cyp7b1 promoter. In vitro, HepG2 hepatocytes were treated with Srebp1 inhibitors fatostatin or betulin to determine whether Cyp7b1 expression and bile acid synthesis can be rescued. Malnourished mice were treated with Srebp1 inhibitors fatostain or betulin (30 mg/kg/day) for 3 days to assess Cyp7b1 protein level and bile acid pool size.

Results: In healthy mouse pups, expression of hepatic Cyp7b1 increased sharply after birth, with peak expression in postnatal week 3. After 6 weeks of life, males sustained higher Cyp7b1 mRNA and protein expression relative to females. Malnutrition dramatically reduced Cyp7b1 transcript and protein and bile acid pool size in males but not females. Expression of Srebp1 was increased 4-fold in malnourished males but was not altered in malnourished females. Srebp1 was enriched 3-fold at the Cyp7b1 promoter in livers from malnourished males compared to controls, while occupancy at the Cyp7b1 promoter of Sp1, a transcription factor that positively regulates Cyp7b1 expression, was decreased. In vitro, Srebp1 inhibitors increased hepatocyte Cyp7b1 expression in a dose-dependent manner without increasing bile acid production. In vivo, treatment of malnourished male mice with Srebp1 inhibitor partially restored Cyp7b1 protein levels but did not increased bile acid pool size.

Conclusion: Decreased bile acid synthesis in malnutrition is associated with transcriptional repression of Cyp7b1 that is mediated by Srebp1 recruitment to the promoter and exclusion of the transcription factor Sp1 in a sex-dependent manner. Srebp1 inhibitor treatment partially rescues hepatic Cyp7b1 expression but not bile acid pool size in malnourished males and immortalized hepatocytes, suggesting that other factors including impaired activity of bile acid synthesizing enzymes may be responsible for decreased bile acid synthesis in malnutrition.

Decoding human norovirus tropism via single-cell RNA analysis

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Human noroviruses (HuNoVs) are the leading viral cause of acute gastroenteritis worldwide in all age groups. Given the substantial healthcare costs and economic burden caused by HuNoV infections, developing effective treatments or vaccines is a priority that requires understanding the mechanisms of HuNoV infection and its interactions with host cells. Cellular tropism is one of the most critical characteristics for viruses to maintain a successful life cycle. However, the cellular tropism for HuNov has not yet been fully elucidated. While several previous investigations have demonstrated that enterocytes are the predominant cell type susceptible to HuNoV infection, one recent study showed that enteroendocrine cells are another permissive cell type for HuNoV infection suggesting that HuNoV may have yet unexplored cellular targets. Notably, these studies used biopsies from immunocompromised transplant patients, leaving the question of cellular targets in immunocompetent patients unanswered.

The development of tissue-stem cell derived organoids (HIOs) as an ex-vivo model of HuNoV cultivation has provided tremendous insights into HuNoV replication and pathogenesis. By incorporating single-cell RNA sequencing (scRNA-Seq) technology, we aim to generate a single cell transcriptomic atlas of HIOs infected by HuNoV to delineate cell type specific responses and cellular factors contributing to their susceptibility to viral infection. In this project, we infected HIOs with a globally dominant GII.4 HuNoV strain and comprehensively profiled transcriptomics of 69,536 individual cells. We have identified unique cell populations that contain GII.4 transcripts indicative of viral infection and replication, as well as key genes and pathways elicited in response to GII.4 infections. We have shown that GII.4 infections change cell type distribution and affect transcription profiles of certain cell types. Furthermore, we have revealed a list of host genes co-expressed with GII.4 transcripts, shedding lights on searching potential viral co-receptors on host cell surface.

Overall, this project has the potential to significantly advance our knowledge of HuNoV infection biology, laying a foundation for future investigations into the development of effective interventions.

Limosilactobacillus reuteri-engineered growth factors to mitigate radiation damage in human intestinal organoids

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We are interested in understanding how the human gut microbiome can be leveraged to aid in gut epithelial response to stresses including damage caused by radiation. The human gastrointestinal epithelium regenerates every 3-5 days, making it a target for DNA damage and cell death following irradiation exposure during cancer radiotherapy and in space. We have access to a repository of human intestinal organoids (enteroids) spanning multiple parts of the GI tract that recapitulate the intestinal epithelium. Within these enteroids, there are non-dividing (differentiated) and dividing (undifferentiated) cells that serve different functional purposes in the epithelium. Interestingly, we found that differentiated cells have increased morphological changes and increased cell death following proton irradiation, while undifferentiated cells remain relatively unperturbed. We determined that radiation protection of undifferentiated enteroids was dependent on WRNE, a rich cell growth medium containing WNT, R-spondin, and EGF. In testing these individual components alone, we determined that the protective effect on the enteroids was mediated by Wnt and R-spondin. RSPOs potentiate Wnt signaling which regulates cell fate determination. We therefore engineered the probiotic bacterium *Limosilactobacillus reuteri* to produce water-soluble Wnt (“skinny Wnt”) and RSPO to protect organoids from cell death following proton irradiation. We are currently testing *L. reuteri*-secreted variants of these human growth factors on irradiated differentiated and undifferentiated organoids to understand the cell biological response to radiation. This work will help us better combat the detrimental effects of radiation on the human gastrointestinal tract.

A novel COX-2-IL-22-dependent crosstalk between eosinophils and type 3 innate lymphoid cells protects against colitis

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Inflammatory Bowel Disease (IBD), including Crohn's disease and ulcerative colitis, affects more than 3 million people in the United States. It is a debilitating disease with very limited and costly treatment options. Accumulation of eosinophils in the inflamed intestinal mucosa of IBD patients has been reported. Our recent pre-clinical study comparing dextran sulfate sodium (DSS)-induced colitis between wild-type (WT) and eosinophil-deficient ΔdblGATA1 mice demonstrated a profound protective effect of eosinophils. RNA-seq analyses of colonic eosinophils revealed a significant upregulation of cyclooxygenase (COX)-2 in colitic mice. To investigate the role of COX-2 in mediating the protective function of eosinophils, we generated a mouse line with eosinophil-specific deletion of COX-2 (COX-$2^{fl/fl}$eoCre$^{+/-}$). We found that similar to eosinophil-deficient mice, the COX-$2^{fl/fl}$ eoCre$^{+/-}$ mice developed exacerbated intestinal inflammation and injury compared with their WT littermates (COX-$2^{fl/fl}$eoCre$^{-/-}$) after DSS treatment. Mechanistic studies combining genetic and in vitro cell culture system identified that Cox-2-deletion in eosinophils impairs IL-22 production in type 3 innate lymphoid cells (ILC3s) through PGE2-EP4 axis. Together, these data provide insight into a mechanism of protective function of eosinophils in IBD and elucidate the underlying mechanism of such function that the eosinophil/COX-2/PGE2 axis could serve as a therapeutic target for IBD treatment.

YAP1 facilitates the re-entry of differentiated cells into the cell cycle during paligenosis through phospho-dependent, non-canonical Hippo pathway regulation

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Unraveling the origins of gastric cancer requires a thorough investigation into the initiation and progression of precursor metaplasias. Our focus lies on the transformation of chief cells in the stomach into proliferative spasmolytic polypeptide expressing metaplasia (SPEM) cells via the process of paligenosis that involves dynamic mTORC1 regulation via 3 stages: Stage 1, chief cells upregulate autophagic activity, as mTORC1 activity decreases; Stage 2, SPEM genes are expressed; and, Stage 3, the mTORC1 activity increases, prompting previously quiescent cells to re-enter the cell cycle. Dysregulated paligenosis emerges as a potential initiator of gastric cancer by enabling mature, non-dividing cells to re-enter the cell cycle. Despite the well-established role of the Hippo pathway in regulating cell proliferation and differentiation via YAP1/TAZ effectors, Hippo involvement in metaplasia and paligenosis remains elusive. After inducing chief cell paligenosis in mice using our well-characterized high-dose tamoxifen (HDT) protocol, nuclear (i.e., active) YAP1 accumulates in paligenotic cells in Stages 1 and 2 with expected induction of canonical YAP1 transcriptional targets. Next we induced expression of YAP1-S127A, which is a constitutively active mutant form of YAP1, in chief cells by first activating rtTA specifically in chief cells via low-dose, non-toxic tamoxifen in *Mist1*$^{CreERT2/+}$*; ROSA26*$^{LSL\text{-}rtTA.IRES.EGFP/+}$*; tetO*^{Yap1S127A} mice followed by doxycycline; or doxycycline alone in or *GIF*$^{\text{-}rtTA}$ *; tetO*^{Yap1S127A} mice. Both approaches led to strong nuclear YAP1 and direct induction of mitosis of previously terminally differentiated chief cells. In contrast, HDT paligenosis was impeded by YAP/TAZ deletion in *Mist1*$^{CreERT2/+}$*; ROSA26*$^{LSL\text{-}Ai9/+}$*; Yap1*$^{flox/flox}$; *Taz*$^{flox/flox}$ mice In HDT-induced paligenosis, bulk transcriptomic analyses indicated increased YAP1 target expression, which was further confirmed to be specifically in paligenotic chief cells, though total YAP1 protein by proteomics and western blot did not show changes, consistent with a shift of YAP1 to the nucleus via dephosphorylation.. Multi-omic data, corroborated by western blot and immunohistochemistry results, suggested a non-canonical Hippo pathway kinase-dependent modulation of YAP1 localization (i.e., paligenotic YAP1 activation did not use the canonical MST1/2→LATS1/2 Hippo pathway). The novel pathway involves loss of the YAP1-deactivating kinas in paligenosis via the massive autophagy in Stage 1, with YAP1 eventually deactivated to end paligenosis via SMAD2/3 effectors of TGF-β signaling. Our findings provide comprehensive insights into upstream kinase regulation and downstream targets associated with YAP1 regulation during paligenosis, contributing to a deeper understanding of gastric cancer initiation and offering potential avenues for targeted therapeutic interventions.

HES1 loss and counteracting ATOH1 up-regulation promotes intestinal stem cell regeneration of ulcerative colitis

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Background: Ulcerative colitis (UC) is associated with a higher risk of colorectal cancer (CRC) and impaired colonic epithelial regeneration. The latter is regulated by the interaction of hairy and enhancer of split-1 (HES1) and atonal homolog 1 (ATOH1). HES1 promotes intestinal progenitor proliferation and absorptive lineage differentiation while also inhibiting ATOH1 from activating secretory lineage differentiation. ATOH1^{+} secretory progenitor cells can exhibit stem cell properties following severe tissue injury. We reported that HES1 loss is frequent in serrated adenocarcinomas and colorectal cancers with KRAS mutation. We also established a mouse model of colitis and colitis-associated CRC (*Fx*$^{-/-}$ mouse) to recapitulate the loss of Notch/Hes1 signaling in colonic epithelium. However, the role of ATOH1 and HES1 in UC remains unknown.

Materials and Methods: We evaluated HES1 and ATOH1 expression in the colonic epithelium of paired UC tissues collected from the quiescent phase and active inflammation by immunostaining. The patients were further grouped into the non-relapsing and the relapsing group to investigate the association between HES1 and ATOH1 with the risk of relapse. The expression of stem cell markers and lineage markers was examined by RT-qPCR. Deletion of *Atoh1* in the intestinal epithelium of *Fx* mice was generated by crossing *CDX2-Cre*/*Atoh1*$^{+/flox}$ mice with *Fx* mice[3]. The effect of *Atoh1* deletion in mice colonic epithelium was assessed with colon histology and ex vivo culture of colonic organoids.

Results: In the colonic epithelium from the quiescent phase of UC, the expression of HES1 and ATOH1 was like that from the healthy control. HES1 displayed a strong nuclear expression in crypts of quiescent UC but showed attenuated expression during active inflammation. In contrast, ATOH1 expression was low in crypts of quiescent UC but increased by 2.5-fold during active inflammation. After treatment, HES1 expression recovered while ATOH1 returned to a lower level. Further, HES1 showed a near-complete loss in the non-relapsing group during active inflammation but had a much higher expression in the relapsing group. However, ATOH1 expression was similar between the two groups. The upregulation of ATOH1 during active inflammation was accompanied with the upregulation of the stem cell genes but not the secretory lineage genes downstream of ATOH1. *Atoh1*$^{-/-}$*Fx*$^{-/-}$ mice colon displayed decreased goblet cell expansion compared to that of *Fx*$^{-/-}$ mice. They showed significantly improved gut barrier integrity and inflammation-associated dysplasia progression (inflammation and dysplasia score both decreased by ~38%). Colonic organoids from *Atoh1*$^{-/-}$*Fx*$^{-/-}$ mice, however, had severely impaired development and regeneration.

Conclusions: In all, we have shown that attenuated HES1 expression is a prominent feature of active UC while upregulation of ATOH1^{+} secretory progenitors in active inflammation is critical for intestinal stem cell regeneration but it may also promote inflammation-associated transformation.

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ACKNOWLEDGEMENTS

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