DDC
Texas Medical Center Digestive Diseases Center

10TH ANNUAL
Frontiers in Digestive Diseases Symposium

Infection and Injury as a Precursor to GI & Liver Cancer

MARCH 2, 2019

ONSTEAD AUDITORIUM
HOUSTON, TEXAS

Texas Medical Center Digestive Diseases Center
10th Annual Frontiers in Digestive Diseases Symposium:
Infection and Injury as a Precursor to GI & Liver Cancer

Saturday, March 2, 2019
Onstead Auditorium, Houston, Texas 77030

About Texas Medical Center Digestive Disease Center

The Texas Medical Center Digestive Diseases Center (DDC) 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. It 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. It is one of only 18 NIH-funded Digestive Diseases Research Core Centers in the country and the only center in the southeast United States. The center was founded by Mary K. Estes, Ph.D., emeritus director and professor of molecular virology and microbiology at Baylor.

The DDC director is Hashem B. El-Serag, M.D., M.P.H., Margaret M. and Albert B. Alkek Chair of the Department of Medicine, and professor of gastroenterology and hepatology at Baylor. Doug Burrin, Ph.D., research physiologist at USDA-ARS Children's Nutrition Research Center and professor of pediatrics at Baylor, is co-director. James Versalovic, M.D., Ph.D., pathologist-in-chief and head at Texas Children's Hospital, and professor of pathology and immunology at Baylor, serves as associate director. J. Marc Rhoads, M.D., gastroenterology division director and professor of pediatric gastroenterology at The University of Texas Health Science Center serves as assistant director. For more information about the center, including membership, events, and funding opportunities, please visit https://www.bcm.edu/research/centers/digestive-disease.

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A G E N D A

7:30 -8:15 AM	**Breakfast**
8:15 - 8:30 AM	**Welcome Remarks** **Hashem El-Serag, M.D., MPH** Director, Texas Medical Center Digestive Diseases Center Chair and Professor, Margaret M. and Albert B. Alkek Department of Medicine Baylor College of Medicine
8:30 - 9:15 AM	**"The role of Inflammation, Injury and Stem Cells in the Pathogenesis of Gastric Cancer"** **Timothy C. Wang, M.D.** Chief, Division of Digestive Diseases Columbia University Medical Center
9:15 - 9:45 AM	**"Helicobacter pylori in Gastric Carcinogenesis"** **David Graham, M.D.** Professor of Medicine Baylor College of Medicine
9:45 - 10:15 AM	**"Hippo/YAP Signaling Pathway in Gastroesophageal Cancer"** **Shumei Song, M.D., Ph.D. (PF awardee)** Associate Professor, Department of Gastrointestinal Medical Oncology The University of Texas MD Anderson Cancer Center
10:15 - 10:30 AM	**Coffee break**
10:30 - 11:15 AM	**"Insights from Antiviral Therapy into Immune Responses to Hepatitis B and C Virus Infection"** **Barbara Rehermann, M.D.** Chief, Immunology Section, Liver Diseases Branch, NIDDK National Institutes of Health
11:15 - 11:45 AM	**"Risk for Hepatocellular Cancer in Patients with Treated Hepatitis C"** **Hashem El-Serag, M.D., MPH** Chair and Professor, Margaret M. and Albert B. Alkek Department of Medicine Baylor College of Medicine
11:45 -12:15 PM	**"Metabolic Pathway Reprogramming - the Chances and Challenges of Genome Engineering in the Liver"** **Karl-Dimiter Bissig, M.D., Ph.D. (PF awardee)** Associate Professor, Molecular and Cellular Biology, Center for Cell and Gene Therapy Baylor College of Medicine
12:15 - 1:00 PM	**"The Microbiome in Colon Cancer: Does it Matter?"** **Cynthia L. Sears, M.D.** Bloomberg-Kimmel Professor of Immunotherapy Johns Hopkins University School of Medicine
1:00 - 2:15 PM	**Lunch / Poster Session**
2:15 - 2:30 PM	**Poster Awards and Concluding Remarks**

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Texas Children's Hospital®

APPROVED CME ACTIVITY
Directly provided by Texas Children's Hospital
Hosted by the Texas Medical Center Digestive Diseases Center
Saturday, March 2, 2019 | 8:00 am – 2:00pm | Onstead Auditorium

"The role of Inflammation, Injury and Stem Cells in the Pathogenesis of Gastric Cancer"
TIMOTHY C. WANG, M.D., Chief, Division of Digestive Diseases, Columbia University Medical Center

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"Risk for Hepatocellular Cancer in Patients with Treated Hepatitis C"
HASHEM EL-SERAG, M.D., MPH, Chair and Professor, Department of Medicine, Baylor College of Medicine

"Metabolic Pathway Reprogramming: Chances and Challenges of Genome Engineering in the Liver"
KARL-DIMITER BISSIG, M.D., PH.D., Associate Professor, Molecular and Cellular Biology, Center for Cell and Gene Therapy, Baylor College of Medicine

"The Microbiome in Colon Cancer: Does it Matter?"
CYNTHIA L. SEARS, M.D., Bloomberg-Kimmel Professor of Immunotherapy, Johns Hopkins University School of Medicine

TARGET AUDIENCE
Internal Audience, Physicians, Specialists Gastroenterology, Research in Digestive Diseases, Medical Students, Residents, Fellows, Any physician or researcher with interest in digestive diseases

EDUCATIONAL OBJECTIVES
At the conclusion of this live activity, participants should be better able to: 1. Define infection and injuries that are a precursor to GI and Liver cancer; 2. Apply best practices and treatments for infection and injury to avoid GI and Liver cancer; 3. Identify opportunities to apply this knowledge to the detection of GI and liver cancer; and 4. Interpret the current research concerning injury and infection as a precursor to cancer for better patient care.

ACCREDITATION STATEMENT
This live activity has been planned and implemented in accordance with the accreditation requirements and policies of the Texas Medical Association through the joint providership of Texas Children's Hospital and Texas Medical Center Digestive Disease Center. Texas Children's Hospital is accredited by the TMA to provide continuing medical education for physicians.

CREDIT DESIGNATION
Texas Children's Hospital designates this live activity for a maximum of *4.25 AMA PRA Category 1 Credit(s)* ™. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

DISCLOSURE
All speakers listed above have reported no relationships with proprietary entities related to the content of this activity. Persons involved in the planning of this activity have reported no relevant financial relationships with any commercial interest.

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Proteomics indicate that 30-Day intermittent fasting increases lean body mass biomarkers

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2. Department of Biochemistry; BCM; Department of Pediatrics, Division of Gastroenterology, Nutrition and Hepatology, BCM; Michael E. DeBakey Department of Surgery, Division of Abdominal Transplantation, BCM

BACKGROUND: Intermittent fasting (IF) results in several metabolic changes including: normal maintenance of glucose concentrations, reduction of glycogen stores, mobilization of fatty acids, ketone generation, circulating leptin reduction, and often the elevation of adiponectin concentration (Johnson et al., 2007; Wan et al., 2010). Behavioral changes that occur during periods of IF include increased arousal and mental acuity (Fond et al., 2013). These responses are thought to be related to the metabolic shift to ketone utilization. Adaptive responses of the brain and autonomic nervous system to food deprivation have been shown to play major roles in the fitness-promotion. Conditions of low glycogen stores increase intramyocellular triacylglycerol (IMTG) consumption in type I fibers. Consequently, lipolysis may be increased after exercise to favor IMTG replenishment, and facilitate muscle adaptations (Vicente-Salar N. et al., 2015). Upregulation of mitochondrial UCP-3, while fasting, is an adaptive response after long-term IF (4-8 weeks) and appears to be a consequence of decreased fat oxidation rate. Protein expression involved in lipid metabolism is known to increase during long-term fasting. It is unclear how IF might affect other aspects of body composition. Proteomics is an emerging investigative venue and evaluates circulating analytes that are components of innumerable biochemical pathways. Specific proteins may be measured using liquid chromatographic tandem mass spectrometry (proteomics). **RESEARCH QUESTION:** Does Ramadan-related dawn to sunset IF change the proteomic biomarkers related to lean body mass? **METHODS:** Fourteen healthy volunteers (13M: 1F; Aged 24-61 years) were recruited. A total of 42 blood samples were collected at 3 study time-points from all subjects: before fasting [pre-fast visit (V2)], after 30 days of Ramadan IF (on-IF V4) and after 1 week of resumed habitual daytime meals (V5). Each subject fasted from dawn to sunset (~15 hrs/day). Periodic plasma samples were banked until batched proteomic analyses were performed. More than 1900 protein gene product groups were identified. The expression data from time-point V2 was compared with the data from time-points V4 and V5. Abundance/depletion values were reported as log2 fold changes (FΔ). **RESULTS:** Upregulation of TPM3 (Gene ID 7170) was observed after 30 days of IF (V2 vs V4; log2 FΔ 2.823, *P*=0.001) which further increased during fed-state V5. This increased expression trend from V2 to V5 was also observed with MYH9 (Gene ID 4627: log2 FΔ 2.639, *P*=0.022); PFN1 (Gene ID 5216: log2 FΔ 6.356, *P*=0.001); ITGB1 (Gene ID 3688: log2 FΔ 2.260, *P*=0.011); TLN1 (Gene ID 7094: log2 FΔ 2.561, *P*=0.016). Decreased expression was observed in MYO9A (Gene ID 4649: log2 FΔ -4.452, p=0.016) and ACTA1 (Gene ID 58: log2 FΔ -7.633, P=0.001). **DISCUSSION:** Our results indicate that dawn to sunset fasting for 30 days significantly increased gene expression related to lean body mass (striated and smooth muscle). IF also increased protein expression related to physical integrity of the cytoskeleton, cytokinesis, and cell adhesion of non-muscle cells. These proteins have roles in homeostasis, tissue repair, and immune response. Collectively, these findings suggest accumulation and/or regeneration of lean body mass after fasting could have benefits in the prevention and treatment of non-alcoholic fatty liver disease. Further studies are indicated.

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Dawn to Sunset Fasting for One Month Induces Myogenic Biomarkers, Decreases Brain-Derived Neurotrophic Factor and Increases Neurotrophin-4 Levels in Healthy Volunteers: Its Clinical Implications in Non-Alcoholic Fatty Liver Disease

Ayse L. Mindikoglu, M.D., M.P.H.[1,2], Mehmet Tosun, M.D.[3,4], Mustafa Abdulsada, MBChB[1]; Antone R. Opekun, M.S., P.A.-C [1,4]; David Y. Graham, M.D.[1], Prasun K. Jalal, M.D.[1,2], Antrix Jain, M.S.[4]; Sung Y. Jung, M.Sc, Ph.D.[4]; Sridevi Devaraj, Ph.D.[6]; Mirjana Maletic-Savatic, M.D., Ph.D.[3]

1. Department of Medicine, Gastroenterology and Hepatology, BCM; 2. Michael E. DeBakey Department of Surgery; 3. Department of Pediatrics, BCM; 4. Department of Pediatrics, Gastroenterology, BCM; 5. Advanced Technology Core, BCM; 6. Clinical Chemistry and Point of Care Technology, TCH

BACKGROUND AND AIMS: Lean body mass provides a ready reserve of essential amino acids to use to mount inflammatory responses and cytokine production. Non-alcoholic fatty liver disease (NAFLD) is associated with sarcopenia (sarcopenic obesity), cognitive dysfunction and altogether poor clinical outcomes. Neurotrophins including brain derived neurotrophic factor (BDNF), neurotrophin-3 and neurotrophin-4 play an important role in muscle regeneration, memory, learning, and regulation of energy metabolism. Elevated BDNF levels were shown to be associated with increased fat mass, obesity, type 2 diabetes, metabolic syndrome and cardiovascular risk factors. Based on these observations, we formulated a hypothesis that dawn to sunset fasting for one month would induce myogenesis, decrease BDNF levels and increase neurotrophin-3 and-4 levels in healthy subjects. **METHODS:** We conducted a pilot study in healthy subjects who fasted 'religiously' (no eating or drinking) from dawn to sunset (approximately 15 hours/day) for 30 days during Ramadan. Serum samples were collected before study initiation, at the end of the month-long fasting period and one week after the completion of fasting. BDNF, neurotrophin-3 and neurotrophin-4 levels were determined by ELISA and one-way repeated measures ANOVA was performed. Untargeted proteomic profiling was also performed using ultra high-performance liquid chromatography/tandem mass spectrometry in serum samples. Significant fold changes in gene protein products were assessed by volcano plot analysis. Gene Set Enrichment Analysis (GSEA) was performed to identify biological pathways involved in 30-day dawn to sunset fasting. **RESULTS:** Fourteen healthy subjects (13M;1F; aged 21-62 years) were studied. BDNF levels significantly decreased one week after study completion (mean 69.17 vs. 56.94 vs. 17.06 ng/ml, *P*=0.033) **(Figure 1**). One week after completion of 30-day of fasting, a significant **increase** in neurotrophin-4 abundance was observed (mean 58.75 vs. 57.49 vs. 61.47 pg/ml, *P*=0.002) when levels were compared with the levels at study initiation and during the intermittent fasting state. (**Figure 2**). Similarly, we found increase in serum neurotrophin-3 (mean 12.11 vs. 13.93 vs 14.37 pg/ml, *P*=0.200) levels one week after completion of 30-day of fasting compared with the levels at study initiation. Volcano plot analysis identified significant log2 fold increases in gene protein products associated with myogenesis after one month of fasting and one week after one month of fasting. **CONCLUSIONS:** This study indicated that 30-day dawn to sunset fasting significantly altered biomarkers of myogenesis, reduced BDNF levels and increased neurotrophin-3 and -4 levels in healthy subjects. Obesity is a risk factor for NAFLD, and our findings suggest another potential metabolic anti-obesity target in need of further studies. Dawn to sunset fasting for one month may have important prevention and treatment implications for obesity and NAFLD.

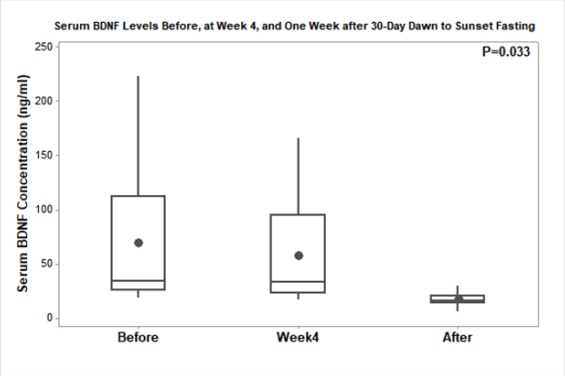

Figure 1. BDNF levels significantly **decreased** one week after study completion (mean 69.17 vs. 56.94 vs. 17.06 ng/ml, *P*=0.033).

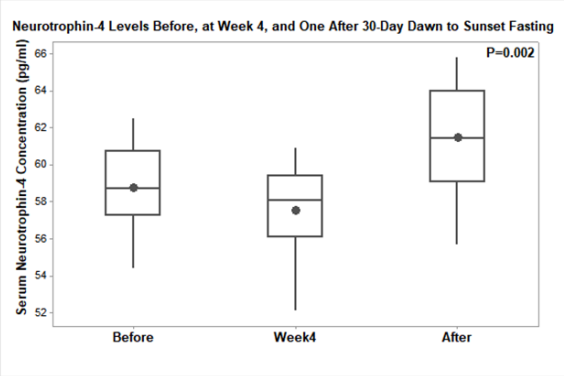

Figure 2. One week after completion of 30-day of fasting, a significant **increase** in neurotrophin-4 abundance was observed (mean 58.75 vs. 57.49 vs. 61.47 pg/ml, *P*=0.002) when levels were compared with the levels at study initiation and during the intermittent fasting state.

The short chain fatty acid acetate plays a central role in regulating inflammation in a model of hypertension

Sriram Ayyaswamy, Yuqing Wang, Sherry Phillips, Arielys M. Mendoza, Robert M. Bryan Jr, and David Durgan

Department of Anesthesiology, Baylor College of Medicine, Houston, Texas

Hypertension is a multi-faceted disease that involves systemic and neuroinflammation. However, for most forms of hypertension the underlying cause of this inflammation is unknown. Our lab has previously demonstrated that gut dysbiosis plays a causal role in the development of hypertension in a rat model of obstructive sleep apnea (OSA). Short chain fatty acids (SCFAs) are produced in the gut by bacterial fermentation of insoluble fiber and have recently been shown to play a role in modulating the immune response in multiple cell types.16S rRNA analysis of the microbiota showed a significant loss of short chain fatty acid–producing bacteria following 2 weeks of OSA. Metabolomics analysis revealed that the concentration of the SCFA acetate was decreased by 48% in OSA, as compared to sham, rats. In this study we hypothesized that impaired acetate production by the dysbiotic OSA microbiota contributes to inflammation both locally in the gut epithelium and systemically. We first exposed HT-29 human colon cancer epithelial cell line to cecal content isolated from sham or OSA rats. OSA cecal content induced a significant increase in gene expression of the pro-inflammatory markers IL-1β and IL-8, relative to sham ($p<0.05$ and $n=3$). Interestingly, when HT-29 cells were supplemented with acetate prior to and during cecal content exposure, this prevented the OSA cecal content induced expression of IL-1β and IL-8 ($p<0.05$ and $n=3$). We next examined the effects of OSA and acetate on the inflammatory response of circulating immune cells. We isolated peripheral blood mononuclear cells (PBMCs) from sham and OSA rats and stimulated these cells with lipopolysaccharide (LPS) in the absence or presence of acetate. PBMCs from OSA rats showed a significant increase in the proinflammtory cytokines IL-6, IL-1β and TNF-α, relative to sham when treated with LPS. Supplementation of these cells with acetate had a protective effect in particular with OSA rats as characterized by the decrease in proinflammtory cytokines IL-6, IL-1β, TNF-α and IL-10 that are involved in systemic inflammation. Using ELISA we further analyzed the expression of TNF-α released in the supernatant by PBMCs. Treatment with acetate markedly reduced the LPS induced expression of TNF-α by PBMCs from OSA animals. Collectively our data suggest that reduced acetate production, following OSA-induced dysbiosis, contributes to gut and systemic inflammation.

A methodical approach to the diagnosis of atrophic gastritis: a retrospective review with clinicopathological correlation

M. Suzanne Bloomquist, MD, Shilpa Jain, MD, Harris Health and Baylor College of Medicine

Background: Atrophic gastritis (AG) is histologically identified by destruction of parietal cells. The two most common etiologies include autoimmune metaplastic AG (AMAG) which is antibody-mediated, and chronic Helicobacter pylori infection. The diagnosis of AG can often be missed in random gastric sampling as the oxyntic mucosa appears to be antralized histologically. AMAG is associated with enterochromaffin-like (ECL) cell hyperplasia which can progress to a neuroendocrine neoplasm. We report a descriptive study of presentation, diagnosis, and complications of AG.

Materials & Methods: A retrospective data search was performed from 1/2014 through 11/2018 for the diagnosis of atrophic or autoimmune gastritis and 111 biopsies were retrieved. The clinical information, pertinent laboratory data, endoscopic reports, and follow-up biopsies and data were collected.

Results: Out of 4947 stomach biopsies performed at our institution, AG was diagnosed in 2.2% (111/4947). Our cohort comprised 86 patients, predominantly Hispanic (66%, 57/86) and black (28%, 24/86), with M:F ratio of 1:3.4, and mean age of 56 (range 29-77). The reason for endoscopy included prior vitamin B12 deficiency or atrophic gastritis in 26% (22/86), unspecified or iron deficiency anemia in 28% (24/86), as well as dyspepsia, abdominal pain, and imaging abnormalities. Laboratory studies showed anemia in 64% (55/86), vitamin B12 deficiency in 39% (28/71) of tested patients, and iron deficiency in 37% (27/73) of tested patients. Please refer to the table for antibody serology, gastrin, and chromogranin A studies. On EGD, atrophic mucosa was noted in 65% (56/86) of patients. Random biopsies (all biopsies in 1 container) were obtained in 22% (19/86), separate antrum and body biopsies in 36% (31/86), gastric mapping in 30% (26/86), body only biopsies in 3% (3/86), and biopsy of specific lesions in 8% (7/86). ECL-cell hyperplasia was diagnosed in 62% (53/86) of patients, with 3% (3/86) showing ECL-cell dysplasia. Neuroendocrine tumors were identified in 12% (10/86) of patients.

AMAG-specific tests	**Positive**
Either anti-parietal OR anti-intrinsic factor antibody positive	57% (20/35)
Both anti-parietal AND anti-intrinsic factor antibody positive	17% (6/35)
Anti-parietal antibody positive	59% (19/32)
Intrinsic factor antibody positive	80% (12/15)
Elevated serum gastrin	83% (15/18)
Elevated chromogranin A	83% (5/6)

Discussion & Conclusion: Although AMAG is precancerous, the issue of surveillance endoscopy for cancer screening is unsettled. Follow-up examination intervals are thus based on histologic abnormalities present on initial biopsy including extent of atrophy, intestinal metaplasia, dysplasia, and neuroendocrine cell hyperplasia. AG is a challenging histologic diagnosis, especially in random gastric sampling. In addition, no single laboratory test is perfectly sensitive or specific in AMAG. A step-wise clinicopathologic approach including a high index of suspicion based on patient demographics, prior or concurrent laboratory testing, and separate sampling of stomach antrum and body are thus crucial in facilitating an accurate and specific diagnosis.

Unexpected prevalence of hepatic fibrosis in urea cycle disorders

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Urea cycle disorders (UCDs) are among the most common inborn errors of liver metabolism. Early diagnosis and improvements in therapies to control hyperammonemia have led to improved long-term survival and have revealed chronic complications, which were previously considered uncommon. Liver disease is one such complication, which can manifest as elevated serum aminotransferases, hepatomegaly, fibrosis, portal hypertension and end-stage liver disease. Additionally, hepatocellular carcinoma may occur and may prove fatal. The causes of hepatopathy in UCDs are unknown. Guidelines for monitoring hepatic disease in UCDs are lacking, and there are no formal recommendations for prevention or treatment of this complication. To estimate the prevalence of hepatic fibrosis and cirrhosis in UCDs, we performed a retrospective histopathology review of liver biopsies performed diagnostically or explants from children with UCDs at Texas Children's Hospital (n=29, ages 6 days to 8 years) over a 21 year period (1995 - 2016). Forty-eight percent (n=14) of the cohort had ≥ F1 fibrosis, and 14% (n=4) had evidence for advanced fibrosis of F3 or higher. All explants or biopsies from individuals with hyperornithinemia-hyperammonemia-homocitrullinuria syndrome (n=1), argininosuccinate lyase deficiency (n=4), arginase deficiency (n=2) and partial ornithine transcarbamylase deficiency (n=3) showed ≥ F1 fibrosis. The highest prevalence of advanced fibrosis was observed in argininosuccinate lyase deficiency (n=3 with ≥F3 fibrosis). Although serum aminotransferases were elevated (> 30 U/L) in 78% of the cohort, there was no significant correlation with severity of fibrosis. Hepatic steatosis and marked hepatic glycogenosis were also common findings. Overall, our histopathologic findings demonstrate that hepatic fibrosis, steatosis and glycogen accumulation may be unrecognized complications in many individuals with UCDs. Further studies are needed to assess the underlying pathogenesis of liver disease in UCDs and to determine the utility of noninvasive biomarkers and imaging for identifying liver disease in individuals with UCDs.

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Exploring therapeutic potential by targeting Gremlin1 in Cerulein-induced mouse chronic pancreatitis

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Background: Gremlin1 (Grem1), an endogenous antagonist of bone morphogenetic proteins (BMPs), promotes fibrosis through inhibition of the anti-fibrogenic BMP/Smad1/5 pathway. We reported that Grem1 is upregulated in human and mouse chronic pancreatitis (CP). To determine the effects of blocking Grem1 in CP, we administered a Grem1 antibody (VelocImmune® Grem1 Ab, Regeneron Pharmaceuticals) to mice under cerulein-induced CP.

Methods: Based on Grem1's natural ability to inhibit BMP signaling, we first characterized the Grem1 Ab in vitro by assessing its capacity to restore the suppressed BMP/Smad1/5 signaling by Grem1. Human pancreatic fibroblasts and mouse pancreatic stellate cells were treated in 4 groups: 1) vehicle, 2) BMP2 (50ng/ml), 3) BMP2 (50ng/ml)+Grem1 (500ng/ml)+IgG isotype (5000ng/ml), and 4) BMP2 (50ng/ml)+Grem1 (500ng/ml)+Grem1 Ab (5000ng/mL). Phospho(p)Smad1/5 levels were assessed by western blotting. For in vivo studies, male C57BL/6 mice were randomly assigned to 3 groups: 1) Control mice receiving normal saline, 2) CP mice receiving cerulein (50μg/kg, 5 ip injections/day, 3days/wk for 4wks)+IgG isotype, and 3) CP mice receiving cerulein+Grem1 Ab (n=3-4/group). IgG isotype or Grem1 AB was given at 10mg/kg, ip, 2days/wk, beginning at the 2ndwk of cerulein injections and ending at wk4. Mice were weighed weekly and pancreata were harvested at day4 post treatment. Histopathologic scores were obtained from H&E stained sections. Sirius red staining and Vimentin immunofluorescence staining were performed for fibrosis assessment.

Results: We confirmed in vitro that Grem1 blocked BMP2-induced pSmad1/5 signaling, which was restored by Grem1 Ab. In vivo, compared to the CP mice receiving IgG isotype, the CP mice receiving Grem1 Ab gained more body weight over the course of the study and had 70% reduced fibrosis at 4wks ($p<0.05$). However, acinar injury scores were similar in the CP mice regardless of treatment.

Conclusions: Blocking Grem1 activity by Grem1 Ab in vitro restores BMP2-induced pSmad1/5 signaling, and in vivo attenuates pancreatic fibrosis. These results imply that Grem1 Ab may have therapeutic potential for CP.

Exploring the role of chromatin modifiers in IDH-mutant intrahepatic cholangiocarcinoma

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Objective: Intrahepatic cholangiocarcinoma (ICC) is highly malignant and accounts for 10-15% of all primary liver malignancies. The global incidence of ICC has been steadily increasing during the last 2 to 3 decades and the grounds for this increase are unclear. Isocitrate dehydrogenase (IDH1/IDH2) genes are mutated in 15-20% of ICC; similarly, ~30-40% of ICC cases have mutations in at least one of the chromatin modifiers as ARID1A, ARID2, BAP1, or PBRM1. However, the exact mechanisms of oncogenesis of either IDH or the chromatin modifiers are unknown. Our aim is to understand how IDH mutations synergize with chromatin modifier mutations by building novel human-based mouse models and establishing new human ICC cell lines from patients. We hypothesize that the molecular analyses of these models will illuminate new translational avenues.

Method: Immortalized human hepatoblasts were engineered to overexpress mutant IDH1 with or without knockdown of the chromatin modifiers ARID1A, ARID2, BAP1, or PBRM1, or the tumor suppressors CDKN2A or TP53. These are all frequently co-mutated with IDH1 in human ICC. We have begun to characterize these cells by using a protein array and by injecting into mice.
Results: Using protein arrays, we found proteins specifically regulated by mutant IDH1, but also others broadly regulated by chromatin modifier knockdown. While the results are still being analyzed, candidate oncogenic and cell proliferative pathways have emerged as likely downstream targets. Intradermal injections of the IDH1 mutant-alone hepatoblasts into nude mice slowly generated tumors, while those with additional chromatin modifier knockdown rapidly generated fast-growing tumors.. We also demonstrate that orthotopic liver tumors generated from the hepatoblast model have immunohistochemical hallmarks of human ICC. Finally, we have established several new ICC cell lines from patients including one with a concomitant IDH1 and ARID1A mutation.

Conclusion: Results from the protein array have helped us identify potential downstream therapeutic vulnerabilities. The novel IDH-mutant iCCA mouse models will continue to be characterized, with a focus on how IDH1, ARID1A, ARID2, BAP1, and PBRM1 induce tumorigenesis. This model, coupled with both established and our new human ICC cell lines, our long-term goal is to test various classes of inhibitors to target the pathways involved, with an eye towards clinical translatability.

Telomere dysfunction is a driver of inflammatory bowel disease

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Summary
The molecular instigators and therapeutic strategies for inflammatory bowel disease (IBD) are limited. Here, telomere dysfunction is shown to activate pAtm/c-Abl-mediated phosphorylation and stabilization of Yap1 up-regulates *pro-IL-18* expression, a major pro-inflammatory factor in IBD. This signaling axis cooperates with gut microbiome which stimulates cytosolic receptors causing activation of caspase-1, which in turn cleaves pro-IL-18 into its mature IL-18 form. Epithelial-derived IL-18 leads to recruitment of IFN□-secreting T cells and other immunocytes to provoke classical IBD pathology. Consistent with a role for DNA damage signaling as a driver of IBD, newly diagnosed IBD patient samples exhibited elevated expression of p□H2AX, YAP1, Caspase-1 and IL-18 along with significantly reduced telomere lengths compared to healthy tissue controls. Alleviation of IBD pathology can be achieved in mice via telomerase reactivation in intestinal epithelium or pharmacological inhibition of Atm, Yap1, or caspase-1 as well as antibiotic treatment – each intervention dramatically reducing pro-IL-18 cleavage and inflammation. Thus, telomere dysfunction-induced activation of the Atm-Yap1-pro-IL-18 pathway identifies DNA damage signaling as a key instigator and promoter of IBD, illuminating several novel therapeutic strategies for disease interception and management.

Rotavirus infection induces intercellular calcium waves through purinergic signaling

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Rotavirus (RV) remains the leading viral cause of diarrheal disease in children worldwide. The life-threatening diarrhea from RV infection is potentially exacerbated by the release of signaling molecules from infected villus epithelial cells. RV increases cytosolic calcium in infected cells, which is necessary for RV replication and activation of secretory pathways. Thus, characterizing RV-induced calcium signaling is needed to understand RV pathogenesis. However, the activation of a calcium signal from infected to uninfected cells has not been directly observed. Therefore the signaling mechanisms that facilitate widespread dysregulation of fluid secretion remain undefined. To address this, we conducted live cell fluorescence imaging in cell lines and jejunum human intestinal enteroids (jHIEs) to measure calcium signaling during RV infection.

We generated African monkey kidney MA104 cells and jHIEs that stably express GFP-based genetically encoded calcium indicators to measure calcium dynamics during RV infection. RV significantly increases the number and magnitude of calcium transients in infected wells. Further, we observed that single RV-infected cells triggered long-distance intercellular calcium waves that encompassed surrounding uninfected cells. Treatment with the ectoNTPase apyrase or the P2Y1 purinergic receptor inhibitor BPTU blocked these intercellular waves and RV replication. We created a genetic knockout of the P2Y1 receptor in MA104 cells using CRISPR/Cas9, and there were reduced calcium waves in RV-infected knockout cells. In RV-infected HIEs, blocking calcium waves with NTPase and P2Y1 blocker BPTU decreased serotonin secretion, which exacerbates RV diarrhea and vomiting *via* the enteric nervous system. Furthermore, these inhibitors also reduced fluid secretion in RV-infected jHIEs in an enteroid swelling assay.

Our results demonstrate that RV-infected cells release extracellular ATP/ADP to cause calcium signaling in uninfected cells important for RV replication and secretory pathways. Our data points to purinergic signaling as a therapeutic target for developing anti-diarrheal drugs. Furthermore, we are the first to show that viruses can exploit purinergic signaling and calcium waves to potentially amplify pathophysiological signaling important for diarrhea.

Dysregulated TGF-β signaling leads to impaired DNA damage repair in alcoholic liver disease

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Background: Alcoholic liver disease (ALD) is a complex process that includes a wide spectrum of interrelated hepatic lesions, from steatosis to cirrhosis and hepatocellular carcinoma.
Exposure to the ethanol-derived toxic metabolite acetaldehyde leads to DNA damage and liver injury. The transforming growth factor beta (TGF-β) signaling pathway is an important negative regulator of cell proliferation and an inducer of apoptosis in ALD. Our recent findings have revealed the novel role of the crucial TGF-β/Smad3/4 adaptor and transcriptional cofactor *SPTBN1* in conferring genomic stability and proper DNA repair by up-regulating the Fanconi anemia DNA repair pathway, in the context of toxin/alcohol-induced DNA damage.

Methods: (1) *SPTBN1* mutant mice were treated with alcohol to determine their susceptibility to aldehyde-induced developmental abnormalities. (2) Genomic instability and sensitivity to DNA damaging agents in primary *Sptbn1*+/+, *Sptbn1*-/- MEFs were determined by clonogenic survival and metaphase chromosome aberrations analysis. (3) ChIP assays were performed to determine the recruitment of *Sptbn1*/Smad3 at FancD2 promoter. (4) We investigated the clinical relevance of altered *SPTBN1* and FancD2 function using immunohistochemical analyses of 20 human liver specimens from alcoholic hepatitis (n=5), alcoholic cirrhosis (n=5), and alcohol-associated liver cancer (n=5), as well as normal controls (n=5).

Results: (1) *Sptbn1*-deficient mice exhibit a phenotype similar to human fetal alcohol syndrome and are sensitive to ethanol exposure. (2) *Sptbn1*-deficient cells exhibit genomic instability and hypersensitivity to DNA damage (3) *Sptbn1*-deficiency delays DNA damage repair. (4) FancD2 ectopic expression rescues the DNA repair defect in *Sptbn1* null cells. (5) SPTBN1 and FancD2 are clinically correlated in alcoholic hepatitis and alcohol-associated HCCs.

Conclusions: Our model proposes that in response to liver toxins such as alcohol, the TGF-β/ *SPTBN1*/Smad3 pathway prevents liver injury and cancer through its direct effects on DNA repair and genomic stability. Thus, characterizing the role of TGF-β in alcohol-induced injury could potentially enhance our mechanistic insight into the basis for therapeutics targeting toxin- induced DNA damage and tumorigenesis.

Human intestinal enteroids with inducible neurogenin-3 expression as a novel model of gut hormone secretion

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Background: Enteroendocrine cells (EECs) are specialized epithelial cells that produce molecules vital for intestinal function and homeostasis. Due to their limited numbers, in-depth analysis and functional studies of EECs remains challenging. Human intestinal enteroids (HIEs) derived from intestinal epithelial stem cells are currently the most biologically relevant *in vitro* model of the intestinal epithelium. Differentiated HIEs contain all major intestinal epithelial cell types; however, like the native intestine, HIEs spontaneously produce few EECs, which limits molecular investigation.

Methods: To increase EEC abundance, we used lentivirus transduction to stably engineer jejunum HIEs with doxycycline-inducible expression of neurogenin-3 (*NGN3*), a transcription factor that drives EEC differentiation. We validated this model with doxycycline induction in 3D HIEs, 2D monolayers, and transwell monolayers to measure increases in the enterochromaffin cell subtype and secretion of serotonin and additional EEC hormones.

Results: Treating tet*NGN3*-HIEs with increasing concentrations of doxycycline induced a dose-dependent increase in *NGN3* and chromogranin A (*CHGA)* expression and the number of ChgA-positive and serotonin-positive cells, making this a system with increased enterochromaffin cell differentiation. Despite increased ChgA-positive cells, other differentiated cell types remained largely unchanged by gene expression and immunostaining. Doxycycline-induced tet*NGN3*-HIEs secreted serotonin, monocyte chemoattractant protein-1, glucose-dependent insulinotropic peptide, peptide YY, and ghrelin in response to norepinephrine and rotavirus infection, supporting the presence of multiple EEC types.

Conclusions: We have combined HIEs and inducible-*NGN3* expression to establish a flexible, stable *in vitro* model system that can increase human EECs in enteroid systems and advance the molecular and physiological investigation of EECs.

Diet-induced evolution of the human microbiome: the impact of dietary sugars on gut health

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Host diet acts as a strong selective pressure on the gut microbiome. Adaptation can occur through taxonomic shifts in the composition of the microbiome and through adaptation of individual microbes producing strain variation, both of which can have profound effects on host-microbe interactions. During the 20th century, Americans increased their consumption of simple sugars, including trehalose and high fructose corn syrup. How gut microbes evolve in the presence of these carbohydrates and what affect adapted strains have on host physiology and metabolism is unknown.

Here I propose to identify the alleles selected for in a microbial population exposed to trehalose or high fructose corn syrup and the phenotypic effect these alleles have on interactions with the host gut. To this end, I will experimentally evolve a defined community of gut microbes in bioreactors in the presence of trehalose or high fructose corn syrup for 100 generations. Sampling will be taken over the course of the experiment and mutations arising in the population will be determined by metagenomics and population level allele determination. The populations will be assessed for their effect on host gut phenotypes using human intestinal enteroids.

Currently, I am establishing the microbial community. The selected microbes are those abundant and prevalent in the human small intestine, co-exist in culture, and can be maintained at a flow rate and population size representative of that in the small intestine. Moreover, this community was able to persist for 150 generations in sucrose media. Ultimately, this research will directly address how changes in dietary sugar affect human health via evolution of the gut microbiome.

Functional dyspepsia and sucrase-isomaltase expression

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Background: Functional dyspepsia (FD) is a common functional gastrointestinal (GI) disorder affecting one of every six Americans. FD is diagnosed by symptom-based the Rome criteria which include epigastric pain or burning, early satiety, and bothersome fullness after eating in absence of structural disease explaining the symptoms. The pathogenesis of FD is multifactorial but the following physiologic disturbances have been implicated: decreased gastric motility, excess acid production, impaired gastric accommodation, and hypersensitivity to gastric distension. However, recent studies suggest duodenal inflammation, namely duodenal eosinophilia, microbiome disruption, and neuroendocrine feedback mechanism dysregulation play a role in FD pathogenesis. Given the known disruptions of duodenal mucosal integrity in patients with FD, the possibility of decreased expression of surface proteins native to duodenal enterocytes should be considered. Among the endogenous intestinal proteins are the disaccharidases, also called brush border enzymes, which digest carbohydrate polymers. Deficiency of one disaccharidase, sucrase-isomaltase (SI), has been implicated in the pathogenesis of irritable bowel syndrome, another functional GI disorder. It is unknown if intestinal SI insufficiency plays a role in the pathogenesis of FD and FD symptoms.

Objective: The purpose of this study was to identify if sucrase-isomaltase expression in duodenal mucosa, as measured by immunohistochemistry assay of endoscopic mucosal biopsies, differs in FD-affected patients and healthy controls. Decreased SI expression in FD-affected patients should further support the need to investigate the relationship between disaccharidase insufficiency and the FD condition.

Materials and Methods: This study was a cross-sectional case control study with prospectively collected symptom questionnaires. Duodenal biopsy samples from twenty patients with FD (by Rome II criteria) and twenty age (±2 years) and gender matched controls were compared. Two negative control samples were provided by patients with complete SI deficiency and two positive control samples were provided by patients with diminished SI activity. SI expression was measured by immunohistochemistry assay targeting a conserved region near the SI amino-terminus. Slide images were captured at 400X magnification using an AmScope digital microscope camera. Three individual villi were selected to represent each patient's duodenal mucosal sample (132 villi total). Quantitative representation of DAB and hematoxylin tissue stains was accomplished via a logarithmic red, green, blue to grayscale conversion program that counts grayscale pixels between two intensity values. Additional slides were stained with hematoxylin and eosin for counting of eosinophils. The eosinophil count was obtained by observing five non-overlapping high power fields at 40X magnification. Statistical analysis using McNemar's test for paired nominal data was performed.

Results: Forty male patients were included with a mean age of 61.1 ± 10.3 years. SI expression scores were similar in FD-affected patients and patients without FD (p=0.14) and regardless of the presence or absence of the FD symptom early satiety (p=0.32), proton pump inhibitor use (p=0.40), or tobacco smoking (p=0.17). SI expression score was associated with pathologist estimated eosinophil count (p=0.03).

Conclusions: SI expression does not differ in FD-affected patients and unaffected controls according to quantitative SI intensity scoring method. SI expression score does not differ based on presence of early satiety, tobacco smoking, or proton pump inhibitor use. The statistically significant relationship found between SI expression and duodenal eosinophilic infiltrate is congruent with recent studies, however critical examination and re-counting of eosinophils within individual villi did not confirm the estimated eosinophil counts made by the reviewing pathologist.

Early postnatal malnutrition induces functional and histologic changes in the colon

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Background: Gastrointestinal (GI) dysmotility is observed in states of protein-calorie malnutrition. Kowalski *et al.* (1967) studied patients with kwashiorkor, marasmus, and mild malnutrition using barium swallows and/or enemas and noted significant damage to the motor function of the GI tract that was dependent on the severity and duration of malnutrition. Subsequently, Redmond *et al.* (1971) performed sigmoidoscopy on 20 children with severe kwashiorkor; gross observation of the bowel wall revealed "hypotonicity and laxity." In other states of malnutrition, such as anorexia nervosa and small-for-gestational age neonates, GI dysmotility is often present in the form of constipation and feeding intolerance. Mechanisms by which malnutrition causes dysmotility remains unclear. **Objective**: To model malnutrition-associated GI dysmotility in mice and to determine whether functional or histologic changes in the colon might provide insight into underlying mechanisms. **Methods**: Early postnatal malnutrition was induced by giving low-protein, low-fat "regional basic diet" (RBD) chow to lactating dams starting on day of life 8. Control litters were given isocaloric chow of normal macronutrient composition. Pups were tested at 2 weeks of age or were weaned to the same RBD or control chow and tested at 2 months of age (as young adults). Upper GI tract motility was assessed by fluorescein isothiocyanate (FITC)-dextran gavage and colonic motility was assessed by rectal bead latency. Proximal and distal segments of colon were evaluated by light microscopy, and mucosa thickness, crypt depth, and muscularis interna and externa thickness were determined. Additional segments of colon were analyzed *ex vivo* by force transduction to determine the response to cholinergic stimulation. Finally, colon contents were analyzed by targeted LC/MS-MS to measure concentrations of pro-kinetic metabolites. **Key Results**: RBD pups and RBD young adult males were underweight and stunted, whereas RBD young adult females were less affected by malnutrition. Malnourished mice demonstrated slow GI motility: the mean geometric center of gavaged fluorescence was more proximal in RBD pups versus control pups, gastric emptying was delayed in RBD young adult females, and a trend towards prolonged bead expulsion time was observed in RBD young adult females. Histologic examination of distal colon revealed increased muscularis externa thickness in RBD pups versus control pups (8.6 vs 5.1 μm, $p = 0.039$), and a trend toward decreased crypt depth in RBD females versus control females (109 vs 132 μm, $p = 0.070$). Force transduction demonstrated an exaggerated response to cholinergic stimulation in RBD young adult mice compared to controls. LC/MS-MS revealed significant decreases in 16 of 19 bile acids in the colon of both RBD pups and RBD young adults versus respective controls. **Conclusions**: In our diet-induced model of early postnatal malnutrition, GI transit is delayed in a sex-specific manner. We observed in the distal colon a paradoxical increase in smooth muscle thickness, which might represent a compensatory change manifest as an exaggerated response to cholinergic stimulation. RBD mice also have decreased quantities of bile acids, potent pro-kinetic agents, in the colon. These data lead us to hypothesize that intestinal bile acid signaling abnormalities, which result from impaired hepatic bile acid biosynthesis in malnutrition, contribute to malnutrition-induced GI dysmotility.

Development of ultrasound 2D-shear wave elastography in mice – utility in detecting sinusoidal obstruction syndrome in the FOLFOX mouse model

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Background: Ultrasound two-dimensional shear wave elastography (US 2D-SWE) has tremendous potential as a noninvasive method of assessing liver disease. Clinical US 2D-SWE technology has yet to be used for studying mouse models of liver disease. We developed a technique for performing US 2D-SWE in mice and used it to assess SOS in a FOLFOX mouse model. Primary aims were to: 1) design and optimize a method for utilizing a clinically available 2D-SWE unit in mice and 2) assess US 2D-SWE's ability to detect early SOS.

Methods: 11-week-old male C57Bl/6J mice received 1x/week intraperitoneal injection of vehicle (n=13) or FOLFOX (5-fluorouracil, folinic acid, & oxaliplatin; n=16). Mice, anesthetized and fasted, were imaged (day 7, 21, or 28)—via previously validated water bath approach—with a GE LOGIQ-E9 ultrasound unit and 9 MHz linear transducer. Images were acquired in cines, with one measurement per image. Liver samples (H&E) were scored semi-quantitatively. All US SWE data collection and H&E scoring was done with investigators masked to treatment groups. As validation, day 21 was repeated. Differences in treatment group median US SWE velocities were analyzed using permutation testing. To analyze semi-quantitative variables, ordinal logistic regression (odds-ratios (OR), 95%CIs) was used. Joint inferences on day 21 experimental replicates were done using Edgington's combining method for p-values.

Results: FOLFOX treatment led to increased sinusoidal dilatation (OR: 3.3 [1.6,7.4], p=0.00091) and hepatocellular necrosis (OR: 3.0 [1.5,6.8], p=0.0022). Hepatic steatosis (OR: 1.0 [0.52,2.0], p=0.97) and central venule (CV) injury, based on the assessment of obstruction (OR: 0.63 [0.38,1.6], p=0.50), endothelial damage (OR: 1.7 [0.84,3.4], p=0.15), and inflammatory infiltrates (OR: 0.75 [0.68,2.7], p=0.40), were comparable between groups. US 2DSWE velocities by timepoint were (FOLFOX vs control): day 7 - 1.44 m/s vs 1.33 m/s (p=0.20, n=6), day 21 - 1.51 m/s vs 1.29 m/s (p=0.0025, n=17), and 28 days - 1.62 m/s vs 1.26 m/s (p=0.10, n=6), respectively.

Conclusions: Our results suggest that sinusoidal dilation and hepatocellular necrosis are early events associated with FOLFOX induced SOS in mice. We have developed a methodology for the use of 2D-SWE to assess liver pathology in mice—which may aid future study of mouse models of human disease. Furthermore, these results suggest that US 2D-SWE may be an important clinical option in the diagnosis and management of SOS in humans.

Sex Differences in Genetic Associations with Barrett's Esophagus and Esophageal Adenocarcinoma

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Background: The incidence of esophageal adenocarcinoma (EA) has increased rapidly in the United States over the past 40 years. EA is characterized by a strong and yet unexplained male predominance (with a male-to-female ratio in incidence of up to 9:1). Genome-wide association studies (GWAS) have identified more than 20 susceptibility loci for EA and its premalignant lesion, Barrett's esophagus (BE). However, the sex differences in genetic associations with these two diseases remain largely unknown.

Material and Methods: We performed the first sex-specific GWAS analysis of EA and BE using pooled data from the International Barrett's and Esophageal Adenocarcinoma Consortium (BEACON), the United Kingdom Barrett's Esophagus Gene Study, and United Kingdom Stomach and Oesophageal Cancer Study. We compared cases (patients from these studies with EA or BE) to population-based controls, and used logistic regression models to estimate odds ratios (OR) assuming an additive genetic model and adjusting for age and top principal components. We further tested joint effects of genetic and gene by sex interactions. To correct for multiple testing, we used $P < 5 \times 10^{-8}$ for statistical significance.

Results: There were 4,660 male cases, 1,710 male controls, 1,122 female cases and 467 female controls. Previously reported associations at chromosome 19p13.11 (rs199620551 and rs10419226) in sex-combined analysis were only significant in females (OR=0.73 and 0.74, $P = 2.04 \times 10^{-8}$ and 3.91×10^{-8}, respectively). Further, we identified a novel association at rs10898938 (11q13.4, OR=1.63, $P = 9.17 \times 10^{-9}$) in males only, and this variant has the strongest interaction with sex (P for interaction= 1.98×10^{-8}).

Conclusions: We observed that the impact of genetic variants on BE and EA risk may differ for males and females. These identified differences could improve our understanding of the genetic architecture of the disease. Further studies are needed to elucidate the biological processes through which this risk is conferred.

The blood pressure lowering effect of every other day fasting involves alteration of the gut microbiota

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The importance of a healthy gut microbiota, and detriment of a dysbiotic microbiota, on host physiology is becoming increasingly evident. We have previously demonstrated that gut dysbiosis is associated with neuroinflammation and hypertension. Furthermore, pre- and pro-biotic treatments to reduce gut dysbiosis mitigated neuroinflammation and prevented hypertension. However, the mechanisms linking gut dysbiosis to neuroinflammation and hypertension are not fully understood. Recent evidence suggests that circulating bile acids have neuroprotective effects. We tested the hypothesis that bile acids represent a novel microbiota-gut-brain axis mediator that when disrupted contribute to hypertension. Bile acids were measured by HPLC-MS/MS in systemic plasma of normotensive WKY and hypertensive SHRSPs. A number of bile acid species were significantly reduced in SHRSPs, including a 59% reduction in ursodeoxycholic acid and a 72% reduction in cholic acid ($p<0.05$, n=4-5), both known to have neuroprotective properties. In addition, the mRNA level of TGR5, one of the major bile acid receptors, was also significantly decreased in the brain of SHRSPs when compared to WKYs ($p<0.005$, n=5). Intermittent fasting has previously been shown to alter the composition of the gut microbiota and increase circulating bile acids. We next investigated the effect of every other day fasting (EODF) on the development of systemic hypertension. Beginning at 5 weeks of age WKY and SHRSP rats were randomized to an ad libitum (control) or EODF feeding regimen for 10 weeks. From 10-15 weeks of age EODF in SHRSP significantly reduced systolic blood pressure (SBP) relative to SHRSP control rats ($p<0.001$, n=7). Two-way repeated measures ANOVA showed no significant difference in SBP of WKY control, WKY EODF, and SHRSP EODF from 10-15 weeks of age. To examine the role of the gut microbiota in the blood pressure lowering effects of EODF, cecal content was isolated from SHRSP control and EODF rats at 15 weeks and transplanted into germ free rats. Germ free rats colonized with SHRSP EODF microbiota exhibited significantly lower SBP compared to those colonized with SHRSP control microbiota ($p<0.01$, n=7-8), similar to the donors. In summary, we have identified significant alterations in multiple components of bile acid signaling that may represent a mechanism linking gut dysbiosis to hypertension. Additionally, we demonstrate that EODF-induced alterations to the microbiota play a beneficial role in lowering SBP.

Fusobacterium nucleatum Bolsters *Clostridium difficile* Biofilms in Intestinal Mucus

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Background: The pathogenesis of *C. difficile* infection (CDI) involves disruption of the host microbiota, allowing vegetative *C. difficile* to associate with intestinal mucus and interact with other mucosa-associated bacteria. However, very little is understood of the pathogenesis of CDI at the mucus layer of the colonic epithelium. We were particularly interested in *Fusobacterium*, a genera known to form multi-species biofilms in other locations of the body and promote the growth of other pathogens. We hypothesized that select mucosa-associated microbes such as *Fusobacterium* would promote *C. difficile* colonization and biofilm formation. **Methods & Results**: To create a robust model of the microbiota and intestinal mucus layer, we developed a novel bioreactor system with mucus coated inserts. Bioreactors were inoculated with healthy human feces, treated with clindamycin and infected with *C. difficile* R20291 to mimic CDI, with the addition of human colonic mucus coated coverslips. *C. difficile* was found to colonize and form biofilms within the provided mucus as demonstrated by fluorescent in situ hybridization (FISH) and staining by Ruby Red Biofilm Tracer and crystal violet. 16S rRNA sequencing revealed a unique biofilm profile, with substantial colonization by *Fusobacterium* (~20%). To define the role of *Fusobacterium* in *C. difficile* mucus colonization, gene expression and aspects of biofilms were examined. RNAseq data demonstrated a significant shift in *C. difficile* gene expression towards colonization-related genes when *F. nucleatum* was present. In terms of adhesion, *C. difficile* alone adhered to human-derived goblet cell mucus coated coverslips, goblet cells lines HT29-MTX and LS174T and human colon enteroid monolayers as determined by CFDA-SE/CMRA fluorescently tagged bacteria, immunostaining, and scanning electron microscopy (SEM). Addition of *F. nucleatum* resulted in pronounced *C. difficile-F. nucleatum* aggregates by 1 hour. In aggregation assays, multiple *C. difficile* strains aggregated with *F. nucleatum*. This interaction was lost in *F. nucleatum* lacking surface adhesion RadD, indicating the requirement of RadD for *C. difficile* interactions. Moreover, WT *F. nucleatum*, but not *F. nucleatum* ΔRadD increased *C. difficile* biofilm formation by crystal violet, Ruby red biofilm tracer, acridine orange staining and SEM. *F. nucleatum-C. difficile* biofilms also exhibited enhanced resistance to biofilm dispersing agents (EDTA, proteinase K, DNAse) and the antibiotic vancomycin compared to *C. difficile* alone. Importantly, a subset of patients with *C. difficile* infection, both primary and recurrent, were found to harbor high *F. nucleatum* OTUs and aggregates of *C. difficile* positive stool demonstrated a physical interaction between the species. Collectively these data point to *F. nucleutum*-adhesion to *C. difficile* and subsequent biofilm formation. **Conclusions:** These results demonstrate the unique role of mucosa-associated bacteria, including *F. nucleatum*, in the mucus colonization of *C. difficile*. Adherence to mucus and biofilm formation may allow *C. difficile* to efficiently deliver toxins in close proximity to the host, thus providing a potential mechanism for persistent colonization and infection. This work points to the potential of targeting the mucosa-associated bacteria and mucus in an effort to minimize *C. difficile* colonization and disease.

The role of Muc2 sulfation in shaping the adherence and colonization of adherent-invasive *E. coli* in the gut

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The intestinal mucosa forms a protective homeostatic barrier between invading bacteria and the underlying epithelium. Alterations in the mucosal composition have been observed in both animal models and patients suffering from inflammatory bowel disease (IBD). The integrity of the layer is attributed to the sulfated mucin, Muc2. Though the physiological importance of the mucus layer is well established, the role of mucin sulfation in protecting against enteric pathogens is unclear.

We have shown that adherent-invasive *E. coli*, found in diseased mucosa of IBD patients, harbor sulfo-glycan binding adhesins that belong to the family of multivalent adhesion molecules (MAMs). Mucin-epithelial competition binding assays demonstrate the presence or absence of MAMs critically affects bacterial attachment to epithelial cells, and binding to epithelial cells is decreased in the presence of sulfomucin. Co-incubation of mucin with a sulfatase-producing gut commensal, *Bacteroides thetaiotaomicron*, decreases binding of *E. coli* to mucin and increases attachment of bacteria to the underlying epithelial surface.

Our data show that interactions between MAM adhesins and sulfated Muc2 inhibit translocation of bacteria to the epithelium, and sulfatases secreted by mucin-foraging bacteria such as *B. thetaiotaomicron* inhabiting the same niche may affect the capacity of the mucus barrier to retain AIEC. How these results translate to a disease setting is subject to our current studies.

Histamine increase after ischemic stroke leads to gut barrier breakdown related post-stroke inflammation in aging

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Abstract: Human aging is characterized by chronic, low-grade inflammation, a phenomenon termed "inflammaging". Aging is a non-modifiable risk factor for stroke and inflammatory processes are a major contributor to ischemic brain damage. Mast cell (MC) degranulation and the subsequent release of histamine (HA) may contribute to chronic inflammation, BBB leakage and neutrophil accumulation. Over the past decade, the histaminergic system has been implicated in the modulation of cell death in brain disorders including stroke. However, the exact mechanisms underlying HA actions on ischemia-induced damage have not yet been defined. Stroke leads to gut dysfunction and dysbiosis, which might trigger MC infiltration. Since previous studies have shown that the gut/microbiome has a significant impact on stroke outcomes, we hypothesized that stroke increases HA levels, histamine receptors (HR) and MC infiltration in the gut leading to gut barrier breakdown further contributing to post-stroke inflammation (PSI) and damage.

Methods: Aged (Ag) C57BL/6 (20-22 months old) and young (Yg) (3 months old) male mice were subjected to 60 min transient middle cerebral artery occlusion (MCAO) or sham surgery. Mice were sacrificed after 6-hours (h), 12-h, 24-h, 3-days (d) and 7-d post-MCAO. Brain, blood, gut and spleen were harvested and used for flow cytometry, mRNA and immunohistochemistry (IHC) before behavior analysis. Luminal contents were collected and subjected to 16s sequencing and metabolomics analysis.

Results: Stroke increased HR expression in the intestine of 7-d PS Ag mice compared to the Ag controls. HA levels in Ag brain and blood was 2-fold higher than in Yg mouse at 6-h after stroke and remained high at 24-h. In addition, locomotor activity was significantly reduced in post stroke mice. Increased MCs were seen in the gut of PS compared to control mice. The protective gut mucus layer was depleted with increased HR activation and with a pro-inflammatory biomarker elevation. This was correlated with imbalanced gut microbiota. This work suggests that there is a role of gut HR and systemic HA in modulating brain health.

Conclusion: Controlling HA release and suppressing HR activation in the periphery especially in the gut PS might improve recovery after stroke.

HIF2-induced Wnt5a in small intestine regeneration

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Radiation-induced gastrointestinal syndrome (RIGS) occurs when the small intestines are exposed to high doses of radiation. Radiation injury to the intestinal stem cell (ISC) and endothelial compartments impair intestinal regeneration, cause loss of epithelial integrity and mucosal barrier dysfunction. This in turn leads to malabsorption, dehydration, electrolyte imbalances, bacterial translocation, sepsis, and often death. Furthermore, radiation therapy for abdominal tumors is challenging because the small intestine is exquisitely radiosensitive. The intestine's self-renewal ability and susceptibility to radiation derive from the rapid-cycling ISCs in the crypts. Unfortunately, there are no therapies to prevent, mitigate, or treat RIGS or even modest intestinal radiation injury. The EGLN family of prolyl hydroxylases are cellular oxygen sensors that regulate cell survival and metabolism through the degradation hypoxia-inducible factors (HIFs). HIFs are known to induce tissue remodeling, increase epithelial integrity, stimulate intestinal angiogenesis, and promote stem cell survival, all of which are essential for response to radiation injury. Our group has shown that stabilization of HIF2, but not HIF1, through genetic or pharmacologic inhibition of the EGLN proteins mitigates and protects against RIGS in mice. To understand the mechanism by which HIF2 mediates this protection, we generated intestinal organoids from mice with a Cre-inducible allele that expresses a non-degradable form of human HIF1 or HIF2 and performed RNA-seq. Whole transcriptomic analysis revealed HIF1 and HIF2 have distinct gene responses in the small intestinal crypt. HIF2 induced the expression of known radiation modulators and genes involved in oxidative damage response, tissue healing, and intestinal homeostasis such as the non-canonical *Wnt5a*. **Thus, we hypothesize that HIF2 induces intestinal regeneration after radiation injury by inducing *Wnt5a* expression to promote ISC survival.** We validated HIF2 drives *Wnt5a* expression in multiple organoid model systems. Our group co-developed the spheroid formation assay (SFA) to test potential radiation modulators, such as Wnt5a, in gastrointestinal organoid cultures. The SFA is a modified *ex vivo* clonogenic assay that allows us to evaluate crypt regeneration following irradiation. Interestingly, pre-treatment with recombinant Wnt5a resulted in higher crypt regeneration after irradiation, as compared to untreated intestinal organoids. We then generated knockouts (KO) of *Wnt5a* or *Ror2*, its cognate receptor, using the Cre/lox system to test if Wnt5a is necessary and sufficient for radioprotection. We found that *Wnt5a* KO organoids had decreased crypt regeneration following radiation. Our preliminary data suggest Wnt5a protects the intestine from radiation by increasing ISC survival. Our future directions are to test if this radioprotective phenotype extends to human intestinal organoids and *in vivo* models.

Comparison of the fecal microbiome among inbred mouse strains with high or low lactation capacity reveals early differences that are lost with maturation and progression through pregnancy and lactation

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Introduction: Prior studies in human populations and in the C57BL/6J strain of inbred mouse suggest that the fecal microbiome changes dramatically with progression through pregnancy. In addition, studies in both mice and human populations suggest that genetic background also influence the composition of the gut microbiota. Prior work in our laboratory has identified inbred mouse strains that display extreme difference in both their ability to rear cross-foster litters and in a number of other lactation-related traits. The QSi5 mouse is a high lactation capacity inbred strain that was developed by positive selection for fecundity for 10 generations. At the other end of the distribution, the PL/J mouse has both poor fecundity and low lactation capacity.

Hypothesis: The fecal microbiota of low or high lactation capacity inbred mice exhibit differential changes during the progression from a non-parous state through pregnancy and lactation.

Experimental Design and Methods: Fecal samples were collected from QSi5 and PL/J females at 4 and 11 weeks of age (non-parous), on the 16th day of pregnancy, and on day 1 and 10 of lactation. A minimum of five samples were collected from each strain and all animals were fed the same standard diet and were maintained in the same environment. Samples were sequenced for hypervariable region IV of the 16S ribosomal RNA gene and data analyzed using QIIME. Taxonomies were assigned using an RDP classifier and aligned with the Greengenes (v. 13.5) core reference database. Alpha diversity and beta diversity indices were calculated from this observed taxonomic unit (OTU) table. The resulting OTU table was further used to predict the functional capacity and pathway enrichment using the PICRUSt and LEfSe.

Results: Comparison of alpha diversity revealed that at four weeks of age, QSi5 was more diverse ($p<0.05$) than PL/J. The beta diversity analysis shows some overlap, but the two strains cluster in different regions of the PCoA plot. At four weeks of age, there were 29 taxa elevated (FDR adjusted $p<0.05$) in QSi5 and 14 in PL/J. Class *Bacilli*, order *Lactobacillales*, family *Lactobacillaceae* and *Rikenellaceae*, and genus *Lactobacillus* were elevated among others in QSi5, while PL/J had elevated levels of the following taxa: class *Deltaproteobacteria*, order *Desulfovibrionales*, family *Streptococcaceae*, *Ruminococcaceae* and genera *Bilophila*, *Oscillospira*, among others. Metabolic pathway analysis showed that ribosome biogenesis, riboflavin metabolism and restriction enzyme pathways were elevated in QSi5, while methane metabolism, electron transfer carriers and pentose glucose interconversions were elevated in PL/J. Comparison of observed OTU among the strains at the later time points revealed that as the animals matured and progressed through pregnancy and lactation, strain-dependent differences were lost despite an overall increase in diversity.

Conclusion: Progression through pregnancy and lactation causes a progressive loss in the strain-dependent difference that occur in these same animals.

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Resolution of intestinal damage by locally delivered therapeutics

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Background: The epithelial layer of the human GI tract serves numerous important functions including the absorption of vital nutrients as well as serving as an important line of defense against potential enteric pathogens. This barrier can be disrupted during inflammatory processes such as intestinal graft versus host disease (**GVHD**) or inflammatory bowel disease (**IBD**). With the breakdown of this vital barrier, the individual becomes susceptible to a variety of infectious etiologies as well as nutrient malabsorption that may severely affect the health and growth of the patient. While most current therapies for treatment of these pathologies focus on systemic immune suppression, few options currently exist for topical therapies targeted towards epithelial wound healing. Our goal is to develop a robust platform for efficient delivery of therapeutic proteins to the GI tract by engineering *Lactobacillus reuteri* (LR) to precisely secrete proteins with therapeutic purposes such as an anti-TNFR1 camelid antibody.

Methods: The protein sequences of four previously characterized anti-TNFR1 camelid antibodies were codon optimized for expression in *L. reuteri*, cloned into an inducible plasmid (pSIP411), and transformed into *L. reuteri*. Protein expression and secretion by engineered *L. reuteri* was confirmed via Western blot. Cell assay using human colonic epithelial cells and human macrophages were used to test the efficacy of the secreted camelid antibodies.

Results: Recombinant strains of *L. reuteri* were successfully generated and shown to be capable of secreting three different anti-TNFR1 camelid antibody constructs. In vitro testing of bacterial supernatants from induced recombinant *L. reuteri* strains on human colonic epithelial cells showed successful inhibition of IL8 gene expression following TNFalpha stimulation suggesting that secreted anti-TNFR1 camelid antibodies are biologically active.

Conclusion: There is increasing interest in the ability to engineer biologically relevant microorganisms to fight diseases of the gastrointestinal tract. Our work shows the successful creation of recombinant *L. reuteri* strains capable of secreting biologically active anti-TNFR1 camelid antibodies.

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Lactobacillus reuteri and a PPI alone provide approximately 12% additive increase in *H. pylori* eradication

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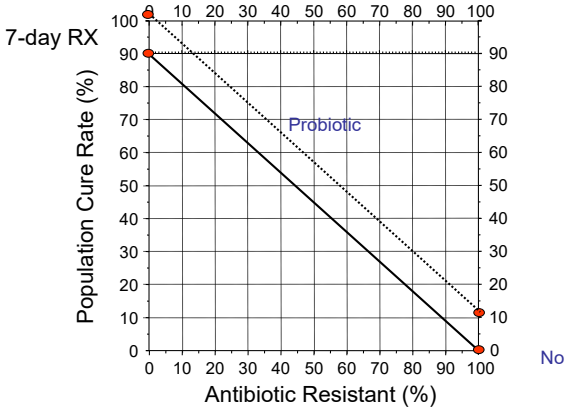
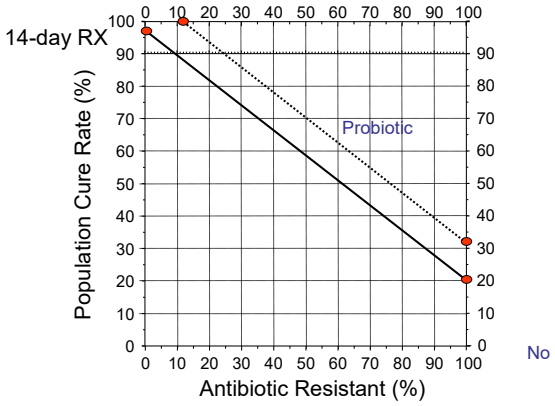
Background: A large number of clinical trials have been performed in adding probiotics to antimicrobial *H. pylori* eradication therapy and meta-analyses involving more than 4,000 subjects have shown a mean increase in intention to treat (ITT) eradication rate of 12.2% (95% CI 9.1-15.3%) among patients in whom antimicrobial resistance had compromised traditional triple therapies. *Lactobacillus reuteri* can inhibit *H. pylori* both in vitro and in vivo. The eradication rate with a *L. reuteri* and a PPI without antibiotics is unknown and that data is needed to predict the expected outcome in any population irrespective of the presence of antibiotic resistance.
Aim: To evaluate whether *L. reuteri* + a PPI will eradicate *H. pylori*.
Methods: A double-blind placebo-controlled randomized 2 site study of *L. reuteri* (Gastrus capsules containing 2 x 10^8 CFU *L. reuteri* DSM 17938 plus 2 x 10^8 CFU *L. reuteri* ATCC PTA 6475) 7 times per day (every 2-3 hours) or matching placebo plus 20 mg pantoprazole b.i.d. for 4 weeks was done. Subjects were *H. pylori* infected (i.e., positive 13C UBT and histology). Those with peptic ulcer, pregnancy or lactation, malignancy, other clinically significant condition, alcohol or drug abuse, history of allergy to pantoprazole or *L. reuteri*, or having taken bismuth compounds, anti-secretory drugs, antibiotics or probiotics during the previous 4 weeks were excluded. Cure was defined as a negative 13C UBT 4 or more weeks after stopping therapy. The short-form Leeds dyspepsia (SF-LDQ) questionnaire was completed at the time of the enrolment and at the final UBT. Sample size was based on need to obtain success with ≥50% cures to be clinically useful as *L. reuteri* monotherapy.
Results: 48 subjects completed therapy (14 men, 34 women, avg. age 49 years). Recruitment was halted after 56 subjects because of low cure rate; there were 8 drop-outs. The cure rates were 3/24 (12.5%; 95% CI 2.6-32%) with *L. reuteri* vs. 4.1% (1/24) with placebo. Side effects (diarrhea was most frequent) occurred in 5/28 in the active group vs. 3/28 with placebo (P = 0.53). There was no difference in SF-LDQ between groups when assessed 4 weeks post therapy.
Conclusion: The cure rate with probiotic therapy was almost identical to that seen with antibiotic (i.e., the effect is additive). In western populations, the addition of *L. reuteri* would increase the cure rate to 90% or greater when clarithromycin resistance was less than 14% with 7 day triple therapy less than 26% with 14 day triple therapy (see figures).





A potential link between *Drosophila* Pngl and AMPK signaling

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Mutations in human *N*-glycanase 1 (*NGLY1*) cause a rare developmental disorder with global developmental delay and a host of other phenotypes including neuropathy, movement disorder, and chronic constipation. NGLY1 is a cytoplasmic deglycosylation enzyme capable of removing *N*-linked glycans from *N*-glycoproteins. To understand the pathophysiology of *NGLY1* deficiency, we have characterized mutations in the *Drosophila NGLY1* homolog, *PNGase-like* (*Pngl*). We previously reported that loss of *Pngl* impairs *Drosophila* BMP signaling during intestinal development (Galeone, Han, et al., *eLife*, 2017). However, loss of BMP signaling can only explain some of the intestinal phenotypes in *Pngl* mutants and is not the primary cause of lethality in these animals. One of the *Pngl* mutant phenotypes that cannot be fully explained by the loss of BMP signaling is that they fail to empty their guts at the end of the larval stage. The resulting food accumulation phenotype is likely to contribute to the developmental delay and lethality of *Pngl* mutant larvae. The activity of the visceral muscle (VM) surrounding the midgut is critical for gut clearance. We did not observe gross morphological defects in the *Pngl* mutant midgut VM. However, *Pngl* mutants showed a significant decrease in intestinal peristalsis. It has previously been reported that mutations in *Drosophila AMPKα* result in a food accumulation phenotype in the larva stage associated with decreased gut peristalsis. Accordingly, we examined whether decreased AMPK signaling can explain the food accumulation and lethality in *Pngl* mutants. I will present genetic and biochemical evidence suggesting a potential link between Pngl and the energy sensor AMPK.

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An all-in-one CRISPR plasmid generates an endogenous *FGFR2-BICC1* fusion model of cholangiocarcinoma

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Background: Cholangiocarcinoma (CCA) is a highly deadly cancer type whose incidence is increasing. CCAs represent 3% of all gastrointestinal cancers, yet, 20% of hepatobiliary cancer related mortalities are caused by CCA, reflected in the dismal 2% 5-year survival rate for late- stage cases. In intrahepatic cholangiocarcinoma (iCCA), *FGFR2* gene fusions are primary contributors that help drive tumor growth and disease outcome. Despite clinical benefit of several FGFR inhibitors such as BGJ398, TAS-120, INCB054828, and ponatinib, only a percentage of patients are responsive and they rapidly acquire drug resistance. The current availability of *FGFR2* altered iCCA preclinical models to address these issues are minimal, with no cell lines and only one reported non-publically available patient-derived tumor xenograft (PDTX) model. Therefore, a primary goal within this study is to develop *FGFR2*-fusion expressing preclinical models to investigate novel therapeutic strategies.

Methods: *FGFR2- BICC1* models will be created in two ways. First, intrahepatic cholangiocytes or cholangiocarcinoma cell lines lacking driver oncogenes will be genetically altered with a CRISPR plasmid to generate an endogenous *FGFR2-BICC1* fusion. Second, we will establish *FGFR2-BICC1* PDTX mouse models. Here, we have taken advantage of the ability of CRISPR to generate large intrachromosomal structural changes to create an endogenous *FGFR2-BICC1* fusion, since both genes reside on chromosome 10 in opposite directions. We created an all-in- one multiplex CRISPR plasmid containing two sgRNAs that target introns in *FGFR2* and *BICC1* to create a 58Mbp inversion on human chromosome 10 leading to the formation of the *FGFR2- BICC1* fusion. This allows an endogenous fusion transcript to express off the endogenous FGFR2 promoter, thus creating more human-relevant models for translational work.

Results: The all-in-one multiplex, mCherry-tagged CRISPR *FGFR2-BICC1* plasmid was transfected into the non-tumorigenic CCA cell lines HUH-28 and CC-SW-1, and the immortalized cholangiocyte cell line MMNK1. Successfully transfected cells were then enriched by fluorescence-activated cell sorting and single sorted into 96-well plates to generate monoclonal cell lines. DNA was isolated from clones and PCR was performed to identify which clones were fusion positive.
Fusion expression was validated at both the RNA and protein levels. These clones were then expanded for functional studies. Preliminary data show an effect on cell proliferation.

Discussion and Conclusion: Since *FGFR2* gene fusions occur in ~20% of iCCA, these models will allow researchers the opportunity to enhance targeted therapy of a substantial portion of CCA patients. In future studies we will compare fusion-carrying and wild-type isogenic clones by performing various functional studies such as growth rates (*in vitro* and *in vivo*), response to FGFR2 therapy, and reverse phase protein arrays. We also plan to assess the role of frequent *FGFR2* fusion co-mutations in our new cell line models via genetic knockdown assays. Results from these studies, along with bioinformatics and genome sequencing will help determine the best therapeutic combinations to test *in vitro* and *in vivo* using PDTX mouse models.

Effects of gut microbiome rejuvenation on Cerebral Amyloid Angiopathy

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Cerebral Amyloid Angiopathy (CAA) is an emerging cause of vascular cognitive impairment in the elderly. CAA is characterized by amyloid-β (Aβ) deposition in the cerebral vasculature and is associated with disruption of blood-brain barrier (BBB) and increased neuroinflammation. To date, there is no definitive answer for whether the observed neuroinflammation is the *result* or the *cause* of CAA pathogenesis. It is known that aging is associated with a state of low-grade, chronic inflammation ("*inflammaging*") in the periphery and in the CNS. However, the source of inflammaging remains unknown. Gut microbiome is a potent modulator of the immune response and age-related changes in gut microbiome may have a differential modulatory effect on the overall inflammatory state. In general, changes in gut microbiome or gut metabolome compared to a healthy, youthful control that may either induce or exacerbate pathology are referred to as "*dysbiosis*." **We hypothesized that age-related gut dysbiosis precedes CAA pathogenesis**, which may contribute to increased neuroinflammation and CAA-related vascular injuries. We used the Tg-SwDI mouse (also referred to as "CAA mouse", harboring Swedish, Dutch, and Iowa mutations of human amyloid precursor protein (APP)) model to test our hypothesis. Tg-SwDI mice develop cerebrovascular Aβ deposits and cognitive deficits beginning at 3-4 months. Our preliminary 16S rRNA sequencing of fecal samples of CAA mice (n=127) compared to wildtype (WT) controls (n=80) showed higher gut microbiome alpha- (or "within-sample") diversity in the fecal samples of CAA mice (Inverse-Simpson diversity score by Mann-Whitney U rank sum test, p=0.036). Upon visualization of beta- (or "between samples") diversity of CAA and WT controls, with weighted-UniFrac-distances by principal coordinate analysis (PCoA), we found a notable clustering effect (n=207, p=0.001, PCoA axes PC1 and PC2 with 34.6% and 26.4% variations explained). To assess the integrity of gut epithelial barrier, we performed bacteria-specific fluorescent in situ hybridization and immunohistochemical staining, which showed significant close proximity of bacterial colonies with gut epithelium, loss of the number of MUC_2^+-mucin producing goblet cells per crypt ($p \leq 0.007$), and reduced E-cadherin expression in the gut epithelial junction in CAA mice. These changes in microbiotal composition accompanied by signs of gut epithelial injury are consistent with gut dysbiosis. Importantly, we detected these gut-related events around the anticipated onset of CAA in Tg-SwDI mice. We then assessed the relative abundance of short chain fatty acids (SCFAs) in fecal samples of symptomatic (~10 months) CAA mice and WT controls. We found that acetate and butyrate levels were significantly higher in CAA (n=30, differential false discovery rate (FDR) <0.25). Both of these SCFAs have been implicated in modulation of multiple neurodegenerative diseases (NDDs). Curiously, isocaproate levels were significantly higher in symptomatic (~10 months) when compared to pre-symptomatic (3 months) CAA mice (differential FDR < 0.25). In conclusion, our findings suggest that gut dysbiosis and shifts in the gut metabolomic profile occur early in CAA pathogenesis and may be responsible for *ongoing* increased neuroinflammation and the immune response to CAA-specific vascular injuries. This work is significant if follow-up studies confirm that changes in gut microbiota can be detected before clinical manifestations of CAA and/or other NDDs. Therapeutic strategies to reverse pathology in CAA and other NDDs may involve manipulation of the microbiome.

In situ molecular architecture of the *Helicobacter pylori* cag type IV secretion system

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Helicobacter pylori is a Gram-negative bacterium that persistently colonizes the gastric mucosa in about 50 percent of humans. Most persons colonized with *H. pylori* remain asymptomatic, but the presence of *H. pylori* is associated with an increased risk of gastric adenoncarcinoma, gastric lymphoma, and peptic ulcer disease. *H. pylori* strains carrying the Cag pathogenicity island (CagPAI) are associated with increased risk of gastric cancer or ulcer disease. The CagPAI codes for the Cag Type IV Secretion System (T4SS) and the CagA 'oncoprotein', whose translocation to human gastric epithelial cells results in a myriad of phenotypic changes associated with *H. pylori* pathogenesis. We seek to fully define the Cag T4SS structure at unprecedented resolution using in situ cryoelectron tomography. To determine 3D structures of the intact Cag T4SS at high resolution, subtomogram averaging was used to analyze 865 T4SS subtomograms visualized on the *H. pylori* cell surface. Individual *H. pylori* cells contain multiple $T4SS_{Cag}$ nanomachines, each composed of an outer membrane complex (OMC) with 14-fold symmetry and inner membrane complex (IMC) with 6-fold symmetry. The wheel-shaped OMC is composed of five subunits, of which CagX, CagY, and CagM form a central hub or channel, and Cag3 and CagT contribute to formation of the peripheral wheel. The IMC, which has never been visualized in detail, is configured as six tiers in cross-section view and three concentric rings surrounding a central channel in end-on view. The IMC contains three T4SS ATPases: i) VirB4-like CagE arranged as a hexamer of dimers at the channel entrance, ii) a hexamer of VirB11-like Cagα docked at the base of the CagE hexamer, and iii) VirD4-like (Cagβ) and other unspecified Cag subunits, associated with the stacked CagE/Cagα hexamer and forming the outermost ring. The Cag_{T4SS} represents a new structural paradigm for the T4SS superfamily.

Awareness of chronic viral hepatitis in the United States: A US population-based study

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Background:
The World Health Organization has set the goal of reducing the hepatitis-related mortality rate by 65% between 2015 and 2030. Diagnosis and awareness of infection is the first essential step towards achieving this goal. Our study examined the current awareness rate of chronic viral hepatitis in the U.S and the potentially associated factors.

Methods:
We obtained data from the National Health Nutrition and Examination Survey 2013-2016. There were 11,488 persons aged ≥20 who participated in the serology testing for HBV and HCV. We defined chronic HBV infection by the presence of hepatitis B surface antigen (HBsAg) and HCV infection by + HCV RNA test. We defined significant fibrosis based on AST to Platelet Ratio Index (APRI) score >0.7. Awareness of viral hepatitis was determined by a question: “Have you ever been told you had hepatitis B or C?” Awareness rate of HBV and HCV infection was estimated, using published weights to account for oversampling and nonparticipation. Survey-weighted generalized logistic regression analyses were used to examine the associations of the awareness with the socio-demographic factors, including age, gender, race/ethnicity, education, birthplace, income, and insurance.

Results:
Awareness of chronic HBV infection, past HBV exposure, and HCV infection were present in 33.9%, 11.7%, and 55.6% of participants, respectively. Among HCV infected baby boomers, the awareness was in 61.5%. The awareness of HBV infection was significantly higher in individuals with high education level. Age group (40-60 years), women, non-Black race/ethnicity, those with high household income who were born in the U.S with insurance plans tend to be aware of their infection. For HCV, awareness was the lowest in Hispanics and Asians, foreign-born who lived below the federal poverty level and low education level. Among HCV infected baby boomers (born between 1945 and 1965), the awareness rate was 61.5%. Awareness among chronic viral hepatitis patients at risk for significant fibrosis was 62.0% in HBV and 38.2% in HCV infection.

Conclusion:
In conclusion, current awareness of chronic viral hepatitis in the U.S remains suboptimal. Active public health policy to identify persons at risk and provide appropriate management is warranted.

Longer-term risk of hepatocellular cancer in HCV patients treated with direct acting antiviral agents

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Background: Sustained virologic response (SVR) after direct acting antiviral agents (DAA) holds promise for reducing subsequent hepatocellular cancer (HCC). Only recently have the treatments been available long enough to estimate the longer term risk reduction.

Methods: We conducted a retrospective cohort study of HCV patients who achieved SVR with DAA in any of the 129 Veterans Health Administration hospitals between 1/1/2015 and 12/31/2015. We calculated the cumulative as well as quarterly HCC incidence rates for HCC. We used Cox regression models to identify demographic, clinical, and behavioral factors associated with incident HCC among patients with SVR. We also examined the effect of regression or progression of fibrosis (using serial FIB-4) on HCC risk using landmark analysis.

Results: Among 19,518 patients with SVR, 544 incident cases of HCC were diagnosed during mean 2.9 (max=3.6) years of follow-up. The cumulative 1, 2 and 3-year risks of HCC were 1.1, 1.9 and 2.8%, respectively. Overall, the quarterly incidence rate of HCC remained stable between 1.00 and 1.23 per 100 PY during follow up. Cirrhosis was strongly associated with HCC risk (adjusted hazard ratio=4.75, 95% CI=3.89-5.79). The quarterly incidence rate of HCC ranged from 1.5 to 2.3 per 100 PY in patients with cirrhosis (p-value for trend <0.05). HCC risk was also higher in older patients, those with alcohol use, and patients previously infected with HCV genotype 3. The risk of HCC was the higher in patients who had persistently high FIB-4 values in both cirrhosis and non-cirrhosis patients. HCC risk fell substantially in cirrhosis patients who experienced regression of FIB-4, yet remained higher than the accepted threshold for HCC surveillance.

Conclusions: Among patients successfully treated with DAA, the HCC risk does not regress after 3.6 years of follow-up. HCC risk remained above the accepted thresholds for surveillance in all patients with cirrhosis, regardless of other factors and change in non-invasive markers of fibrosis.

Reducing colonoscopy no-show rates at Ben Taub General Hospital through the use of a short message service

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Background

Missed appointments, or "no-shows", are a common occurrence in clinical practice and result in significant loss to the healthcare system. Nationwide, no-show rates in gastrointestinal endoscopy units vary from 4% to 23%. No-shows in endoscopy units result in decreased efficiency, longer wait times for patients, and higher use of hospital resources. Therefore, preventing no-shows is an important target in improving patient care and lowering costs in gastrointestinal endoscopy practices.

Aims

Our aim is to reduce colonoscopy no-show rates through the utilization of an automated short messaging system (SMS) sent one week prior to a colonoscopy appointment as a reminder of their upcoming procedure. As a result, we hope this will ultimately help with reducing colonoscopy wait times.

Methods & Analysis

Preliminary analysis showed that at the Ben Taub General Hospital endoscopy unit in 2018, the no-show rate has varied from 9 to 14%.For twelve months, we will send out reminders to patients at one week prior to their colonoscopy appointment in the form of an automated SMS. The patients will be prompted to respond with a "yes" or "no" text message. If there is no response or if they respond with "no", they will be removed from the schedule on their designated day and another patient will be moved into their spot. The primary outcome will be rate of no-shows to colonoscopy appointments. We will compare this intervention with the previous system in which no reminder was sent to the patient prior to their colonoscopy. The effect of the intervention and impact of other predictors of no-shows will be analyzed in the pre-intervention and post-intervention patient cohorts using paired t-test analysis.

Household income and education correlates with pediatric inflammatory bowel disease incidence in a large metropolis

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Background: The incidence of pediatric inflammatory bowel diseases (PIBDs: Crohn's disease [CD], ulcerative colitis [UC], and IBD-unclassified [IBD-U]) is on the rise worldwide. Yet, the critical risk factors for this rising incidence are not well understood. Demographic characteristics of PIBD may improve our understanding of their developmental origins and aid in prevention.

Methods: We conducted a geographic study on all 488 PIBD patients diagnosed between 2010 and 2015 at Texas Children's Hospital within 13 counties around Houston. An annual incidence map by ZIP code of residence at diagnosis was created using ArcGIS and the American Community Survey (ACS) estimate of population between 2011 and 2015. Univariate and multivariate correlation between demographic variables and PIBD incidence was examined. A model that incorporated health data on the county level from ACS 2011-2015 and US Health Data 2015 was created with Backward Elimination Stepwise Regression in R. Statistical analyses were performed on GraphPad Prism V7 and R. Statistical significance was set at $p<0.05$.

Results: The PIBD population included 272 Non-Hispanic Whites (55.7%), 84 Hispanics (17.2%), 78 African-Americans (16.0%), 28 Asians (5.7%), and 26 Other (5.3%). There were 266 CD cases (54.5%), 147 UC (30.1%), and 75 IBD-U (15.4%). Fischer's Exact test revealed that Hispanic children were more likely to be diagnosed with UC ($p<0.01$) and IBD-U ($p<0.03$) compared to other races. A significant positive correlation ($r=0.35$, $p<0.0001$) between median household income and PIBD incidence by ZIP code was observed. ZIP codes with majority college educated adults had a higher incidence of PIBD than ZIP codes with majority high school educated adults ($p<0.0001$) and this was true for pediatric CD also ($p<0.0001$). Pediatric UC, however, was more common in ZIP codes where the majority of adults were high school educated ($p = 0.0036$). When a multivariate analysis was conducted, none of the demographic factors were significant. A multivariate model with county-associated health factors identified that UC and CD were predictive of each other and limited healthy food was the demographic factor with the strongest positive correlation in both. Median household income was a stronger correlate of CD than UC.

Conclusions: This is the largest regional demographic study of PIBD. Our findings support earlier observations that Hispanic children more commonly present with UC and IBD-U in the USA. A significant positive correlation between PIBD incidence and household income was observed, which seemed to be the strongest demographic variable. Interestingly, adult education differentially correlated with pediatric CD and UC incidence. We speculate that income and education-related environmental and dietary differences may be important in the developmental origins of PIBD in large metro areas.

Stochastic microbiome development associates with acute mammalian colitis severity

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Introduction: Inflammatory Bowel Diseases (IBDs) are characterized by chronic intestinal inflammation in genetically susceptible individuals. The etiology and pathogenesis of IBD are not clearly understood, including the fact that genetically identical twins exposed to similar environments from conception frequently do not share the disorders. Therefore, we underscore that the high monozygotic twin discordance may originate from randomly established IBD susceptibility. The gut microbiome is thought to play an important role in the development of IBD and has been observed to establish in a stochastic fashion in mammals. We hypothesized that stochastic microbiome variation plays a role in IBD development and severity, and examined this hypothesis in mice as a model.

Methods: We studied how "spontaneous" microbiome variation in genetically identical C57BL/6J mice influenced acute colitis severity in the dextran sulfate sodium (DSS) model. In a discovery and validation cohort of animals, we compared fecal microbiomes between DSS sensitive (DSS-S: severe colitis) and DSS resistant (DSS-R: mild colitis) mice prior to DSS.

Results: Richness and alpha-diversity of the microbiomes of DSS-S and DSS-R groups did not differ, but distinct separation between these two groups was found with beta-diversity analyses. The genus *Faecalibaculum* was more abundant in DSS-S mice ($p<0.01$), while *Blautia* and *Lactobacillus* were less common ($p<0.05$) in these animals. In the validation cohort, the separation between DSS-S and DSS-R mice was not as distinct as in the discovery cohort. However, the genus *Lactobacillus* was again found to have a similar trend towards associating with DSS-R groups ($p=0.20$).

Conclusions: Small vivarium-based breeding decreased stochastic microbiome variation and associated colitis susceptibility in mice. Modest bacterial taxonomy variation based DSS susceptibility was detected whereby *Lactobacillus* was consistently increased in the DSS-R mice. Our findings suggest *Lactobacilli* as a potential protectant against mammalian colitis and lends support for our ongoing research towards probiotic based prevention of IBD.

Comprehensive analysis for genomic features of stem cell-like hepatocellular carcinoma

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Background: Hepatocellular carcinoma (HCC) is lethal malignancy with second highest worldwide cancer mortality. Cancer stem cell have been regarded as a major cause of tumorigenesis and metastasis. However, genomic features of stem cell-like cancer cells contributing aggressive tumor biology and therapeutic resistance in HCC remains unclear.

Objective: The aim of this study is to develop novel prediction models for stem cell-like hepatocellular carcinoma (scHCC) in clinical HCC cohorts and understand underlying biology associated with HCC stemness by integrating multi-platform data including the genome, epigenome, transcriptome, proteome.

Methods: Hepatic stem cell (HSC) signatures were extracted by analyzing gene expression data from human early human hepatoblasts (10 week in gestation), late human hepatoblasts (17 week) and mature hepatocytes. By using Bayesian compound covariate predictor algorithm, HSC signatures were then applied to gene expression data from HCC tumors (n = 1550 in 7 cohorts) to stratify tumors according to stemness of cancer cells. Multi-platform analysis were also performed to reveal molecular mechanisms for HCC stemness by integrating stemness score from prediction model with multi-layer molecular data including DNA mutation, copy number alteration, DNA methylation, miRNA, mRNA, and protein expression.

Results: HCC patients were stratified to 3 subtypes according to HSC signatures (high, moderate, and low stemness). High stemness in HCC tumors were significantly associated with overall survival of HCC patients after definitive treatment for HCC. In correlation analysis, HSC signature was strongly associated with pre-existing HCC genomic signatures including NCI, HS, SNUR, R65 signatures. Immune cells including CD4 memory activated T cells and activated NK cells were highly enriched in high stemness subtype and naïve B cells, M1 macrophage in low stemness subtype. In metabolic analysis, high stemness subtype showed metabolic dependency on tricarboxylic acid cycle, vitamin, and amino acids metabolism. TP53 and RB1 were highly mutated in high stemness subtype. By applying *in-silico* analysis with genomic drug sensitivity data we identified several small molecules including fatty acid amide hydrolase inhibitor, CXCR2 antagonist, NMPRTase inhibitor, and thioredoxin-1 inhibitor.

Conclusion: Stemness in HCC is not only associated with clinical outcomes but also with correlated with multiple genomic and proteomic traits in tumors. Our findings may offer the foundation of clinical trials for new therapeutic approaches to refractory HCC patients.

De facto Targets of Histone Deacetylase Inhibitors in liver cancer

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Histone deacetylase inhibitors (HDIs) are promising epigenetic drugs against a variety of cancers. Despite the universal anticancer efficacy, the mechanism of action is not clear. Our previous findings suggest that the anti-cancer effects of HDIs may be independent of HDACs. HDIs chelate the zinc ion in the catalytic pocket of histone deacetylase (HDACs) and could target hundreds of other metalloproteins. Using the CRISPRa SAM library, we performed a genome-wide gain-of-function screen for genes related to HDIs-mediated cytotoxicity in human liver cancer cell line Hep3B in the presence of HDIs SAHA and MS275. The BCL2 family emerged as the top enriched genes with the anti-apoptotic members MCL1 and BCL2L1 positively enriched in HDIs-treated cells while pro-apoptotic members such as BIM, PUMA, BAX, BAK, and NOXA negatively enriched. EGFR, a known positive regulator of anti-apoptotic BCL2 family members, was also positively enriched. In contrast, HDACs did not show significant enrichment. These results were further validated using stable cell lines. In addition, we took advantage of multiple available human cancer datasets and performed systemic non-biased bioinformatics analysis regarding the role of HDACs and BCL2-related genes in carcinogenesis and HDIs sensitivity. These results suggest that, rather than HDACs, BCL2 family members and their related modulators could be the de facto targets of HDI for their anti-cancer efficacy in liver cancer cells.

Rev-erb in GABAergic neurons controls circadian hepatic insulin sensitivity

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Liver plays an essential role in nutrient metabolism, insulin sensitivity, and diabetes. Elevated basal glucose levels in the early morning is associated with elevated sensitivity to insulin-mediated suppression of hepatic glucose production in healthy individuals. The mechanism underlying the timing of the counterbalance is poorly understood. Here we show that the circadian clock in the brain plays an essential role in the process. Nuclear receptor Rev-erb is a key component of the molecular clock machinery. We demonstrated in mice that Rev-erb in the suprachiasmatic nucleus GABAergic (SCN^{GABA}) neurons controls diurnal variation of hepatic insulin sensitivity through the sympathetic nervous system in a manner that independent of the circadian consummatory or locomotor behaviors. Rev-erb dictates the excitability of the SCN^{GABA} neurons. Depletion of Rev-erb in GABAergic neurons made mice resistant to chronodisruption-induced glucose intolerance, while restoration of the temporal firing pattern of the SCN^{GABA} neurons rescued the glucose intolerance caused by Rev-erb dysfunction or chronodisruption. These findings provide molecular and cellular insights into the physiological circadian rhythm in hepatic insulin sensitivity and the pathogenesis underlying chronodisruption-induced glucose intolerance.

Human breast milk promotes the secretion of potentially beneficial metabolites by probiotic *Lactobacillus reuteri* DSM 17938

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Background: *Lactobacillus reuteri* DSM 17938 (LR) inhibits pathogen growth and modulates the immune system in newborns. In neonatal rats, we observed that LR in combination with dam's breast milk or human breast milk (HBM) (but not formula) increased the percentages of intestinal Foxp3$^+$regulatory T cells. This suggested a beneficial effect of breast milk on LR-mediated immunomodulation. We also found that when cultured, LR demonstrated better growth in HBM than in formula.

Objectives: To investigate the effects of HBM on LR-associated proteins and metabolites in culture, in order to provide mechanistic insights into the health benefits of LR.

Methods: LR was cultured anaerobically for 16 hours in HBM from 6 mothers and in 2 commercially-available cow-milk based formula. The fermented supernatants were collected, and global metabolomics were analyzed by Metabolon, Inc. Bacterial pellets were sonicated and bacterial lysates were collected to analyze proteins by using liquid chromatography–mass spectrometry at the U.T. Proteomics Center.

Results: Metabolomics: Principle component analysis and hierarchical clustering showed a major segregation among formula-cultured and HBM-cultured LR, with major differences in biochemical composition of the fermented media. Compared to LR cultured in formula, LR cultured in HBM (HBM+LR) produced significant changes in amino acids of different subpathways. We detected 261 of 452 metabolites that were up regulated, and 191 metabolites that were down regulated ($p<0.05$) in HBM+LR compared to formula+LR. Several metabolites that increased by greater than 5-fold in HBM+LR were involved in glyoxylate cycle (succinate), urea cycle (citrulline), methionine methylation (cysteine, N-acetylcysteine), and polyamine synthesis (spermidine). The glyoxylate cycle, similar to human tricarboxylic acid cycle, generates adenosine triphosphate (ATP) to provide energy through the oxidation of acetyl-CoA. Citrulline and polyamines have protective effects following intestinal mucosal injury. Proteomics: We found that 11 proteins were upregulated and 19 proteins were downregulated when LR was cultured in HBM. Among the proteins, pyruvate dehydrogenase E1 component, enolase, and 2-oxoisovalerate dehydrogenase subunit beta were upregulated, each of which is involved in acetyl-CoA formation. The upregulated methionine-tRNA ligase is involved in cysteine formation. Cysteine, a product of the methionine pathway, is a precursor to the antioxidant glutathione.

Conclusions: We found significant increases in LR-associated metabolites related to ATP production and antioxidant status promoted by HBM in this study. Our previous work linked ATP-derived adenosine/inosine to immune modulation in mice with autoimmune disease. These metabolites may be linked to the mechanism of action of *L. reuteri*.

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Demographic, lifestyle, and dietary risk factors for gastric intestinal metaplasia among US veterans

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Background: The risk of non-cardia gastric cancer is increased in the presence of gastric intestinal metaplasia (GIM). The aim of this study was to determine risk factors for the presence and severity of gastric intestinal metaplasia among a non-immigrant United States population.

Methods: We used data from a cross-sectional study of 2244 patients randomly sampled and invited to endoscopy from primary care clinics (n=569) and endoscopy clinics (n=1675) at the Michael E. DeBakey VA Medical Center in Houston, Texas between 2/2008 and 8/2013. All patients completed standardized lifestyle and symptom questionnaires, a 110-item Food Frequency Questionnaire and underwent endoscopy with gastric mapping (5-7 standardized biopsies). Presence and severity of GIM was determined by two GI pathologists and defined as intestinal metaplasia on any non-cardia gastric biopsy; extensive gastric intestinal metaplasia was defined as involving both antrum and corpus. Multivariate logistic regression models were used to examine the determinants and magnitude of associations among demographic, lifestyle, and dietary factors with the presence and severity of gastric intestinal metaplasia.

Results: We identified 431 cases with gastric intestinal metaplasia and 1813 controls without GIM. The prevalence of gastric intestinal metaplasia was 21.6% and 18.4% among participants recruited from primary care clinics and endoscopy clinics, respectively. Compared to controls, cases were older on average (62.1 vs. 59.9 years), more likely to be male (97.0% vs. 90.8%), current smokers (34.5% vs. 28.1%), *Helicobacter pylori* positive (52.9% vs. 25.0%) but less likely Non-Hispanic White (NHW) (41.1% vs. 61.0%). Older age (per 1 year increase, AdjOR, 1.04; 95%CI, 1.03-1.06), male sex (AdjOR, 2.13; 95%CI, 1.17-3.88), non-White race/ethnicity (vs. NHW: Hispanic, AdjOR, 2.40; 95%CI, 1.67-3.46; Black, AdjOR, 1.88; 95%CI, 1.46-2.42), current smoking (AdjOR, 1.93; 95%CI, 1.39-2.67), and *H. pylori* infection (AdjOR, 2.89; 95%CI, 2.30-3.63) were independently associated with risk of gastric intestinal metaplasia. We did not find significant associations with dietary intake of fruits (tertile 3 vs 1 OR, 1.41; 95%CI 0.96-2.08), vegetables (OR, 1.02; 95%CI 0.69-1.52), fat (OR, 0.83; 95%CI 0.51-1.35), sodium (OR, 0.92; 95%CI 0.56-1.50), or vitamin C (OR, 1.18; 95%CI 0.79-1.79) with gastric intestinal metaplasia. In a subset of 116 patients with extensive gastric intestinal metaplasia, the strength of association was stronger with older age, male sex, race/ethnicity, and smoking.

Conclusion: Older age, male gender, race/ethnicity, and smoking were the non-endoscopic factors associated with gastric intestinal metaplasia in a non-immigrant US population, while dietary factors were not. These factors remained significant after adjusting for *H. pylori* status.

Analysing colonoscopy wait times at Ben Taub General Hospital

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Background/Aim

From April 2017 to April 2018, the wait time for patients to undergo a screening colonoscopy increased from an average of 5 months to 12 months. Given the severe morbidity and mortality than can result from delayed colonoscopies, minimizing wait times is essential. The aim of this study was to use process analysis to identify the cause(s) of significant increase in wait times.

Methods

A retrospective chart review of 310 patients who underwent direct colonoscopy at Ben Taub General Hospital was conducted. 100 patients underwent colonoscopy in April 2017 and 210 patients in April 2018. All participants were adults (>18 years) who were referred for colonoscopy from a primary care clinic. Screening colonoscopies conducted (for being age >50) were excluded. Patient demographics and indications for colonoscopy were recorded. Thorough examination of each patient's chart confirmed if the indication for colonoscopy was appropriate.

Results

April 2017

Of 100 patients, average age was 59 years and 59% were female. Indications for needing colonoscopy were positive FIT testing (48%), personal history of colon cancer (20%), rectal bleeding/blood in stool (13%), family history of colon cancer (12%), iron deficiency anemia (IDA, 10%), or abnormal imaging (1%). Note, 4 patients had more than one indication. Overall, 81% of patients had the correct indication for colonoscopy. Those with 'IDA' and 'rectal bleed/blood in stool' did not meet those indications 70% and 30.8% of the time, respectively. Specifically in patients with the 'IDA' indication, those that did not actually have IDA did have either a positive FIT test or a urinalysis with red blood cells.

April 2018

Of 210 patients, 137 (65.2%) were female and the average patient age was 58. Interestingly, 88% of patients met the criteria for their colonoscopy indication. 63% of IDA patients were correctly categorized, twice as many as in 2017. Additional analysis on patients' American Society of Anesthesiologists (ASA) physical status classification was conducted. One third of patients had a misclassified ASA. In the majority of these cases, ASA III patients were incorrectly classified as ASA II as their Body Mass Index (BMI) was not taken into account.

Conclusion

Incorrect categorization of both ASA status and indication for colonoscopy are likely contributing to the increase in wait times for direct colonoscopy. Misclassification of ASA status delays care for patients who, when moving from class II to III, require a change in type of anesthesia. Patients who have an incorrect indication for colonoscopy- namely 'IDA' or 'rectal bleed' - have delayed procedures due to not having appropriate lab work completed before the day of colonoscopy.

Emerging *Clostridium difficile* ribotypes exhibit unique carbon substrate utilization profiles

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Clostridium difficile is a gram-positive spore-forming pathogen that recently has become the most common nosocomial infection in the developed world. *C. difficile* is a genetically diverse species and distinct ribotypes have been shown to be overrepresented in both human outbreaks and in animals. Mass use of trehalose in food manufacturing coincided with the emergence of several epidemic ribotypes (RT017, RT027, & RT078), which have evolved a heightened ability to utilize this sugar as a carbon source. However, the contribution of the diet to the emergence of additional ribotypes is poorly understood. Using a Biolog Phenotype Microarray carbon source plates, we have profiled carbon substrate utilization of clinical isolates from a variety of ribotypes collected through an active surveillance network based in Texas. Our analysis suggests that the utilization of trehalose, leucine, melezitose, and sorbitol are highly variable traits between ribotypes but reproducible within closely related isolates. We also show that ribotype 255, an emerging strain in the state of Texas, exhibits broadly enhanced ability to utilize non-traditional carbon substrates. Ongoing work will continue to profile additional isolates and validate substrate-based fitness advantages using genomic verification, molecular characterization, and competition assays.

Risk of Cirrhosis and HCC in Patients with Steatosis and Normal Aminotransferase

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BACKGROUND: Nonalcoholic fatty liver disease (NAFLD) affects 20-30% of the U.S. population. Guidelines define NAFLD based on presence of hepatic steatosis on imaging or biopsy. In clinical practice, many patients are diagnosed with hepatic steatosis in the absence of markers of liver inflammation. The relationship between hepatic steatosis with normal aminotransferases and long-term risk of progressive disease is unclear. We sought to examine the long-term incidence of cirrhosis and hepatocellular cancer (HCC) in patients with hepatic steatosis and normal aminotransferases. **METHODS:** We conducted a retrospective cohort study using national Veterans Health Administration (VHA) data. We identified patients with at least 1 ALT test in the VHA 1/1/2004-12/31/2008. We excluded patients with positive serologic testing for HBV/HCV or alcohol (ICD-9 codes/positive AUDIT-C scores) any time prior to or during study follow up. We applied a previously validated natural language processing algorithm to identify hepatic steatosis for a subset of patients. We identified 3 groups: patients with steatosis and persistently normal ALT (men:<40 IU/ml, women:<31 IU/ml); those with steatosis and evidence of abnormal ALT (positive control); and those with no steatosis nor abnormal enzymes (negative control). Patients were followed from the first ALT date until 12/31/2015 or diagnoses of HCC, cirrhosis, or death. Diagnosis of HCC was based on VHA Cancer Registry/chart review; cirrhosis was based on ICD 9/10 codes; these were excluded if before or within 1 year of index. We examined incidence rates for cirrhosis/HCC across these groups. We also conducted Cox proportional hazards models adjusting for demographic differences to determine the independent effect of hepatic steatosis on the risk of HCC. **RESULTS:** We identified 11,415 patients with hepatic steatosis and persistently normal ALT; the mean age was 56.3 (SD=10.3); 66% were white. We identified 42,901 positive controls and 24,645 negative controls. There was a higher proportion of patients with diabetes, hypertension, and dyslipidemia in the 2 steatosis groups vs. those without steatosis (p<0.001). The annual incidence rates for cirrhosis and HCC were 2.5 (95% CI: 2.2-2.9) and 0.16 (0.09-0.27) per 1000 person years in patients with steatosis and normal ALT. These rates were similar in negative controls and statistically significantly lower than the incidence in positive controls; these trends did not change after adjusting for demographic differences in the 3 groups (Table). **CONCLUSION:** Individuals with hepatic steatosis and normal transaminases have similar risk of liver-related outcomes as those in general clinical population without hepatic steatosis.

	Steatosis /Elevated Liver Enzymes N=42901	Steatosis /Normal Liver Enzymes N=11415	No steatosis/Normal Liver Enzymes N=24645
Age	53.3 (SD 12.1)	56.3 (SD 10.3)	58.1 (SD 10.7)
Incidence Rate per 1000 person years (95 % CI)			
Cirrhosis	4.93 (4.70-5.17)	2.50 (2.19-2.85)	2.40 (2.19-2.64)
HCC	0.49(0.42-0.57)	0.16(0.09-0.27)	0.13 ((0.08-0.20)
Hazard Ratio (95 % CI)			
Cirrhosis	2.3(2.0-2.5)	1.1(0.9-1.3)	Ref
HCC	4.6 (3.0-7.0)	1.3 (0.7-2.5)	Ref

Examining the role of gut dysbiosis in hypertension and cerebral small vessel disease

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Cerebral small vessel disease (CSVD) includes hypertension, vessel remodeling, blood brain barrier (BBB) breakdown and neuroinflammation. Recent studies suggest that alterations in the gut microbiota are linked to hypertension and stroke. We have demonstrated a causal role for gut dysbiosis in the development of hypertension in the spontaneously hypertensive rat (a model of hypertension).Thus we hypothesized that gut dysbiosis may contribute to the development of CSVD. To test this hypothesis, we changed the gut microbiome of WKY (control, normotensive rats) and stroke prone spontaneously hypertensive rats (SHRSPs; a model of CSVD and hypertension) pups by fostering them on mothers of the same or opposite strain. Upon weaning, fostered animals were co-housed with animals of the same strain of their fostered mother (4 groups: wWKY, wSHRSP, sWKY, sSHRSP; w and s indicate the strain of foster mother). Feces was collected after each systolic blood pressure (SBP) measurement to evaluate gut microbiota using 16S rRNA sequencing. At 20 weeks of age, rats were sacrificed, brain was collected to assess for CSVD phenotypes, and ileal tissue was taken to assess local gut gene expression for inflammatory markers and toll-like receptor signaling. Repeated measures two-way ANOVA of SBP from 6-20 wks showed a 6 mmHg increase in sWKY compared to wWKY ($p<.05$), and a 22 mmHg decrease in wSHRSP compared to sSHRSP ($n=6$-8, $p<.01$). Independent of genotype, rats nursed by SHRSP mothers had evidence of gut inflammation compared to animals nursed by WKYs, which include increased expression of Il-1a, Il-6 and TLR2 ($n=6$, $p<.01$). Fecal 16S rRNA data showed that the gut microbiota of offspring was altered by cross-fostering to closely resemble the foster mother. Interestingly, rats fostered by SHRSP mothers demonstrated a significant decrease in *Akkermansia*, a genus associated with improving gut barrier integrity ($n=6$-8, $p<.05$). To confirm the effect of gut microbiome switching in the cross-fostering study, we gavaged male Germ Free (GF) rats (13wks) with cecal content from either WKY or SHRSP animals (GF-W and GF-S). GF-S exhibited a 19 mmHg increase in SBP (18-25 wks; $n=7$-8, $p<.05$). Furthermore, we observed significantly lower abundance of *Akkermansia* in GF-S, as compared to GF-W (18-23 wks; $n=7$-8, $p<.05$). Finally, to test if *Akkermansia* could play a therapeutic role against CSVD, we administered either *Akkermansia muciniphila* (Akk, $\approx 10^9$ colony forming units, $n=7$) or vehicle to SHRSP rats weekly (oral gavage, starting at age of 3 weeks). This study is currently underway. We conclude that gut dysbiosis contributes to the onset and development of CSVD possibly by inducing inflammation in the gut, and that probiotic treatment may be a potential therapeutic approach to attenuate gut inflammation and hypertension in CSVD.

Characterizing the role of rapid intestinal epithelial cell loss and immune surveillance in promoting pathogenic infection using zebrafish (*Danio rerio*) as a model host

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Cellular extrusion is a mechanism by which the epithelium eliminates dying or crowded cells to maintain homeostatic conditions. The dysfunction of extrusion may be relevant in understanding intestinal disorders such as inflammatory bowel diseases and diarrheal diseases. Previous observations in cultured cells have shown that the rapid loss of multiple cells by extrusion can lead to breaches in cell-cell contacts, potentially exposing structural weaknesses or paths to luminal pathogens, immune cells, and small molecules entering the basolateral compartment[1].

The objective of this study is to determine how compromised barrier function may drive pathogenesis and inflammation in epithelial tissues. The interactions between immune cells and luminal bacteria during intestinal epithelial extrusion represent some of the earliest events during infection, yet have not been well characterized, as an in vivo model has yet to be established that would facilitate dynamic high-resolution imaging. To overcome this limitation, we have developed tools and imaging methods for the zebrafish (*Danio rerio*) to develop an in vivo intestinal epithelial extrusion model and address questions of how pathogens may capitalize on transient loss of barrier function during induced cell loss. Further, our novel approach revealed an increased presence of neutrophils at sites of epithelial extrusion. Our preliminary data suggests that increased amounts of cell elimination by extrusion may disrupt barrier function and allow pathogens to invade.

Given that zebrafish have a similar intestinal structure to mammals, and many epithelial signaling processes and developmental pathways are conserved between fish and mammals[2], this work will uncover novel mechanisms pathogens use to invade host tissues and promote intestinal disorders.

The prevalence of *Helicobacter pylori* related gastric cancer is decreasing

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Background: Gastric cancer is the fifth leading cause of cancer-related mortality globally. Gastritis related to *Helicobacter pylori* (*H. pylori*) infection is thought to be the most important causal factor for non-cardia gastric cancer. *H. pylori* negative gastritis has been described, but its prevalence among patients with gastric cancer is unknown. The aim of this study was to examine the prevalence of *H. pylori* infection among those with newly diagnosed gastric adenocarcinoma.

Methods: This was a retrospective cohort study of consecutive, newly diagnosed patients with non-cardia gastric adenocarcinoma at the Michael E. DeBakey VA Medical Center in Houston, Texas from 2007 to 2018. We performed a structured review of the medical records to verify the diagnosis of gastric adenocarcinoma on histopathology and the date of diagnosis. *H. pylori* infection positivity was defined by the presence of *H. pylori* bacteria on histopathology without or with special staining (i.e., Genta, Giemsa, and immunohistochemical staining), positive serum *H. pylori* antibody serology, positive stool *H. pylori* antigen, or positive urea breath test at the time of, prior to, or after gastric adenocarcinoma diagnosis. We used Poisson regression to examine the number of cases of non-*H. pylori* related non-cardia gastric cancer based on year of diagnosis (2007-2010, 2011-2014, and 2015-2018).

Results: There were 91 consecutive patients with confirmed incident non-cardia gastric adenocarcinoma diagnosed between November 2007 and October 2018. Most were men (N=87, 95.6%) and black (N=47, 51.6%), and the mean age at diagnosis was 68.0 years (SD 10.8). In addition to gastric cancer biopsy histopathology, 73 patients had at least one additional test for *H. pylori* infection; these included *H. pylori* antibody serology (n=31) and non-cancer gastric biopsy histopathology with (n=41) or without special staining (n=25). None had testing by stool *H. pylori* antigen or urea breath test. The overall prevalence of *H. pylori* infection in the study cohort was 35.2% (n=32). Among the 73 patients with an additional test for *H. pylori* infection, the prevalence of *H. pylori* was 43.8% (n=32). Prevalence rates of *H. pylori* related gastric cancer decreased slightly from 42.1% in 2007-2010 to 40.0% in 2011-2014 to 26.8% in 2015-2018 (p-value for trend=0.44).

Discussion: The prevalence of *H. pylori* infection among patients with non-cardia gastric adenocarcinoma is relatively low (35.2%-43.8%). This finding suggests there may be other important causal factors for gastric adenocarcinoma (i.e. *H. pylori* negative) in a US population.

Targeting *Poglut1* with an antisense oligonucleotide improves liver function in a mouse model of Alagille syndrome

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Haploinsufficiency of jagged1 *(Jag1),* a component of Notch signaling pathway, causes Alagille syndrome (ALGS) a multisystem disease which is mainly characterized by bile duct paucity. We have previously shown that removing one copy of *Jag1* on a C57BL/6 background recapitulates the ALGS liver phenotypes. Furthermore, we have shown that removing one copy of the glycosyltransferase *Poglut1* improves the phenotypes of *Jag1* heterozygous animals. POGLUT1 adds glucose residues to epidermal growth factor-like (EGF) repeats harboring the C^1-X-S-X-(P/A)-C^2 consensus motif. All Notch receptors and most canonical Notch ligands harbor such EGF repeats. Indeed, our mass spectrometry data on human JAG1 protein shows that all target sites of POGLUT1 on JAG1 are efficiently glycosylated. The genetic rescue of the *Jag1+/–* liver phenotypes by decreasing *Poglut1* gene dosage prompted us to examine the therapeutic potential of *Poglut1* suppression for treatment of ALGS liver disease. To this end, we have identified a number of antisense oligonucleotides (ASOs) that can specifically decrease *Poglut1* mRNA levels in mouse cell lines and *in vivo*. ASO-mediated *Poglut1* reduction from P1 results in a significant increase in bile duct numbers in *Jag1* heterozygous mice at the age of P12. Furthermore, examining serum chemistry markers in *Jag1+/–* animals treated with anti-*Poglut1* ASO demonstrates an overall improvement of liver function compared to *Jag1+/–* animals treated with a control ASO. Additional genetic experiments indicate that removing one copy of *Poglut1* can significantly improve the survival of *Jag1+/–; Notch2+/–* double heterozygous animals, which show broader and more severe phenotypes compared to *Jag1+/–* mice. Collectively, our findings suggest that decreasing *Poglut1* levels by antisense oligonucleotide approach is a viable strategy for treatment of ALGS phenotypes and possibly other diseases like non-small cell lung cancer that are associated with increased POGLUT1 expression levels.

P2X2 Purinergic receptor signaling promotes hepatocellular injury and inflammation in non-alcoholic steatohepatitis

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Background. P2X2 purinergic receptors are ligand (ATP) gated ion channels expressed in hepatocytes and non-parenchymal cells. Cellular stress and injury lead to ATP release into the extracellular milieu which, in turn, via activation of cell-surface P2 purinergic receptors, influence diverse cellular functions. Previous studies suggest that excessive fatty acid exposure triggers ATP release from hepatocytes *in vitro*. However, the impact of purinergic signaling on the induction of endoplasmic reticulum (ER) stress and pathogenesis of non-alcoholic steatohepatitis (NASH) remains unknown. The purpose of this study was to determine the significance of P2X2 purinergic receptor signaling in the induction of hepatocellular injury and inflammation in the methionine and choline deficient (MCD) diet mouse model of NASH. MCD diet causes defective triglyceride secretion, elevated ER stress and hepatocellular injury in mice. **Methods.** Wild type (WT) and P2X2 purinergic receptor knockout (KO) mice had free access to MCD or control diet for 3 weeks. Formalin-fixed, paraffin embedded liver sections were analyzed by light microscopy after H&E staining (hepatocellular injury, lipid droplet morphology), and immunostaining for CD45 and F4/80 (inflammatory cell infiltration). Total liver homogenates were analyzed by Western blotting for the induction of ER stress (phospho and total IRE1a, Grp78), and inflammatory cell activation (MMP9). Serum analysis included markers of liver injury (ALT) and lipid secretion (Triglyceride, VLDL, Cholesterol). Mouse primary hepatocytes were isolated and treated with ATPgs (100 mM, 4 h) *in vitro* with or without pre-treatment with SP600125 (SP, JNK inhibitor; 30 mM). Total protein extracts were analyzed by Western blotting for the induction of ER stress (phospho and total IRE1a, Grp78). **Results.** MCD diet-induced hepatocellular injury was attenuated in the KO, as compared to WT (WT v KO, ALT, 279 v 182 U/L, $p<0.05$). Serum lipid profiles were comparable between the WT and KO (Triglyceride, 55 v 59; VLDL, 11 v 12; cholesterol, 35 v 40 mg/dL, n.s.). While the MCD diet-induced lipid accumulation within hepatocytes was comparable, the lipid droplet composition was markedly different between the genotypes, with predominantly microvesicular steatosis in the WT and macrovesicular steatosis in the KO. Suggesting a role for P2X2 signaling in inflammatory cell infiltration and activation, WT livers had increased CD45 (leukocytes) and F4/80 (macrophages) positive cell infiltration and extravasation into hepatic parenchyma and MMP9 protein expression (Fold change, WT v KO, 2.7 v 1.7, $p<0.05$). Most notably, the number of CD45+ inflammatory foci was elevated in response to MCD diet in the WT, as compared to KO (WT, 121; KO, 42; $p<0.05$). MCD diet induced significant ER stress in the WT livers which was attenuated in the KO (WT v KO, Fold change, Phospho-IRE1a: 1.7 v 1.2; Total IRE1a: 2.0 v 1.2; Grp78: 2.9 v 0.9, $p<0.05$). Extracellular ATP treatment alone was sufficient to induce phosphorylation of IRE1a, which was dependent on intact JNK signaling in hepatocytes *in vitro* (Fold change, Un v ATPgS, SP, SP+ATPgS: 1.0 v 1.5, $p<0.05$, 0.8 (n.s), 1.2 (n.s). **Conclusions.** These results suggest that extracellular ATP treatment alone is sufficient to induce ER stress and activation of the IRE1a arm of the unfolded protein response in hepatocytes *in vitro,* and MCD-diet induced NASH livers *in vivo*. P2X2 purinergic signaling promoted hepatocellular injury via its effects on lipid droplet morphogenesis and inflammatory cell infiltration in a dietary model of experimental NASH. These findings highlight an unrecognized role for extracellular ATP and P2X2 purinergic signaling as key mediators of hepatocellular injury and inflammation in the pathogenesis of NASH.

MicroRNA profiling of primary and peritoneal metastases of Gastric Cancer identified miR650 as a novel metastatic mediator that suppresses invasion

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Background: Gastric cancer (GC) remains one of the major cause of death worldwide, being the fifth most incident tumor in the word. Peritoneal carcinomatosis (PC) is the most common type of metastasis within GC patients, which is associated with decreased median survival rates to less than 1 year. There is an urgent need to deepen the knowledge on molecular biology of this disease, opening new opportunities to develop life-extending therapies. MicroRNAs (miRNAs) plays a role in the modulation of gene expression by binding in specific mRNA, regulating important pathways in cancer such as cell differentiation, proliferation and progression by mRNA inhibition or degradation. The aims of this study was to investigate the microRNA expression profile in primary GC tumors (GC-P) and matched normal tissue (GC-N) and ascites cells (GC-A) and identify key mediator for PC metastases. **Methods:** We performed a multiplex microRNA panel array (Agilent) with 10 samples from each type of samples, mormal (GC-N), matched primary tumors (GC-P), and metastatic ascites cells (GC-A) from GC patients collected at MD Anderson Cancer Center. Functional studies were performed using miR650 mimic and inhibitors in GAC cell lines and our patients' derived GAC cells. mirRNA profile from these samples were analyzed statistically. **Results:** mirRNA expression profile were analyzed to identify mirRNAs that were significantly lower expressed in GC-A related to GC-P, but also significantly lower expressed in GC-A when compared to GC-N. The rationale is that we wanted to identify mirRNAs with a continuous decrease in expression along the GC disease state (GC-N > GC-P > GC-A). After filtering according to fold change (FC) (FC>2) and p-value ($p<0.05$), we found 1 mirRNA (microRNA-650) with the expected expression pattern (GC-A vs GC-T FC = -4; GC-T vs GC-N FC = -5.7; GC-A vs GC-N FC = -22). Next, we validated the expression of miR-650 in an independent cohort performed by 20 metastatic samples and 20 non-metastatic samples matched with normal samples using qPCR and we confirmed our array data. We examined the microRNA expression in primary and metastatic cell lines, including one metastatic ascites cell line established in our laboratory (GA051816). Finally, to study the functional importance of this miRNA on GC cell invasion and modulation of epithelial–mesenchymal transition (EMT) process, we transfected miR-650 mimic in two metastatic cell lines (Kato III and GA51816). Upregulation of miR650 significantly decreased GC cell invasion and colony formation, while inhibition of miR650 in high expressed GC cells (AGS and MKN45) using miR650 inhibitors, we observed that GC cells were demonstrated increase in cell colony formation and invasion upon inhibition of miR650 and accompanying of increase in EMT properties. **Conclusion:** In this study, we were able to profile and identify an important miRNA (miR650) that might have an important role in modulating invasiveness and EMT process in gastric cancer. Further experiments should be performed and confirmed our preliminary findings.

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Intestinal stem cell responses to fecal microbial communities

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The microbiome plays key roles in intestinal homeostasis and function. However, the mechanisms by which commensal communities impact host gut physiology and function remain largely undefined. Published data as well as our preliminary findings indicate that intestinal microbial organisms influence epithelial proliferation and differentiation.

The objectives of this research project are to identify interactions between specific commensal microorganisms and the epithelium of the gastrointestinal tract, and to elucidate how these interactions modulate the intestinal stem cell (ISC) response. To begin to study microbiome/ISC interactions, I am using an innovative approach that combines mini bioreactor assays (MBRAs) to propagate and study complex commensal communities from human stool under anaerobic conditions coupled with novel *in vitro* organoid models of the human intestinal epithelium derived from adult ISC cells (human intestinal organoids, HIOs). Treatment with products from a subset of MBRA samples, stimulates HIO proliferation. This proliferative response is accompanied by an induction of WNT signaling, a well characterized pathway that plays a key role in stem cell activation. Additionally, this exposure also stimulates Paneth cells, a driver of stem cell proliferation by WNT signaling. These responses occur in HIOs across multiple segments of the intestine and with microbial communities cultivated from several donor stools. Unexpectedly, I have isolated specific facultative anaerobes from the MBRA cultured communities that induce the proliferative effect. Currently, I am focused on cultivating and characterizing bacterial strains that induce ISC proliferation.

Understanding how bacterial communities interact with and stimulate ISC responses will aid in the development of commensal communities as a potential therapeutic in response to intestinal damage.

Nutrient-sensing nuclear receptors regulate coagulation

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Background: Malnutrition affects 320,000 live births in the United States each year. Malnourished small-for-gestational-age (SGA) versus appropriate-for-gestational-age neonates are coagulopathic, with elevated international normalized ratio (INR) and increased risk of hemorrhage, including intraventricular hemorrhage that can lead to death or long-term disability including cerebral palsy. Although vitamin K deficiency can cause coagulopathy, vitamin supplementation fails to normalize INR. Vitamin K-refractory coagulopathy is also observed in adolescents with anorexia nervosa and in children with chronic malnutrition. Why coagulopathy occurs in malnutrition is unknown, although recent evidence implicates the nutrient-sensing nuclear receptors farnesoid X receptor (FXR) and peroxisome proliferator-activated receptor (PPAR)α, which competitively bind DNA regulatory elements with opposite transcriptional and functional outputs. In the fed state circulating bile acids activate FXR to suppress gluconeogenesis and autophagy, while in the fasted state products of lipolysis activate PPARα to stimulate fatty acid oxidation and autophagy. Infants with mutations in the gene encoding FXR have vitamin K-refractory coagulopathy, with suppressed production of the FXR-dependent genes including fibrinogen. We hypothesized that altered PPARα/FXR signaling mediates coagulopathy in malnutrition.

Methods: We modeled early postnatal malnutrition by administering low-protein, low-fat chow or isocaloric control chow to C57BL/6 wild type, $FXR^{-/-}$, or $PPAR\alpha^{-/-}$ mice from 2-7 weeks of life. We assessed coagulation indices in plasma, and gene expression and nuclear receptor binding by RNA-seq and ChIP-qPCR in whole livers and nuclear extracts.

Results: Compared to controls, malnourished mice are 30% underweight and have decreased plasma fibrinogen with 1.3-fold increased INR, mimicking the coagulopathy of SGA neonates, acutely malnourished infants, and children with chronic malnutrition. FXR target genes were repressed as expected, given our recent report demonstrating decreased bile acid synthesis in malnutrition. RNA-seq revealed strong hepatic PPARα activation, with induction of known PPARα targets and decreased transcription of two coagulation factors, fibrinogen and factor 11, in wild type and $FXR^{-/-}$ (but not $PPAR\alpha^{-/-}$) malnourished mice. Near the transcriptional start site of both genes, we identified a region of co-localization between PPARα and nuclear corepressors, suggesting the possibility of corepressor recruitment by activated PPARα. Activated FXR and its steroid response coactivators can occupy the same site, suggesting potential competition between PPARα and FXR in the malnourished and healthy states, respectively. Indeed, ChIP-qPCR confirmed enrichment of PPARα and multiple corepressors at PPAR-response elements in malnourished livers.

Conclusion: Our data support a novel mechanism of transcriptional regulation of coagulation factor synthesis by the nutrient-sensing nuclear receptors PPARα and FXR and their nuclear co-regulator proteins.

Modeling the thrifty phenotype: early postnatal malnutrition produces long-term weight gain, persistently altered hepatocyte DNA methylation and metabolic pathways in males

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Background: Malnutrition contributes to more than 3.1 million global child deaths per year; 96 million children under age five years (14%) are underweight and 159 million (24%) are stunted. The response to malnutrition is sex-specific, with boys more likely than girls to be underweight and stunted. Multiple large studies have now linked nutrient deprivation in the prenatal or early postnatal periods with obesity, type 2 diabetes mellitus, cardiovascular disease, and other risk factors for mortality decades later. Although mechanisms linking early undernutrition to long-term non-communicable diseases are poorly understood, it has been hypothesized that nutrient deprivation early in life confers a "thrifty phenotype" mediated by epigenetic changes, specifically, by altered patterns of DNA methylation. We sought to model the thrifty phenotype in mice and use genome-wide DNA methylation and transcriptional profiles to understand how early undernutrition leads to persistent metabolic abnormalities.

Methods: We modeled early postnatal malnutrition by administering low-protein, low-fat chow or isocaloric control chow to male and female C57BL/6 mice from 2-7 weeks of life. Starting at 8 weeks of life, all mice were switched to 45% kcal high-fat diet, and re-fed for 6 months. Biweekly body composition was quantified by DEXA, and DNA methylation and transcription were assessed genome-wide with DNA bisulfite-seq and RNA-seq, respectively, from hepatocytes isolated during acute malnutrition and after 6 months of refeeding.

Results: After 6 weeks of malnourished diet, young adult males were more profoundly affected than females with respect to underweight and stunting. Malnutrition caused both sexes to have decreased lean body mass, bone area, bone content, and bone mineral density. After 6 months of refeeding, formerly-malnourished males, but not females, had profoundly increased weight gain, as well as increased bone area, bone mineral content, and bone density. Hepatocytes from malnourished males contained twice as many differentially expressed genes (3,265) than malnourished females (1,690), with a larger fraction of differentially methylated regions demonstrating hypomethylation (87% vs 66%). After refeeding, hepatocytes from formerly-malnourished males contained 225 differentially expressed genes, compared to 828 genes in formerly-malnourished females, with nearly 20% of the sites differentially methylated during malnutrition remaining differentially methylated after refeeding. Among the key metabolic pathway alterations was mTORC1 signaling, which was strongly downregulated in male and female malnourished mice and re-fed female mice, but strongly upregulated in re-fed males.

Conclusion: Early postnatal malnutrition produces sex-specific underweight, stunting, and DNA hypomethylation, which could be due to methyl donor deficiency during malnutrition. Despite 6 months of refeeding, changes to the DNA methylome and transcriptome persist. Activation of mTORC1 signaling suggests that increased protein synthesis is formerly-malnourished males could underlie the sex-specificity of the thrifty phenotype.

A novel animal model of gastroparesis

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Background: Gastroparesis, or delayed gastric emptying, occurs in 24.2 per 100,000 adults. Although the prevalence is unknown in the pediatric population, the cost of hospital care for children with gastroparesis has risen dramatically over the last decade. Gastroparesis is observed in a broad range of undernourished states including small for gestational age neonates, children with acute and profound weight loss, and adolescents with anorexia nervosa. Undernutrition creates a cycle of physiologic disturbances, including delayed gastric emptying, that make catch-up growth difficult. Animal models are needed to further elucidate causes of and potential therapies for malnutrition-associated gastroparesis. We aimed to characterize a novel model of gastroparesis induced by early postnatal malnutrition.

Methods: Malnutrition was induced by timed maternal separation (TmSep) of pups from lactating dams for 12 hours per day. Control mouse pups nursed uninterrupted. On day-of-life 15, a gastric gavage of fluorescein isothiocyanate-conjugated dextran was administered, and the gastrointestinal tract was harvested 30 minutes later. Percent gastric emptying was determined by quantifying fluorescence throughout the gastrointestinal tract. H&E-stained sections were imaged on an Eclipse 90i microscope, and thickness of muscularis propria and mucosa was measured by a blinded observer using NIS Elements (Nikon).

Results: Compared to control pups, malnourished mice were moderately underweight, mean 4.45 ± 0.1 g versus 6.96 ± 0.2 g ($p < 0.0001$). On gross examination, stomachs of TmSep mice were strikingly distended compared to control mice. Gastric emptying was impaired in TmSep versus controls (87.3% versus 97.0%, $p=0.023$). The gastric smooth muscle layer was thinner in TmSep mice versus controls, mean 16.9 ± 5.3 μm versus 30.4 ± 8.8 μm ($p=0.03$).

Discussion: We present a novel model of malnutrition-associated gastroparesis induced by timed separation of mouse pups from lactating dams. We further highlight thinning of the gastric smooth muscle layer as a potential etiology that warrants further investigation, although we cannot yet rule out potential contributions from an altered enteric nervous system, gut microbiota, or neuro-hormonal signaling pathways. Defining the underlying pathophysiology may create opportunities for new therapeutic interventions for gastroparesis in children.

Upper gastrointestinal tract motility in mice is sexually dimorphic and dependent on age, duration of fast, and time of day

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Background: An important limitation of gastrointestinal motility testing is high variability. Conditions that could contribute to variability, including the time of day testing is performed and the duration of fasting prior to testing, are rarely reported and have not been examined systematically. This study aimed to explore whether these conditions, as well as sex and age, affect the results of a standard laboratory test of upper gastrointestinal tract motility.

Methods: Male and female 8-week-old mice received a gastric gavage of fluorescein isothiocyanate (FITC)-conjugated dextran. Distribution of FITC-dextran was measured 30 minutes later. Mean geometric centers (MGC) were calculated to determine the effect of short versus prolonged fasting and of morning versus afternoon testing. The influence of age was assessed in 2, 4, 6, 8, and 10 week old animals.

Key Results: At 8 weeks of life, motility was sexually dimorphic, with MGC progressing 17% further in males versus females when mice were tested in the morning following a short fast. Similar trends were observed following a prolonged fast with morning or afternoon testing. Motility in males was unaffected by time of day; however, MGC progressed 29% further in females tested in the afternoon versus in the morning following a short fast. Testing in neonatal mice revealed strikingly low variability and no sex differences.

Conclusions & Inferences: Age, sex, duration of fast, and time of day all influence the results of upper gastrointestinal tract motility testing in mice. Sex differences are not present in neonates, but develop shortly after weaning.

Mutant *Kras* promotes intrahepatic cholangiocarcinoma from cholangiocytes

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Cholangiocarcinoma (CCA) is the most common biliary duct malignancy and is classified as an epithelial cancer of cholangiocyte origin. Despite this classification, the majority of mouse models have expressed oncogenic mutations from Albumin expressing hepatic progenitor cells, and generate both CCA and hepatocellular carcinoma, limiting the understanding of how differentiated cholangiocytes transform. CCA is characterized into 3 different subtypes; intrahepatic (iCCA), perihilar (pCCA) and distal (dCCA). The prognosis for this disease is very poor and surgical resection remains the most favorable treatment. Mutations in *KRAS* are found in 22-53% of pCCA cases and 9-17% of iCCA cases. We have developed a transgenic mouse model harboring a tamoxifen inducible $Kras^{G12V}$ mutation restricted to Hnf1b+ mature cholangiocytes. Using a lineage tracing approach, we show induction of $Kras^{G12V}$ promotes an intrahepatic cholangiocarcinoma phenotype. In contrast to other models of iCCA, we do not observe tumors arising from hepatocytes. Pathologic analysis revealed iCCA had increased signaling through EGFR and PI3K. Preliminary studies inhibiting phosphorylation of Akt significantly reduced tumorigenesis in vivo. Future studies will include RNA sequencing of isolated cholangiocytes as well as the incorporation of hepatic injury models to better understand the biology of iCCA.

Screening for behavioral health needs in a gastroenterology clinic

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In pursuit of improved treatment of gastrointestinal (GI) conditions, a growing number of psychologists are serving as integrated members of gastroenterology care teams.

Recently, the general GI clinic at our institution implemented screening of behavioral health problems via self-report, with the goal of identifying patients who would benefit from psychological interventions. New patient intake forms now include a 1-page behavioral health screening tool that includes the PHQ-4 questionnaire items assessing functional impairment and sleep quality, and a checklist of nine common behavioral health concerns. Our aim was to identify the outcomes of screening in practice and opportunities to refine the process.

Chitinase 3-like-1 Promotes Intrahepatic Activation of Coagulation Through Induction of Tissue Factor in Mice

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Coagulation is a critical component in the progression of liver disease. Identification of key molecules involved in the intrahepatic activation of coagulation (IAOC) will be instrumental in the development of effective therapies against liver disease. Using a mouse model of concanavalin A (Con A)-induced hepatitis, in which IAOC plays an essential role in causing liver injury, we uncovered a previously unknown procoagulant function of chitinase 3 like 1 (Chi3l1). Chi3l1 expression is dramatically elevated after Con A challenge, which is dependent on Con A-induced T cell activation, and the resulting IFN-γ and TNF-α productions. Compared to wild type mice, Chi3l1$^{-/-}$ mice show less IAOC, reduced tissue factor (TF) expression and attenuated liver injury. Reconstituting Chi3l1$^{-/-}$ mice with recombinant TF triggers IAOC and augments liver injury. Conclusion: Our data demonstrate that Chi3l1, through induction of TF via MAPK activation, promotes IAOC and tissue injury.

Longitudinal psychological health in veterans with inflammatory bowel disease

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Background: Psychological and mental health play a critical role in inflammatory bowel disease (IBD), with literature demonstrating how biopsychosocial factors contribute to the expression and maintenance of IBD symptoms. However, research evaluating the psychological aspects of IBD in Veteran populations is notably sparse. Given that Veterans have a higher incidence of mental health disorders than do civilians, understanding IBD manifestations in Veterans is essential to minimizing disease burden, number of hospitalizations, and maximizing health related quality of life. The aim of this study was to prospectively evaluate the association of Veterans' mental health and IBD disease course.

Methods: A prospective, longitudinal observational study was conducted on at the IBD clinics at the Michael E. DeBakey VA Medical Center from 8-29-14 to 6-22-18. Data collected included demographics, dietary restrictions, work status, tobacco use, IBD subtype, and measures of depression and anxiety (PHQ-8 and GAD-7). For PHQ-8 and GAD-7, we evaluated maximum scores, persistence of abnormal scores (2 or more consecutive visits), and proportion of visits with abnormal scores. The primary outcomes of interest were changes in symptoms of anxiety and depression over time. Paired t-test was used to assess for associations between clinical factors and mental health outcomes.

Results: A total of 165 patients with 1 to 12 encounters with psychological assessments were included in this study. 84% of patients were male, 59% were Caucasian, 50% worked part of full time,18% used tobacco, and 25% reported dietary modifications. Of the 79 patients with 3 or 4 visits, 45 patients had CD and 34 UC. GAD_{max} was greater in CD vs UC (9.0 vs 4.0, $p = 0.001$). PHQ_{max} was greater in patients with ileocolonic vs ileal disease (11.9 vs 6.8, $p = 0.047$) and PHQ_{prop} was greater in ileocolonic vs colonic disease (0.7 vs 0.1, $p = 0.009$). Maximum PHQ-8 and GAD-7 scores scores were greater in patients with dietary modifications compared to those without (13.6 vs 8.7, $p = 0.022$) and those who worked full-time or part-time compared to those who were retired, disabled, students, or retired (8.0 vs 11.6, $p = 0.022$). No significant associations were found with abnormal PHQ-8 and GAD-7 scores in 2 or more consecutive visits.

Conclusions: This study demonstrates how CD location, UC location, and lifestyle factors (tobacco use, work, diet) are associated with anxiety and depressive symptoms in patients with IBD. Maximum scores were most significantly different between groups assessed, while persistence of abnormal depression and anxiety scores was not significant between any groups. Further study of the psychological aspects of IBD and associations with disease activity over may shed important light on the impact of psychological health over the continuum of care in IBD.

Genomic and molecular characteristics of gastric cancer associated with expression of long non-coding RNAs

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Background: Gastric cancer is globally the third leading cause of cancer-related death and known to be heterogeneous molecularly and clinically. However, its heterogeneity has not been sufficiently disclosed. Accumulating evidence highlights the involvement of long non-coding RNAs (lncRNAs) in cancer development and progression.

Objective: The aim of this study is to discover novel molecular subtypes of gastric cancer according to lncRNA expression pattern and to understand underlying biology associated with subtypes by integrating multi-platform data including the genome, epigenome, transcriptome, and proteome data from gastric cancer.

Methods: Using the data of 258 stomach adenocarcinoma (STAD) from The Cancer Genome Atlas (TCGA) project, we performed unsupervised clustering of lncRNA to uncover subtypes. Bayesian prediction model was constructed to validate subtypes in independent cohorts and examine clinical significance of lncRNA subtypes. Gene mutations, copy number alterations, differential expression, and differential methylation pattern in each lncRNA subtypes were analyzed, as well as their correlation with subtype specific lncRNAs.

Results: Six major clusters (L6A~L6F) were discovered by the hierarchical clustering analysis of lncRNA. Interestingly, L6A, which is subset of TCGA chromosomal instability (CIN) subtype, showed significantly poor overall survival. L6B was enriched with CIN subtype, indicating the highest copy number alterations. L6C was correlated with microsatellite instability subtype, demonstrating the highest mutation burden. L6E was completely composed of Epstein-Barr virus subtype, exhibiting global hypermethylation pattern. L6F, which is enriched with genomically stable subtype, showed significantly poor overall survival along with L6A and the most distinct expression pattern of miRNA and protein. Moreover, we identified several unreported lncRNAs specific to each subtype. Those lncRNAs were correlated with biological and clinical phenotypes of gastric cancer, including overall copy number alteration, global hypermethylation, immune signature, and mesenchymal phenotype.

Conclusion: This study unveils novel subtypes of gastric cancer associated with distinct clinical phenotypes, which can be applied to the precision medicine. Furthermore, this offers the framework for identifying oncogenic lncRNAs and further testing them as therapeutic targets for treatment of gastric cancer patients in future.

Ras Activity Levels in Pancreatic Ductal Cells Control Tumor Progression and Phenotype

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Oncogenic mutations in *KRAS* are found in multiple human malignancies including lung, thyroid, colon, liver and pancreatic ductal adenocarcinoma (PDAC). In PDAC, greater than 93% of patients have a mutation in the *KRAS* gene. Thus, *KRAS* is hypothesized to be the initiating mutation for the majority of PDAC patients. Recently, scientific advances have shown allelic imbalance of mutant *KRAS* and wild type *KRAS* is responsible for tumor development and biology of cancer and higher levels of mutant *KRAS* results in aggressive undifferentiated phenotypes in human cancers (Mueller et al, 2018). We hypothesized this was occurring in ductal derived PDAC. To test our hypothesis, we employed a mouse model where we modified dosage of mutant *KRAS* expressed in pancreatic ductal cells.

To test our hypothesis, we took advantage of the tamoxifen inducible CreER mediated LoxP recombination system to express mutant *Kras* in ductal cells. Hnf1b is a transcription factor with expression only in pancreatic ducts, so we are able to use an Hnf1b:CreER to express or delete genes of interest in ductal cells. The nomenclature for animals expressing $Kras^{G12V}$ in pancreatic ducts is $Hnf1b{:}Kras^{G12V}$. We used 1, 5 and 10 mg of tamoxifen dosage to achieve low, moderate and high levels of CreER recombination and subsequently Ras activity in ductal cells. We then monitored the mice for disease progression, survival and tumor development. Mice with high levels of Ras activity in ductal cells needed to be euthanized two weeks after tamoxifen injection and manifested a cancer cachexic phenotype. Histological analysis of these mice revealed poorly differentiated aggressive tumor with high grade intraductal neoplasia in large ducts and periductal invasive carcinoma involving all smaller ducts. Mice with moderate levels of Ras also developed cachexic phenotype and had to be euthanized at 10 weeks' time. On histology analysis, these mice showed different grades of pancreatic cancer precursor lesions, Pancreatic Intraepithelial Neoplasia (PanIN1, PanIN2 and PanIN3) and scattered tumors in the pancreas. This phenotype is most relevant to human cancer as it depicts the full spectrum of PanIN-PDAC seen in human disease. Mice with low Ras levels were euthanized at 16 weeks and the pancreas appeared normal with scattered ductal cell proliferation and ductal dilation. Western blot analysis of these pancreata confirmed increasing levels of Ras activity in mice from 1 to 10 mg of tamoxifen dosage. Proteomics analysis on these pancreata reveled significant differences in PTEN/AKT and MAPK pathway between 5 and 10mg mice. Thorough analysis of these pathways in cell of origin context can provide significant insight developing targeted therapy for PDAC.

Hippo Coactivator YAP1 is Essential for Peritoneal Metastases in Gastric Adenocarcinoma and Targeting YAP1 is a Novel Therapeutic Strategy

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Background and Aims: Gastric Adenocarcinoma (GAC) remains a major health burden in the US and worldwide. GAC with peritoneal carcinomatosis (PC) is common affecting ~45% of patients during the course of GAC and leads to poor survival. The molecular events leading to PC are not known. YAP1 oncogene has emerged in many tumor types but its expression and function in PC are unknown. We sought to determine the role of YAP1 in mediating PC and explore whether it could serve as a therapeutic target.

Methods: Patients' derived PC cells and patients' derived xenograph (PDX) and orthtopic (PDO) models were used to study the function of YAP1 in PC *in vitro* and *in vivo*. Immunofluorecenct and IHC staining as well as western blot were used to determine the expression of interested genes. YAP1 knockout in PC cells were generated using LentiCrisp/Cas9 system. RNA Seq and Single cell RNA Seq (sc-RNA Seq) were performed in PC samples to validate the tumor cell heterogeneity and association between YAP1 and CSCs.

Results: YAP1 is overexpressed in human PC cells. sc-RNA Seq revealed human PC cells are highly heterogeneous that further confirmed by dual immunofluorescent staining of YAP1 and EpCAM. Further analyses by RNA Seq and PDX models showed that YAP1^{high} PC cells have an aggressive phenotype, CSC-like properties, and easily formed tumors, and PC in mice irrespective of EpCAM expression or tumor purity. Genetic or pharmacologic inhibition of YAP1 reduced tumor growth in PDX model and PC metastasis in an orthotopic model of human-derived PC.

Conclusion: YAP1 is highly expressed and promotes peritoneal carcinoma formation/progression that can be reversed by pharmacologic and genetic manipulations. Thus, YAP1 could be a valid target in gastric cancer patients with peritoneal carcinomatosis.

Key words: Hippo/YAP1, Gastric Adenocarcinoma, peritoneal metastasis, PDX, CSCs, RNA-Seq

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Development and Characterization of patients' derived Gastric Cancer Cell Lines and Orthotopic Mouse Model from Ascites for Preclinical Research

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Background: Gastric adenocarcinoma (GAC) is a major health problem in the US and globally. GAC with peritoneal carcinomatosis (PC) is common affecting ~45% of the population during the course of GAC and the survival for patients with PC is less than 9 months. Little, in terms of molecular biology, is known about the cells that populate the peritoneal cavity. There is an urgent need to develop metastatic GC cell lines and animal models for uncovering molecular mechanism and preclinical model for PC study. Here, we sought to develop and characterize patients' derived GAC cell lines and orthotopic mouse model from ascites cells.

Methods: The malignant ascites cells from the patients with gastric cancer were cultured and passaged for 5~6 weeks. We immortalized them using hTERT and SV-40 Large T antigen for generation of cell lines. The cells were then transduced with lentivirus expressing CRB-mCherry-Luciferase. The tumorigenicity of these cells was investigated through subcutaneous injection into NOD-SCID mice. Using a modified surgery technique, 1×10^5 mCherry-luciferase tagged cells in matrigel (30 µl) were injected into the serous side of the stomach of NOD-SCID mice for generation of patients' derived orthotopic (PDO) model. Tumor growth and metastasis were monitored by *in vivo* bioluminescence imaging. G-band analysis and RNA-sequencing were performed to investigate the genetic and RNA expression profile of primary ascites cells and ascites from mouse model. Expression of oncogenic proteins and cancer stem cell markers by western blot and flow cytometry and compared to that are commercial available gastric cancer cell lines (MKN45 and SNU1).

Results: We established three cell lines (i.e., GA051816, GA080417, and GA100218) directly from patient-derived ascites cells and characterized them *in vitro*. The doubling times of the cell lines are 22, 39, and 33 hours for GA51816, GA80417, and GA100218, respectively. The expression of oncogenic proteins, including EGFR, HER2, and Myc, and cancer stem cell markers, including CD44 and Aldh1A1, are quite different among these cell lines as well as with other commercially available ones (e.g., Snu-1, and MKN45). Although all the ascites cell lines are able to generate PDX tumors in SCID mice by subcutaneous inoculation, only GA51816 cells are capable to have quick peritoneal metastases (<two weeks) after intragastric injection of 0.1 million cells using our improved orthotopic mouse model. The morphology of ascites from mice with intragastric injection of GA51816 cells are quite similar to that from patients' ascites. The ascites cells from both the mouse model and patients share similar gene expression profiles through RNA-seq analysis and IHC staining of cell blocks from both mouse model and patients.

Conclusion: We have successfully established three patient-derived ascites cell lines and developed an improved orthotopic metastatic mouse model (GA51816) that is highly metastatic to peritoneum. These novel ascites cell lines and orthotopic metastatic mouse model are great resources for investigating the mechanisms of PC and for our preclinical testing for novel targets and immune therapies.

Exploring GLP2 activity and cellular targets in human intestinal enteroids and organoids

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Background: Intestinal failure remains a clinically significant problem, incurring over $500,000 in costs per patient in the first year. While parenteral nutrition has decreased morbidity and increased survival, it is insufficient in some. However, therapeutic advancement, such as glucagon-like-peptide-2 (GLP2), may improve outcomes. While GLP2 is known to promote intestinal growth by stimulating epithelial proliferation and inhibiting enterocyte apoptosis, the exact mechanism of GLP2 is unknown. GLP2-receptor (GLP2R) has been reported in epithelial, mesenchymal, and neuronal cells. HIEs contain only epithelium. HIOs include both epithelial and mesenchymal cells but do not contain neurons. Transplanted HIOs (tHIOs) have a more robust mesenchyme and less fetal-like transcriptome. Therefore, we hypothesized that more *GLP2R* would be present in HIOs vs HIEs and tHIOs vs HIOs and therefore, proliferation (*PCNA*) and size (max diameter) would be greater in HIOs and tHIOs treated with GLP2.

Methods: HIEs and HIOs were generated *in vitro* from endoscopic biopsies or human embryonic stem cells. After 7 or 28 days of growth, the HIEs and HIOs were collected for RNA. In addition, 30-42 day old HIOs were transplanted into the kidney capsule of immunocompromised (NSG) mice. tHIOs were harvested for RNA after 8-10 weeks. RT-qPCR was performed for *GLP2R*. HIEs were grown under proliferative (undifferentiated) or growth- suppressive (differentiated) conditions and treated with GLP2 for 2 days. After 21 days of *in vitro* HIO growth, R-spondin was removed from the media, and GLP2 or vehicle was then given for 3 days (day 25-27). Tissue was harvested and then RT-qPCR was performed for *PCNA* on HIEs, HIOs, and tHIOs, as a measure of proliferation. Additionally, the maximum diameter of the HIOs was measured before and after GLP2 or vehicle treatment and the percent change calculated.

Results: All HIEs demonstrated an absence of *GLP2R* expression. However, tHIOs demonstrated a 41-fold increase in the level of *GLP2R* expression normalized to *GAPDH* (14.13±3.441) when compared to HIOs (0.3419±0.1074), $p<0.0001$ (Figure 1). However, despite high *GLP2R* expression in tHIOs, the HIEs, HIOs, and tHIOs treated with GLP2 all demonstrated no significant difference in proliferation as measured by *PCNA* expression versus control (Figure 2), nor was there a difference in HIO nor tHIO maximum diameter % change between GLP2 treatment and control (Figure 3).

Conclusion: *GLP2R* is present in greatest quantity in tHIOs, followed by HIOs, and is undetectable in HIEs. We suspect that this is due to the increased maturity of the mesenchyme in tHIOs versus HIOs, whereas HIEs have no mesenchyme. Although HIOs and tHIOs demonstrated more *GLP2R* than HIEs, there was no difference in proliferation (*PCNA*) after GLP2 treatment. We suspect that HIOs and tHIOs with an incorporated enteric nervous system (enteric neural crest cells) may demonstrate increased proliferation with GLP2 treatment, but these studies are currently ongoing. A better understanding of the mechanism of action of GLP2 will help optimize its use as a treatment for intestinal failure.

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Development of new mouse models for lysinuric protein intolerance to investigate disease mechanisms and potential therapies

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Introduction: Lysinuric protein intolerance (LPI) is a severe inborn error of metabolism, characterized by osteoporosis, growth failure, urea cycle dysfunction, immune dysfunction and renal disease. LPI is caused by biallelic pathogenic variants in *SLC7A7*, which encodes for the light subunit of the y^+LAT1 transporter, essential for intestinal absorption and renal reabsorption of arginine, lysine and ornithine (cationic amino acids). Perinatal lethality of the only *Slc7a7*$^{-/-}$ mouse model has hindered *in vivo* studies investigating the mechanisms of LPI phenotypes.

Objective: To develop new mouse models of LPI to investigate disease mechanisms of LPI.

Methods: Using CRISPR technology, we developed a *Slc7a7*$^{-/-}$ mouse model on the 129/C57Bl/6J F2 background, which has a deletion of exons 3 and 4 resulting in a premature frameshift mutation. Male and female *Slc7a7*$^{-/-}$ and wild type (WT) mice were dissected at 14-18 days of age. To assess the biochemical phenotype of LPI, plasma and urine concentrations of the cationic amino acids were measured. To evaluate renal architecture, hematoxylin and eosin (H&E) and electron microscopy staining were performed. Micro computed tomography was performed on the spines and femora to estimate bone mass. We also generated a conditional *Slc7a7* knockout mouse model (C57BL/6 background) using embryonic stems cells harboring the conditional knockout allele with exons 3 and 4 of *Slc7a7* flanked by loxP sites. For the global conditional knockout mouse model of LPI, we generated male and female *Slc7a7*$^{fl/fl}$ and tamoxifen-inducible *Gt(ROSA)26Sor* (ERT2) Cre+; *Slc7a7*$^{fl/fl}$ mice and treated them with 75 mg/kg tamoxifen or corn oil via oral gavage for 5 days at 8-9 weeks of age.

Results: Consistent with the biochemical LPI phenotype, *Slc7a7*$^{-/-}$ mice showed increased plasma concentrations and reduced urinary concentrations of the cationic amino acids compared to WT littermates. Consistent with the human LPI disorder, *Slc7a7*$^{-/-}$ mice demonstrated growth failure and reduced survival. H&E stained renal sections from *Slc7a7*$^{-/-}$ mice demonstrated overall loss of the renal cortex, vacuolation and loss of brush border. Electron microscopy of the renal cortex revealed increased lipid vacuolation and secondary lysosomes in *Slc7a7*$^{-/-}$ mice. The histological alterations in the renal cortex and the generalized aminoaciduria observed in *Slc7a7*$^{-/-}$ mice are suggestive of proximal tubular dysfunction.
Slc7a7$^{-/-}$ mice demonstrated reduced bone mass in the L_4 vertebrae and femora. *ERT2* Cre$^+$; *Slc7a7* $^{fl/fl}$ mice treated with tamoxifen lost 11-16% of body weight 5 days post-treatment and showed reduced RNA expression of *Slc7a7* in the proximal small intestines compared to *Slc7a7*$^{fl/fl}$ mice, thereby confirming *Slc7a7* deletion.

Conclusions: The *Slc7a7*$^{-/-}$ mouse model demonstrated various phenotypes that are consistent with the human LPI disorder. We have developed an adult global conditional knockout mouse model of LPI to investigate potential therapies, such as dietary protein restriction and L-citrulline supplementation.

Using artificial intelligence in diagnosis of esophageal squamous cell neoplasia: a post-hoc study of high-resolution microendoscopy (HRME) image interpretation

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Background: High-resolution microendoscopy (HRME) is a low-cost, portable device producing subcellular epithelial images to detect esophageal squamous cell neoplasia (ESCN). A limitation of any microendoscopic technology in underserved settings with high rates of ESCN (e.g., China, Iran, South Africa) is the need for expert interpretation. We developed a fully automated HRME image analysis software to distinguish neoplastic from benign mucosa. This study aims to evaluate the accuracy and confidence of expert and novice endoscopists in HRME image interpretation with and without input from an automated software to detect ESCN.

Methods: High quality HRME images from 130 consecutive patients in the US and China undergoing ESCN screening and surveillance were used. All HRME images were interpreted by the software as neoplastic or non-neoplastic using an abnormal nuclear density cut-off of 218. All endoscopists (6 experts, 7 novices) underwent standardized training in HRME image interpretation and were tested on 199 HRME images. They interpreted the image as neoplastic or non-neoplastic (pre-software read) and reported the confidence level of their interpretation. All endoscopists were then given the automated software interpretation and again asked their interpretation (post-software read) and confidence level. The endoscopists were aware of the software performance throughout the testing (sensitivity 73%, specificity 80%). All HRME imaging sites were biopsied and consensus histopathology was reached by two expert GI pathologists blinded to the HRME results. Diagnostic accuracy of pre- and post-software reads were calculated and mean difference between the two reads was compared using paired t-test.

Results: Overall, the endoscopists had a pre-software read sensitivity of 84.3% (95% CI 79.5%-89.1%), specificity 75.0% (95% CI 71.9%-78.1%), and accuracy 81.1% (95% CI 78.0%-84.2%). On the post-software read, the endoscopists had a sensitivity of 84.8% (95% CI 82.1%-87.5%; change from pre- to post-software read p=0.75), specificity 80.1% (95% CI 77.6%-82.6%; p=0.002), and accuracy of 83.1% (95% CI 81.9%-84.4%; p=0.13). There was **no significant difference** in pre-and post-software read among experts, **but a significant increase in specificity was seen among novices**. When endoscopists had high confidence, there was no significant change in sensitivity and specificity pre- and post-software. Whereas with low confidence, the specificity increased from 58.0% to 71.0% (p=0.004) without significant change in sensitivity.

Conclusion: We found that integrating an automated software image analysis increased the specificity of novice endoscopists in detecting ESCN. Given the high sensitivity but low specificity of Lugol's iodine screening for ESCN, our study suggests that software-assisted microendoscopy may be useful for screening in underserved settings.

Missed opportunities for screening or surveillance among patients with newly diagnosed non-cardia gastric adenocarcinoma

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Background: There are known risk factors for non-cardia gastric adenocarcinoma, and endoscopic surveillance of preneoplastic lesions has been associated with the early detection of gastric adenocarcinoma. We determined possible missed opportunities for the detection and subsequent surveillance of preneoplastic conditions in a cohort of patients with gastric adenocarcinoma.

Methods: We conducted a retrospective cohort study among consecutive, newly diagnosed patients with non-cardia gastric adenocarcinoma from 11/2007 to 10/2018 at the Michael E. DeBakey VA Medical Center in Houston, Texas. We performed structured medical record review for gastric adenocarcinoma risk factors (non-White race [i.e., Black, Hispanic, Asian], smoking, alcohol, *Helicobacter pylori* infection, gastric ulcers, family history of gastric cancer), past endoscopic and gastric biopsy history and histopathological findings. We evaluated the indications for the gastric adenocarcinoma diagnosing endoscopy (diagnostic, surveillance, incidentally found) and identified the proportions of all patients with missed opportunities for screening and surveillance based on risk factors and presence of preneoplastic lesions. Associations between receipt of prior endoscopy and cancer-related outcomes (cancer stage, receipt of treatment, survival) was determined using logistic regression models.

Results: Among 91 patients diagnosed with gastric adenocarcinoma, 87 (95.6%) were men and 29 (31.9%) were White, with mean age at diagnosis of 68.0 years (SD 10.8). The cancer diagnosing endoscopy was done for diagnostic indications in 89.0%, surveillance of preneoplastic gastric lesions in 2.2%, and cancers were found incidentally in 8.8%. Dyspepsia (29.6%), iron deficiency anemia (27.2%) and gastrointestinal bleeding (27.2%) were the most common diagnostic indications. Most patients had at least one risk factor for gastric cancer (N=79, 86.8%), and 42 (46.2%) had 2 or more risk factors. The most common risk factors included smoking (76.9%), non-White race (67.0%) and alcohol use (59.3%). Twenty patients (22.0%) had at least 1 endoscopy performed at a median 2.4 years prior to gastric cancer diagnosis. Of 14 patients who had previous gastric biopsies, 7 had high risk lesions (6 intestinal metaplasia; additional 1 gastric ulcer) but only 2 underwent surveillance endoscopy with gastric biopsies. Receipt of prior endoscopy was not associated with significant differences in cancer stage, receipt of treatment, or survival.

Conclusion: Most patients with gastric adenocarcinoma had at least 1 known risk factor but never had prior screening/surveillance endoscopy and therefore could represent missed opportunity for prevention or early detection. Among the few with known prior preneoplastic lesions, endoscopic surveillance with gastric biopsies was not consistently performed, representing another missed opportunity.

Bile-acid treatment of human intestinal enteroids leads to ceramide production that enhances GII.3 human norovirus infection

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Human noroviruses (HuNoVs) are a major cause of viral gastroenteritis worldwide. Understanding mechanisms of HuNoV replication remained has been limited due to the prior lack of robust in vitro cultivation systems. We have overcome this limitation by demonstrating that stem cell-derived human intestinal enteroid (HIE) cultures support replication of multiple HuNoV strains. Unexpectedly, replication of some HuNoV strains (e.g., GII.3) depends on the presence of bile. The active component(s) in bile that enhances GII.3 replication are heat- and trypsin-resistant non-proteinaceous molecule(s). We hypothesized that bile acids (BAs), one of the major components of bile, are involved in GII.3 replication in HIEs.

To evaluate whether BAs affect GII.3 replication, we added unconjugated-, and conjugated-BAs individually to the culture medium during GII.3 infection. Hydrophobic BAs that were non-cytotoxic at a concentration of 500 μM enhanced replication; a positive correlation was observed between the fold-increase of virus replication and BA hydrophobicity. To determine the mechanism of BA-mediated GII.3 infection, we tested known bile acid pathways and functions. Agonists of the farnesoid X receptor (FXR), which regulates BA homeostasis, did not support GII.3 virus replication. Hydrophobic BAs can induce endosomal acidification leading to activation of the acid sphingomyelinase (ASM). We found that inhibitors of endosomal acidification and ASM significantly suppressed GII.3 replication. ASM converts sphingomyelin in cellular membranes to ceramide. Bypassing ASM activation by adding soluble ceramide exogenously showed GII.3 could replicate in the absence of BA. Treatment of cells with both BA and ceramide synergistically enhanced virus replication. Increased cell surface levels of ceramide are detected using a ceramide-specific antibody within 10 minutes after treatment BA-treatment. Hydrophobic BAs are critical for GII.3 replication and the mechanism of action is through activation of endosomal ASM leading to formation of ceramide which is important for infection.

Prior diagnosis of Barrett's esophagus in patients with esophageal adenocarcinoma is infrequent, but associated with improved survival

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Background: The current approach to reducing mortality from esophageal adenocarcinoma (EA) relies on upper endoscopy to identify Barrett's esophagus (BE), followed by endoscopic surveillance of BE to identify patients with neoplastic progression before invasive EA occurs. We sought to determine the frequency of prior diagnosis of BE in patients with EA, and to evaluate the impact of a prior BE diagnosis on EA stage, receipt of treatment, and survival.

Methods: A retrospective cohort study of all patients diagnosed with EA (defined by ICD-O-3 primary site code C15 in combination with ICD-O-3 histology codes M8140-M8575) in the VA Central Cancer Registry during 2002-2016. We identified EA patients with a prior BE diagnosis (defined by ICD-9 code 530.85 in combination with an endoscopy within 1 year before or after the date of the first BE code) >6 months prior to their EA diagnosis date as far back as October 1999. BE index date was the date of the endoscopy. Mortality data were obtained from the VA Vital Status files; cancer-specific deaths were defined as those with underlying cause of death ICD codes 150.0-151.9. We compared the distributions of EA stage and receipt of treatment between EA patients with and without a prior BE diagnosis. Cox proportional hazards regression models compared mortality risk (all-cause and cancer-specific) and included age, race, BMI, alcohol use, tobacco use, and total primary care and GI visits during follow-up. We additionally adjusted for EA stage, grade and treatment to assess their impact on any survival differences between patients with and without a prior BE diagnosis.

Results: Among 9737 patients with EA, only 445 (4.57%) had a prior diagnosis of BE. Of those with >5 years of administrative data available prior to EA diagnosis, the proportion with a prior diagnosis of BE increased from 3.55% in EA patients diagnosed during 2005-2007 to 7.24% in EA patients diagnosed during 2014-2016. Patients with a prior BE diagnosis were more likely to be diagnosed with earlier stage tumors (65.6% vs. 30.2% had stage 1 or 2 disease; $p<0.001$), and were more likely to undergo surgery (56.9% vs. 26.5%; $p<0.001$). A prior BE diagnosis was associated with a decreased risk of all-cause mortality (hazard ratio [HR] unadjusted for stage=0.66, 95% CI 0.58-0.75), which was largely explained by the earlier stage of EA at the time of diagnosis (HR adjusted for stage=0.97, 95% CI 0.80-1.19). There was no association with all-cause mortality in analyses stratified by EA stage (stage 1, HR=0.99, 95% CI 0.79-1.25; Stage 2, HR=0.94, 95% CI 0.69-1.26; Stage 3-4, HR=0.96, 95% CI 0.74-1.25). Similar results were observed for cancer-specific mortality.

Conclusions: The proportion of EA patients with a prior diagnosis of BE remains low. However, prior diagnosis of BE is associated with better survival, largely due to earlier stage at EA diagnosis.

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Using natural language processing to accurately identify dysplasia in pathology reports for patients with Barrett's esophagus

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Objectives: We aimed to develop a natural language processing (NLP) algorithm to identify esophageal dysplasia in patients with Barrett's esophagus (BE) on histopathology reports with varying report formats in a large integrated electronic medical records (EMR) system.

Background: Identifying dysplasia status for the purposes of research on BE and esophageal adenocarcinoma in electronic data repositories often requires manual data abstraction. NLP is a branch of computer science and linguistics that creates structure to unstructured free text. This automated method can reliably analyze large amounts of data, and has been successfully employed in identifying key clinical information from EMR; however, not in the context of BE.

Methods: We randomly selected 600 patients with suspected BE from the national VA administrative databases. BE diagnosis was ascertained by the presence of ICD-9-CM code 530.85, combined with at least one EGD test (CPT codes 43200-43259, excluding 43246) within 12 months of the initial BE diagnosis date. The presence of BE (intestinal metaplasia) and presence and grade of dysplasia was verified/classified by manual review of the pathology report coinciding with the EGD code. NLP software (CLAMP) was used to develop an algorithm to identify dysplasia. Using findings from manual review as the reference standard, algorithm performance characteristics were calculated as recall (also known as sensitivity), precision (also known as positive predictive value), accuracy (proportion of tests that are either true positive or true negative), and F-measure (harmonic mean of precision and recall) of identifying dysplasia.

Results: Among the 600 patients, 561 had a pathology report available. On manual review, 104 patients (18.5%) were found to not have BE on pathology. Among the 457 patients with confirmed BE, 400 (87.5%) had no mention of dysplasia or were described as having non-dysplasia Barrett's esophagus (NDBE), 34 (7.4%) had low-grade dysplasia, 16 (3.5%) were indefinite for dysplasia and 7 (1.5%) had high-grade dysplasia (HGD). The NLP algorithm was highly accurate in identifying dysplasia from the 561 pathology reports. Compared with manual review, the NLP algorithm could identify dysplasia with 97.1% accuracy, 77.2% recall (44 of 57) and 93.6% precision (44 of 47), with an F-measure of 84.6%. Among the 7 patients with HGD, all were classified by the algorithm as having dysplasia. Only 3 of 504 patients (0.6%) with no BE, with no mention of dysplasia, or with NDBE on the pathology report were incorrectly classified by the algorithm as having dysplasia.

Conclusion: NLP yielded a high degree of accuracy for identifying dysplasia from diverse types of pathology reports for patients with BE. This algorithm can be applied and would facilitate research and possible clinical care in EMR system with text reports in large data repositories.

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Role of Donor Human Milk and Probiotics in Prevention of Necrotizing Enterocolitis and Shaping the Gut Microbiome in Preterm Piglets

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Background: Necrotizing Enterocolitis (NEC) is a devastating inflammatory disease in preterm infants. Human breast milk protects infants against NEC, and probiotic bacteria such as *Bifidobacterium longum* subsp. *infantis* (*B. infantis*) are now administered with formula to reduce NEC risk. The human milk oligosaccharides (HMO) found in breast milk serve as prebiotic substrates for the growth of *Bifidobacterium* sp., especially *B. infantis*. However, formula lacks HMO, limiting its efficacy in supporting colonization by probiotic *B. infantis*.
Objective: Determine if supplementation with *B. infantis* in the presence or absence of a specific HMO can prevent NEC in formula-fed preterm pigs and influence the gut microbiome compared to pasteurized donor human milk (DHM).

Design/Methods: Preterm pigs (N=10 group) were delivered by cesarean section and given total parenteral nutrition plus orogastric feeds of either commercial preterm infant formula (PF), formula with *B. infantis* (10^9 CFU/d) (BI), formula with HMO (1.2 g 3'-sialyllactose/ kg) (HMO), formula with both *B. infantis* and HMO (BI-HMO) or pasteurized DHM for 7 days after birth. Piglets were monitored for clinical NEC symptoms and rectal swabs taken to evaluate fecal microbiome composition using 16S rRNA gene sequencing. Gut tissues were scored for histopathology and gut contents were sequenced to assess differences between the intestinal and fecal microbiota.

Results: The NEC incidence was 90, 72, 70, 60, and 33% for PF, BI, HMO, BI-HMO and DHM, respectively ($p<0.05$). Overall, gross NEC severity scores were higher in the ileum and colon than jejunum. The PF group had the highest NEC severity scores while DHM had the lowest ($p<0.05$). Colonic histological NEC scores were also highest in PF and lowest in DHM. Villus length was higher in DHM vs PF in proximal jejunum, but not different in distal ileum. Dietary treatments led to global changes in microbiome structure (PERMANOVA of weighted UniFrac distance, $p<0.001$). Rectal swabs generally recapitulated the microbiome of intestinal tissues, though some genera, such as *Lactobacillus*, were primarily identified in gut contents. Treatment with *B. infantis* did not reduce NEC incidence; however, the genus *Bifidobacterium* was enriched in rectal samples from piglets with no, mild or moderate NEC compared to severe NEC. BI treatment successfully enriched *Bifidobacterium* while depleting the NEC-implicated genus *Clostridium*; however, these changes did not occur in the BI-HMO group. Interestingly, *Bifidobacterium* was also enriched in rectal samples from piglets given DHM compared to PF, in the absence of *B. infantis* treatment.

Conclusion: As in human infants, our results in preterm piglets confirm the protective effect of pasteurized donor human milk in reducing NEC incidence and severity compared to infant formula. Additionally, treatment with *B. infantis* may have beneficial effects on the microbiome of preterm pigs. However, *B. infantis* and the HMO 3-SL did not reduce NEC incidence. Our results suggest that while 3-SL failed to ameliorate NEC or support *Bifidobacterium* colonization in a preterm piglet model, other factors from human milk may facilitate both of these goals. DHM combined with *B. infantis* treatment could offer a promising option to further reduce the risk of NEC.

Streptococcus gallolyticus adopts a novel mechanism to promote tumor growth – involvement of transforming growth factor β and the extracellular matrix

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Streptococcus gallolyticus subsp. *gallolyticus* (*Sgg*) is a gram-positive opportunistic pathogen that causes bacteremia and endocarditis. It is also known to have a strong clinical association with colorectal cancer (CRC) as shown in epidemiological studies over the past several decades. Patients with bacteremia/endocarditis due to *Sgg* have a much higher risk of having CRC compared to the general population. The role of *Sgg* in the development of CRC was unknown until recently. Studies from our group and others using independent mouse models of CRC demonstrated that *Sgg* actively promotes the development of CRC. However, the mechanism underlying the tumor-promoting activity of *Sgg* is poorly understood.

We found that *Sgg* stimulates the proliferation of CRC cells. Mass spectrometry proteomics analysis was performed to investigate changes in CRC cells following exposure to *Sgg*. The results showed that several types of collagen were upregulated by *Sgg*, with type VI collagen (ColVI) having the highest relative abundance. We confirmed that the level of ColVI was elevated in CRC cells co-cultured with *Sgg* and in colon tissues of mice treated with *Sgg*. To understand the functional relevance of upregulation of collagen by *Sgg*, we generated stable ColVI knockdown CRC cells. Knockdown of ColVI abolished the ability of *Sgg* to stimulate cell proliferation. We further showed that *Sgg* upregulation of ColVI was mediated by *Sgg* activation of TGFβ. Inhibitors of TGFβ ligands or receptors abolished the ability of *Sgg* to stimulate CRC cell proliferation.

The ECM regulates fundamental cell behaviors such as cell proliferation. TGFβ is also able to enhance the proliferation of cancer cells. To further understand the contribution of activation of TGFβ and upregulation of collagen to *Sgg*-mediated CRC cell proliferation, we investigated the activity of decellularized matrix (dc-matrix) prepared from CRC cells co-cultured with *Sgg* or control bacteria and in the presence or absence of an inhibitor of TGFβ signaling. The results suggest that the ECM is the downstream mediator of TGFβ in the context of *Sgg* stimulation of CRC cell proliferation. Thus, our results suggest a model in which *Sgg* activates TGFβ, which leads to upregulation of ECM molecules such as collagen. Increased collagen deposition in the matrix then upregulates the proliferation of CRC cells. This is a novel mechanism by which bacteria utilize to stimulate CRC cell proliferation.

Intergenerational effect of inorganic arsenic on liver glucose metabolism

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Inorganic arsenic (iAs) is a widespread environmental toxin. Its effect on metabolic disorders such as diabetes has started to be recognized. Little is known about its potential effect on offspring's metabolism. Here, we use mouse models to explore the effect of iAs on glucose metabolism across generations (F1). Adult C57BL/6 mice exposed to iAs (0.25 ppm) or vehicle in drinking water for 8 weeks were bred with non-exposed females to generate F1-iAs and F1-control mice, respectively. F1-iAs females showed glucose intolerance while F1-iAs males did not, when compared with F1-control females and males, respectively. F1-iAs females displayed impaired pyruvate tolerance while F1-iAs males displayed normal pyruvate tolerance. These results indicate that the endogenous glucose production contributed to glucose intolerance in F1-iAs females. Liver is the major organ for endogenous glucose production. RNA-seq identified that glucose-6-phosphotase catalytic subunit (G6pc) was up-regulated in female F1-iAs livers compared to F1-control. Foxo1 and Smad3, key regulators of G6pc expression, showed reduced and increased phosphorylation, respectively, in F1-iAs female liver compared to F1-control. Inhibin beta A (Inhba), an upstream regulator of the Foxo/Smad signaling, showed altered gene expression in F1-iAs female liver and altered DNA methylation patterns in the sperms of F0-iAs males. These findings delineate an epigenetic mechanism for Intergenerational and sex-specific effect of iAs on glucose metabolism.

Spontaneously hypertensive rats is susceptible to bacteria translocation from gut to peripheral tissues

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Cerebral small vessel disease (CSVD) is an inflammatory disease characterized by blood-brain barrier (BBB) breakdown and neuroinflammation. We previous observed that spontaneously hypertensive stroke-prone rats (SHRSPs) exhibit gut dysbiosis. Furthermore, transplanting SHRSPs with control gut microbiota significantly reduced makers associated with inflammation. Since the gut microbiome is a potential source of inflammation, we hypothesize that bacteria translocating from gut to brain induce neuroinflammation in CSVD. To test our hypothesis, we determined the efficiency of fluorescent-labeled bacteria (GFP-*E. coli* JJ1901, chloramphenicol resistant) translocation in WKY and SHRSP rats. A single dose of *E. coli* JJ1901 [≈10^9 colony forming units (CFUs)] was administered to WKY and SHRSP rats (11-15 weeks old) through oral gavage. Peripheral tissues, brain, and cecum content were collected 2 hours later, homogenized, and plated on culture plates. The number of CFUs was counted 24 hours later. When compared to WKY rats, SHRSP showed significantly increased bacteria translocation to peripheral tissues (liver, spleen, and abdominal adipose tissue; n=7, $p<0.05$). In addition, more bacteria translocation incidents were found in SHRSPs compared to WKY rats (5/7 in SHRSP vs. 3/7 in WKY). However, similar level of acute bacteria translocation to brain was observed in both groups. To assess translocation rates of the chronically colonized bacteria, as opposed to the acute effects following gavage, colonization was established in newborn rat pups by administrating *E. coli* JJ1901 (≈10^9 CFUs) to pregnant mother rats through oral gavage. Bacteria colonization in the offspring of the gavaged rats was confirmed by plating diluted fecal samples on culture plates. Colonized SHRSPs (15 weeks old) showed significantly increased bacteria translocation to peripheral tissues when compared to WKY controls. Additionally, SHRSPs demonstrated a trend towards increased translocation of *E. coli* JJ1901 to the brain than WKY rats. We conclude that translocation of gut bacteria can be an underlying source responsible for inflammation in pathological states.

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Infection and Injury as a Precursor to GI & Liver Cancer

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Texas Medical Center Digestive Diseases Center
10th Annual Frontiers in Digestive Diseases Symposium:
Infection and Injury as a Precursor to GI & Liver Cancer

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