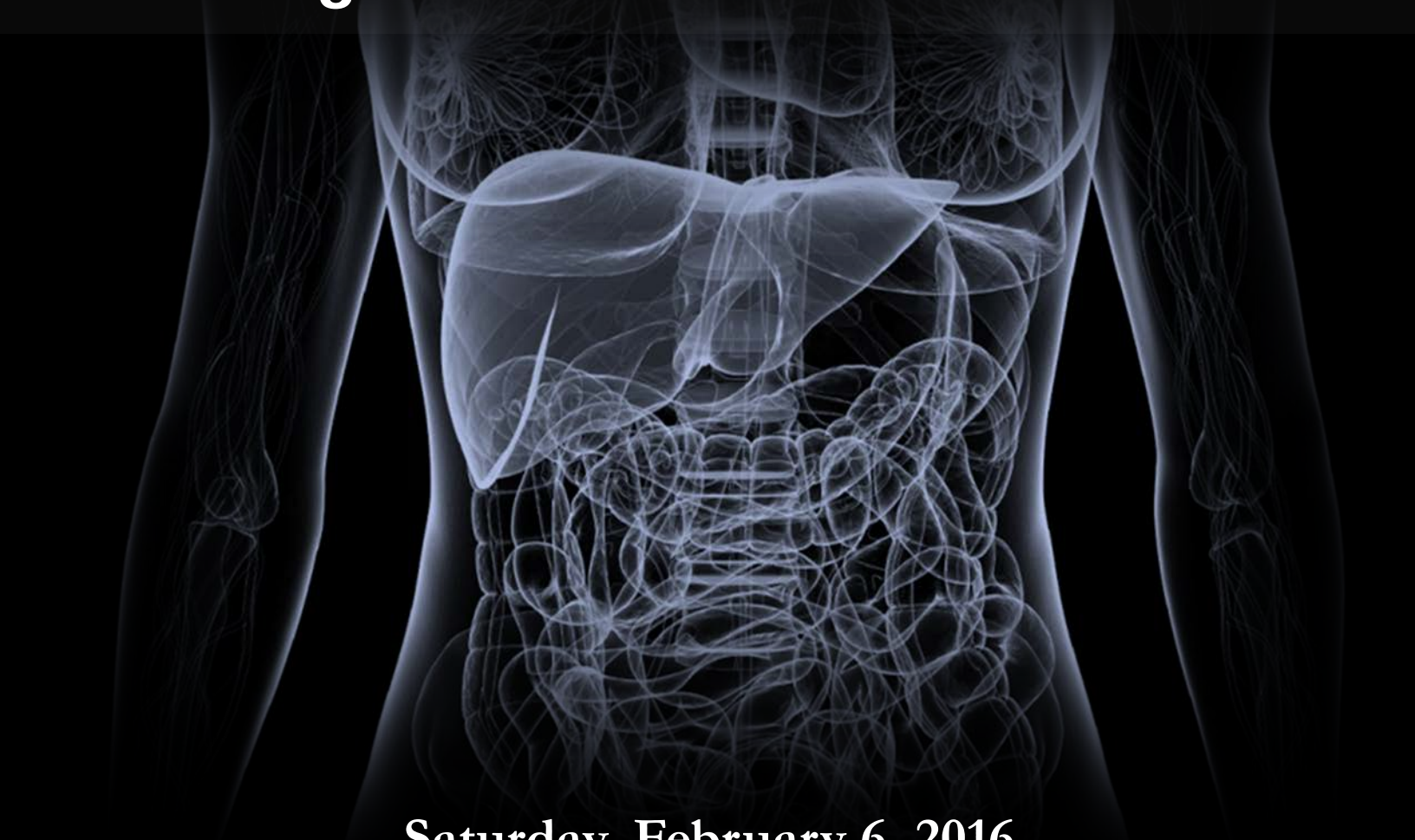
Texas Medical Center Digestive Diseases Center (TMC DDC)

7th Annual Frontiers in Digestive Diseases Symposium

"Frontiers in Regenerative Medicine in Digestive and Liver Disease"



Saturday, February 6, 2016

Onstead Auditorium
6767 Bertner Avenue
Houston, Texas

Texas Medical Center Digestive Diseases Center
7th Annual Frontiers in Digestive Diseases Symposium:
Frontiers in Regenerative Medicine in Digestive and Liver Diseases

Saturday, February 6, 2016
Onstead Auditorium, Houston, Texas 77030

Table of Contents 1

Agenda 2

CME Activity 3

List of Abstracts ..... 4 - 6

Abstracts ..... 7 - 64

TMC DDC Leadership 65

List of Participants ..... 66 - 70

Acknowledgements ..... Back Cover

The TMC DDC is a federally funded center (NIH P30DK58338) 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 University of Texas M.D. Anderson Cancer Center. The TMC DDC is one of only 18 Digestive Diseases Research Core Centers in the country and the only center in the southeast United States.

The TMC DDC supports three basic science cores: Cellular and Molecular Morphology, Functional Genomics and Microbiome, Integrative Biology; and one clinical core: Study Design and Clinical Research. For more information, visit https://www.bcm.edu/research/centers/digestive-disease.

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Frontiers in Regenerative Medicine in Digestive and Liver Diseases

A G E N D A

7:45-8:30 AM	**Breakfast**
8:30 AM – 8:40 AM	**Welcome Remarks** HASHEM EL-SERAG, M.D., M.P.H. Director, Texas Medical Center Digestive Diseases Center
SESSION I	Moderator: SUNDARARAJAH THEVANANTHER, PH.D.
8:40-9:30 AM	**"Regenerative Medicine for Liver Diseases"** MARKUS GROMPE, M.D., PH.D. Director of the Papé Family Pediatric Research Institute, Oregon Stem Cell Center
9:30-10:05 AM	**"Gender-specific responses of p53 during liver regeneration"** MICHELLE BARTON, PH.D. Professor, The University of Texas MD Anderson Cancer Center
10:05- 10:25 AM	**"Balancing Self-renewal & Genome Preservation in Liver Regeneration and Cancer"** ROBERT TSAI, M.D., PH.D. Associate Professor, Texas A&M Health Science Center
10:25-10:35 AM	**Coffee break**
SESSION II	Moderator: NOAH SHROYER, PH.D.
10:35-11:10 AM	**"Modeling the human intestine"** MICHAEL HELMRATH, M.D. Surgical Director, Intestinal Rehabilitation Program, Cincinnati Children's Hospital
11:10-11:30 PM	**"An emerging role for epithelial cell extrusion in pancreatic regeneration and early neoplasia"** JENNIFER BAILEY, PH.D. Assistant Professor, The University of Texas Health Science Center
11:30-11:50 PM	**"Sounding the Alarm: IL-33 mediated inflammation and intestinal tumorigenesis"** JASON HEANEY, PH.D. Assistant Professor, Baylor College of Medicine
11:50-12:25 PM	**"Wnt/Beta-catenin Signaling in Liver Regeneration"** PAUL MONGA, M.D. Professor of Pathology, University of Pittsburgh School of Medicine
12:30-2:00 PM	**Lunch / Poster Session**
2:00-2:10 PM	**Poster Awards and Concluding Remarks**

Texas Medical Center Digestive Diseases Center
7th Annual Frontiers in Digestive Diseases Symposium:
Frontiers in Regenerative Medicine in Digestive and Liver Diseases

Texas Children's Hospital®

APPROVED CME ACTIVITY
Hosted by Texas Medical Center Digestive Disease Center
Sponsored by Texas Children's Hospital
Saturday, February 6, 2016 | 8:00 am – 2:00pm | Onstead Auditorium

MARKUS GROMPE, MD
Oregon Stem Cell Center

MICHELLE BARTON, PhD
UT MD Anderson Cancer Center

ROBERT TSAI, PhD
Texas A&M Health Science Center

MICHAEL HELMRATH, MD
Cincinnati Children's Hospital

JENNIFER BAILEY, PhD
UT Health

JASON D. HEANEY, PhD
Baylor College of Medicine

SATDARSHAN (PAUL) S. MONGA, MD
University of Pittsburgh

Educational Objectives: At the conclusion of the lecture, the participants should be better able to:
• Define the availability of state-of-the-art regenerative medicine in the field of liver and digestive diseases.
• Apply best clinical practices concerning regenerative medicine for patients dealing with liver and digestive diseases.
• Identify opportunities to apply regenerative medicine in the treatment of liver and digestive diseases and the outcomes.
• Interpret the current regenerative medicine research concerning treatment of liver and digestive diseases.

Target Audience: Physicians – gastroenterology / research in digestive diseases, medical students, residents, fellows, and other healthcare professionals

Accreditation/Credit Designation:
Texas Children's Hospital is accredited by the Texas Medical Association it to provide continuing medical education for physicians.

Texas Children's Hospital designates this live activity for a maximum of **3** ***AMA PRA Category 1 Credit™.*** Physicians should claim only the credit commensurate with the extent of their participation in the activity.

Disclosure:
Drs. Grompe, Barton, Tsai, Helmrath, Bailey, Heaney, and Monga 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.

Statement of Commercial Support:
No commercial support was used in this activity.

Texas Medical Center Digestive Diseases Center
7th Annual Frontiers in Digestive Diseases Symposium:
Frontiers in Regenerative Medicine in Digestive and Liver Diseases

LIST OF ABSTRACTS

(Listed in alphabetical order by author.)

Page:	Name and Institution:	Abstract Title:
7.	Jennifer M. Auchtung, PhD Baylor College of Medicine	P-1. Investigating microbial community dynamics and function in fecal minibioreactor arrays
8.	Christopher Ayoub, BA Baylor College of Medicine	P-2. Responding to gut dybiosis: changes in *E. coli* metabolism and virulence as a function of antibiotic resistance
9.	Lori Banks, PhD Baylor College of Medicine	P-3. Neonatal rotavirus infection alters the adult gut microbiome in mice
10.	Douglas Burrin, PhD Baylor College of Medicine	P-4. Enteral obeticholic acid prevents hepatic cholestasis in total parenteral nutrition-fed neonatal pigs
11.	Lee Call, BS Baylor College of Medicine	P-5. Microbial and metabolic signatures of necrotizing enterocolitis in formula-fed piglets
12.	Yanna Cao, MD, MSc University of Texas Health Science Center	P-6. Gremlin Acts Downstream of TGF-β/Smad3 Signaling to Induce Pancreatic Fibrosis
13.	Alexandra L. Chang-Graham, BA Baylor College of Medicine	P-7. Store-operated calcium entry activates calcium-activated chloride channels in rotavirus-infected cells
14.	Min-Shan Chen, GS Baylor College of Medicine	P-8. The role of Gfi1 transcription factor in colorectal cancer progression and metastasis
15.	Guolian Ding, MD, PhD Baylor College of Medicine	P-9. Gender-specific effects of arsenic exposure in hepatic glucose and lipid metabolism
16.	Jessica Donnelly, PhD Baylor College of Medicine	P-10. Modeling *NGLY1* deficiency using human intestinal organoids
17.	Marcin Duleba, PhD University of Texas Health Science Center	P-11. Reconstructing the origin and stages of progression to esophageal adenocarcinoma from patient-matched stem cells
18.	David J. Durgan, PhD Baylor College of Medicine	P-12. Gut dysbiosis is involved in the development of hypertension
19.	Ismail Elbaz-Younes, MD Baylor College of Medicine	P-13. Uptake and circulatory characteristics of the plant-based small RNA MIR2911
20.	Melinda Engevik, PhD Baylor College of Medicine	P-14. Alterations in adherent tumor mucin protein and glycans by probiotic *lactobacillus reuteri* promotes tumor chemotherapy uptake
21.	Tatiana Fofanova, PhD Baylor College of Medicine	P-15. Complex and defined bacteriotherapy can inhibit acute colitis in mice
22.	Chunxu Gao, PhD Baylor College of Medicine	P-16. Histamine-generating *Lactobacillus reuteri* in the intestinal microbiome attenuates inflammation-associated colonic carcinogenesis in a histamine-deficient mouse model
23.	Song Gao, PhD University of Houston	P-17. Recycled locally bioavailable COXIBs for the treatment of inflammation-associated diseases in the colon
24.	Gregory Guthrie, PhD Baylor College of Medicine	P-18. Rifampicin treatment reduces parenteral nutrition associated liver disease in neonatal piglets
25.	Anne Hall, BS, MS Baylor College of Medicine	P-19. Eric2 regulates histamine production machinery via an ion-transport dependent mechanism in *Lactobacillus reuteri*

Page:	Name and Institution:	Abstract Title:
26.	Faith Ihekweazu, MD Baylor College of Medicine	P-20. The natural history of pediatric ulcerative colitis in the era of biologic therapy
27.	Michal Jandzinski, BS University of Texas Health Science Center	P-21. Manipulation of the microbiome improves functional recovery after ischemic stroke
28.	Xiqian Jiang, GS Baylor College of Medicine	P-22. Quantitative real-time monitoring of glutathione
29.	Yanjun Jiang, PhD Baylor College of Medicine	P-23. Enteral obeticholic acid Promotes intestinal growth in total parenteral nutrition-fed neonatal pigs
30.	Li Jiao, MD, PhD Baylor College of Medicine	P-24. Fecal microbiota and risk of colorectal tubular adenoma
31.	Umesh Karandikar, PhD Baylor College of Medicine	P-25. Detection of human norovirus in intestinal biopsies from immunosuppressed transplant patients
32..	Takahiro Kodama, MD, PhD The Methodist Hospital	P-26. Two-step forward genetic screen in mice identifies the Ral pathway as a potential new drug target in hepatocellular carcinoma
33.	Baijun Kou, MD, PhD Baylor College of Medicine	P-27. Unmasking hidden norovirus epitopes to improve diagnostics
34.	Fan Li, MD, PhD M.D. Anderson Cancer Center	P-28. YAP1 mediated CDK4/6 Activation Confers Radiation Resistance in Esophageal Cancer
35.	Yuying Liu, PhD University of Texas Health Science Center	P-29. Impact of oral feeding *Lactobacillus reuteri* DSM17938 on microbial composition of feces and $CD62L^{+}T$ cells in intestinal mucosa of healthy breast-fed mouse pups
36.	Yuan-Hung Lo, MS Baylor College of Medicine	P-30. SPDEF shifts the transcriptional targets of activated β-catenin to enforce tumor quiescence
37.	Berkley Luk, BS Baylor College of Medicine	P-31. Colonization with *Bifidobacterium dentium* alters colonic mucus and serotonin production in a germ-free mouse model
38.	Farida Malik, MD M.D. Anderson Cancer Center	P-32. Fecal Indole Correlates With Loss of Microbiome Diversity in Hematopoietic Stem Cell Transplant (HSCT) Recipients With and Without Intestinal Graft Versus Host Disease (iGVHD)
39.	Janielle P. Maynard, BS Baylor College of Medicine	P-33. P2X3 purinergic receptor overexpression is associated with poor recurrence-free survival in patients with hepatocellular carcinoma
40.	Christina Morra, BS Baylor College of Medicine	P-34. Regulation of histamine production by *Lactobacillus reuteri* by other bacterial metabolites
41.	Kosuke Murakami, PhD Baylor College of Medicine	P-35. Replication of GII.3 human norovirus in human intestinal enteroids
42.	James W. Nelson, BS Baylor College of Medicine	P-36. Alterations in the gut microbiota can elicit hypertension in rats
43.	Antone R. Opekun, PA-C Baylor College of Medicine	P-37. The Effect of Flavonoids Luteolin and Quercetin on Colon Cancer Cells in Vitro
44.	Nina M. Poole, PhD Baylor College of Medicine	P-38. FimH mediates extraintestinal pathogenic *E. coli* invasion and translocation

Page:	Name and Institution:	Abstract Title:
45.	Geoffrey A. Preidis, MD, PhD Baylor College of Medicine	P-39. Mouse models of early-life protein-energy undernutrition reveal striking bile acid dysregulation, coagulopathy, steatosis, intestinal dysmotility, and microbial dysbiosis
46.	Karen Queliza, PhD Texas Children's Hospital	P-40. Granulomatous changes in upper gastrointestinal tract biopsies of pediatric ulcerative colitis patients.
47.	Jason Robinson, PhD Baylor College of Medicine	P-41. Dysregulated arginine metabolism is linked to necrotizing enterocolitis in the premature piglet
48.	Narayan P. Sastri, PhD Baylor College of Medicine	P-42. Characterization of oligomer/conformation specific monoclonal antibodies to the rotavirus nsp4 coiled-coil domain
49.	Kapil Saxena, BA Baylor College of Medicine	P-43. Human intestinal epithelial response to human rotavirus virus infection using enteroids
50.	Laura Schaefer Baylor College of Medicine	P-44. Development of *Lactobacillus reuteri* as a biotherapeutic delivery system
51.	Henry Shiau, MD Baylor College of Medicine	P-45. The unique inflammatory bowel disease phenotype of pediatric primary sclerosing cholangitis
52.	Shumei Song, MD, PhD M.D. Anderson Cancer Center	P-46. PPARδ mediates its oncogenic function in gastric cancer through interaction and activation of Hippo coactivator YAP1
53.	Jennifer K. Spinler, PhD Baylor College of Medicine	P-47. Synbiotic targeting of *Clostridium difficile*
54.	Melissa Suter, PhD Baylor College of Medicine	P-48. Maternal BMI is significantly correlated with endotoxin core IgM antibodies in both maternal and neonatal serum
55.	Shingo Tanaka, MD, PhD Baylor College of Medicine	P-49. Analysis of autophagy related genes in patients with *Helicobacter pylori* infection
56.	Nabihah Tayob, PhD M.D. Anderson Cancer Center	P-50. Improved detection of hepatocellular carcinoma using a longitudinal α-fetoprotein screening algorithm
57.	Takahiro Uotani, PhD Baylor College of Medicine	P-51. *Prostate stem cell antigen* gene polymorphisms as a predictive factor for *H. pylori* infection associated intestinal metaplasia
58.	Xia Wang, PhD University of Houston	P-52. Intestinal stem cells from IBD patients: insights into etiology and therapeutics
59.	Fan Zhang, PhD Baylor College of Medicine	P-53. Natural influence: investigating the impact of microbes on host health using *C. elegans*
60.	Wenli Yang, PhD University of Texas Health Science Center	P-54. The effects of apoptosis inhibition on pulmonary alveolar type 2 epithelial cell alterations in experimental hepatopulmonary syndrome after common bile duct ligation
61.	Mingkun Zhao, GS Baylor College of Medicine	P-55. *In vivo* Gene Delivery to Lymphocytes Using Novel Biodegradable Nanoparticles
62.	Wei Zhao, PhD M.D. Anderson Cancer Center	P-56. Galectin-3 Mediates Tumor-stromal interaction by activating Pancreatic Stellate Cell and promoting Cytokine/Chemokine production
63.	Yong Zhou, PhD University of Texas Health Science Center	P-57. Phospholipid spatiotemporal dynamics mediate bile acid-stimulation of EGFR-dependent signaling and pro-inflammatory response.
64.	Winnie Y. Zou, BS Baylor College of Medicine	P-58. The Plasticity of the Intestinal Stem Cell Compartment and its Induction Following Rotavirus Infection

Investigating microbial community dynamics and function in fecal minibioreactor arrays

Jennifer M. Auchtung, Sarah E. Blutt, Catherine D. Robinson, Mary K. Estes, and Robert A. Britton
Department of Molecular Virology and Microbiology, Baylor College of Medicine

Although it is clear that the microbiome plays an important role in human health, determining factors that contribute to disease can be challenging due to the complexity of host-microbiome interactions. Cultivation of fecal communities in continuous-flow bioreactors is one method that has shown some success in elucidating functions of the gastrointestinal microbiome. However, these experimental setups are often cumbersome and expensive, limiting replicate experiments. We developed small, simple fecal mini-bioreactor arrays that allow for anaerobic, continuous-flow cultivation of up to 96 communities simultaneously. We used these reactors to study *Clostridium difficile*, an important nosocomial pathogen whose persistence is inhibited by a healthy microbiota. We established reproducible conditions where unperturbed communities were resistant to *C. difficile* and disruption promoted invasion. Further, we identified members of the microbiota with potential to inhibit *C. difficile* and are beginning to investigate how these microbes inhibit *C. difficile* in reactor and mouse models of disease. We have also begun investigating how differences between fecal donors and cultivation conditions impact microbial community structure and function and have identified specific communities that promote proliferation and barrier function in small intestinal enteroid cultures. These studies are part of ongoing work to develop new models to investigate interactions between the human GI tract and its commensal microbiome.

Responding to gut dybiosis: changes in *E. coli* metabolism and virulence as a function of antibiotic resistance

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Alkek Center for Metagenomics and Microbiome Research, Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston TX

Animals have evolved intimate symbiotic relationships with a complex consortium of gut microbes (microbiota) that represent a functional extension of the host genome and also exert influence upon host health. When well-tended, the microbiota provides many benefits, like resistance to pathogens and immune boosting, but under imbalanced conditions, like persistent infection and related treatment, some communities (or specific members) can bring about harm. *E. coli*, normally a rare member of the microbiota, is often the cause of the most common bacterial infections in humans, including UTIs, bacteremias, and infectious diarrhea. It can exhibit broad antibiotic resistance (AR) profiles and bloom under these dysbiotic conditions in the microbiome. These 'escapers' of the intestinal environment have become a worldwide scourge. Specifically, the fluoroquinolone resistance-associated clade of extraintestinal pathogenic *E. coli* clonal group (ExPEC ST131-*H*30Rx) is now the most prevalent pathogenic *E. coli* isolate and cause of AR infections in the U.S. Thus, it is of significant global health importance to understand the rise of these pathogens.

We hypothesize that increased fitness within the gut habitat and expanded metabolic capabilities and/or virulence profiles have contributed to the prevalence of ST131 isolates. Utilizing the high-throughput amenable and microbially 'tuned' (both diet and microbiome) nematode, *Caenorhabditis elegans*, we aim to screen thousands of strains in our collections to test this hypothesis. *C elegans* is especially well-suited for these experiments as it is (i) exquisitely sensitive to differences in microbial metabolism and virulence; (ii) easy to make 'germ-free'; (iii) highly conserved in its molecular response pathways; and (iv) structurally and functionally similar in its intestinal environment. Additionally, *Enterobacteriaceae* related to *E. coli* are the most abundant members of the natural *C. elegans* microbiome, and greatly influence its health. As a pilot, we have selected 24 independent clinical isolates of varying AR profiles to feed germ-free worms in liquid culture. By measuring optical density over a time course, we can monitor *C. elegans* growth rates and progeny production in response to the metabolic fitness and virulence of the particular bacterial strain with which it is cocultured. Results have shown *E. coli* strains with greater AR slow *C elegans* development more than those with greater antibiotic sensitivity (AS), suggesting that adaptations enabling AR may indeed have a metabolic impact.

Further, when cultured in a medium that mimics the extraintestinal environment, such as BHI, these *E. coli* strains become more virulent, tending to further delay worm development. 21 of 24 bacterial strains slowed worm development when grown in BHI, increasing the time to laying eggs by an average of 78% relative to worms fed the same strains cultured in LB medium. Excluding the 3 strains that were unaffected, the time to reach adulthood for worms fed BHI-cultured bacteria was increased by anywhere from 23 to 200%, suggesting that different AR adaptations can have varying impacts on virulence. These results demonstrate just how effective the *C. elegans* system will be for the screening of both diverse and related bacterial isolates. Further work includes developing genetic models of host gut dysbiosis by decreasing gut motility and increasing colonization levels in the worms. This will allow us to measure and compare *E. coli* fitness and ability to colonize in a normal gut versus a dysbiotic gut, and will add to our toolbox for dissecting the broad influence of the gut microbiome as well as its specific mechanisms of action.

Neonatal rotavirus infection alters the adult gut microbiome in mice

Lori Banks[1,2], Sarah E. Blutt[1], Joseph M. Hyser[1,2], and Margaret Conner[1]
[1]Department of Molecular Virology and Microbiology, [2]Alkek Center for Microbiome and Metagenomics Research, Baylor College of Medicine, Houston, TX

Rotavirus (RV) is a major cause of acute gastroenteritis in children, leading to severe dehydration and often death. Additionally, repeated bouts of gastroenteritis impair cognitive and physical development for survivors, demonstrating acute diarrhea can have lasting sequela. RV diarrhea involves cross-talk between viral factors, the host cells, and the intestinal microbiome, yet little is known about whether changes to the microbiome during acute diarrhea cause shifts in the microbiome's composition that persist into adulthood. To investigate the long-term effects of early childhood RV infection on the gut microbiome, we infected 5 day old BALB/c pups with rhesus rotavirus (RRV), and measured diarrhea severity over 4 days. To determine acute changes in the small intestinal microbiome upon initiation of diarrhea, intestinal tissue samples were collected 1 day post-infection from a subset of the animals. From the remaining animals, we collected stool samples at 6 and 12 weeks post-infection (wpi) to determine the fecal microbiome, and intestinal scrapings from each segment at 12 wpi to determine the mucosal microbiome. Samples were analyzed by 16S DNA analysis for bacterial composition. During acute diarrhea, we observed significant changes in Firmicutes, Cyanobacteria, and Verrucomicrobia, with changes in Firmicutes driven largely by the replacement of Lactobacillus with Streptococcus. In 6 and 12 wpi samples, we detected no difference in the alpha diversity between compartments, however, significant differences were observed in the relative abundance of Lachnospiraceae and Ruminococcaceae species in the cecum, and Prevotella in the distal colon. We also observed significant changes for Allobaculum and Anaerotruncus in the ileum, and Lachnospiraceae in the proximal colon. Ongoing experiments seek to delineate changes associated with the onset of, peak disease, and recovery from rotavirus diarrhea. The role and identity of species maintained throughout disease progression remains poorly understood, but represent potential therapies to improve recovery from diarrheal diseases.

Enteral obeticholic acid prevents hepatic cholestasis in total parenteral nutrition-fed neonatal pigs

Douglas Burrin[1], Yanjun Jiang[1], Zhengfeng Fang[2], Barbara Stoll[1], Greg Guthrie[1], Bryan Tackett[1], Alex Wang[3], Ignacio Ipharraguerre[4, 5], Jose Pastor[4]
USDA Children's Nutrition Research Center, Department Pediatrics, Baylor College of Medicine, Houston, TX, United States. Animal Nutrition Institute, Sichuan Agricultural University, Chengdu, China. Pediatric Gastroenterology, Hepatology, Nutrition, Department Pediatrics, Baylor College of Medicine, Houston, TX, United States. Lucta SA, Barcelona, Spain. Institute of Human Nutrition and Food Science, University of Kiel, Kiel, Germany.

Total parenteral nutrition (TPN) is a vital support for neonatal infants with congenital or acquired gastrointestinal (GI) disorders and requiring small bowel resection. An adverse outcome associated with prolonged TPN use is parenteral nutrition associated cholestasis (PNAC). We previously showed that enteral chenodeoxycholic acid (CDCA) treatment reduced PNAC. We hypothesized that the protective effects of CDCA were mediated by modulation of FXR-target genes involved in bile acid homeostasis. The aim of the current study was to compare the physiological effects of a selective FXR agonist, obeticholic acid (OCA) vs CDCA on hepatic bile acid homeostasis in TPN-fed piglets. Term, newborn pigs were assigned to receive complete TPN (PN), TPN + enteral CDCA (30 mg/kg), or TPN+enteral OCA (0.5, 5, 15 mg/kg) daily for 19 d. The daily parenteral lipid was Intralipid given at 10 g/kg. Endpoints of PNALD and bile acid homeostasis were measured. We found that, compared to PN pigs, treatment with high dose of OCA (OCA5 and OCA15), but not CDCA and low dose of OCA (OCA0.5), reduced serum PNAC markers including bilirubin, gamma-glutamyl transferase (GGT), total bile acid, triglyceride, and very-low-density lipoprotein. Compared to PN, OCA 5 and 15, but not OCA 0.5 or CDCA, reduced the total plasma bile acid concentration by increasing the proportional transfer of hepatic bile acid into the gallbladder, suggesting increased bile flow. TPN-induced ductopenia as measured by percentage of intact bile ducts per portal tract was prevented by OCA suggesting preservation of bile ducts. The major bile acids in plasma were glyco-conjugated forms of CDCA, hyocholic acid and hyodeoxycholic acid. OCA5 and OCA15, but not CDCA, suppressed hepatic expression of CYP7A1, while CYP27A1 and CYP8B1 mRNA remained unchanged. The bile acid detoxification enzyme CYP3A29 mRNA was inhibited by both CDCA and OCA treatments. OCA, but not CDCA upregulated hepatic mRNA involved in hepatobiliary bile acid and bilirubin transport into bile including bile salt export pump (BSEP), multidrug resistance protein 1(MDR1), and multidrug resistance protein 4 (MRP4). OCA5 and OCA15 induced hepatic and ileal FGF19 expression more than CDCA in pigs. We further found that OCA5 and OCA15, but not CDCA, inhibited the hepatic expression of inflammatory marker interleukin-8. Contrary to our previous study, we found that CDCA did not prevent PNAC. We suspect that this was due to the higher lipid load infused. We conclude that enteral OCA is more effective than CDCA in prevention of PNAC via upregulation of FXR-target genes involved in preservation of hepatobiliary transporters and bile duct function.

Microbial and metabolic signatures of necrotizing enterocolitis in formula-fed piglets

[1]Lee Call, [2]Douglas Burrin, [2]Barbara Stoll, [3]Adesola Akinkuotu, [3]Oluyinka Olutoye, [4]Nadim Ajami, [4]Joseph Petrosino, [6]Anja Wittke, [6]Rosaline Waworuntu, [5, 6]Brian Berg
[1] Interdepartmental Program in Translational Biology and Molecular Medicine, Baylor College of Medicine, [2] USDA-ARS Children's Nutrition Research Center, Dept. Pediatric, Gastroenterology and Nutrition, [3] Division Pediatric Surgery, Baylor College of Medicine, [4] Center for Metagenomics and Microbiome Research, Baylor College of Medicine, [5] Division of Nutritional Sciences, University of Illinois at Urbana-Champaign, [6] Mead Johnson Pediatric Nutrition Institute, Evansville

Background: Major risk factors for necrotizing enterocolitis (NEC) include premature birth, formula feeding, and microbial colonization of the gastrointestinal tract. We previously showed that feeding formula composed of lactose vs corn syrup solids protects against NEC in preterm pigs, however the microbial and metabolic effects of different formula carbohydrates has not been explored in detail.

Objective: Our objective was to characterize the effects of lactose- and corn syrup solids-based formulas on the metabolic and microbial profiles of preterm piglets and to determine whether unique metabolomic or microbiome signatures correlate with severity or incidence of NEC.

Design/Methods: Preterm piglets (103d gestation) were given total parenteral nutrition for 2d followed by enteral formula feeding from 3-7d. Pigs were fed formulas matched in nutrient content but containing either lactose (LAC), corn syrup solids (CSS) or 1:1 mix (MIX). Cecal contents and plasma were analyzed by LC/GC mass spectroscopy. Gut contents and mucosal samples were collected for DNA and RNA isolation. The V4 region of the 16S rRNA gene was sequenced, and bacterial load was measured by qPCR.

Results: NEC incidence was 14%, 42%, and 45% in the LAC, MIX, and CSS groups, respectively. Ileum inflammatory markers (IL-8, IL-6, and IL1b) were highest in CSS vs MIX and LAC groups and also correlated with NEC. TCA cycle intermediates were increased in the plasma of CSS and MIX vs LAC pigs; bile acids were decreased. Markers of glycolysis (lactate, pyruvate) were increased in the cecal contents of CSS vs LAC pigs and in plasma of NEC pigs. The dominant classes of bacteria were bacilli, clostridia, and gammaproteobacteria. NEC vs healthy phenotype and CSS vs LAC formula both showed decreased bacterial diversity. The abundance of Clostridium was increased, whereas that of Escherichia-Shigella was decreased in CSS vs LAC and NEC vs healthy pigs. Bacterial load was not different between either the diet groups or healthy vs NEC groups.

Conclusions: We conclude that lactose-based formula protects against inflammation and NEC and that this correlates with key changes in energy, glucose, and bile acid metabolism. Feeding formula containing lactose vs corn syrup solids selects for greater microbiota diversity and is associated with decreased Clostridium and lower NEC incidence.

Gremlin Acts Downstream of TGF-β/Smad3 Signaling to Induce Pancreatic Fibrosis

Ka Liu, Yanna Cao, Tien C. Ko
Department of Surgery, UTHealth-McGovern Medical School, Houston, Texas

Introduction: Gremlin (Grem1) is a highly conserved glycoprotein and has been reported to play a key role in multiple organ fibrosis. We have reported that Grem1 is elevated in human and mouse chronic pancreatitis (CP). *In vitro*, Grem1 can be induced by the major pro-fibrogenic factor transforming growth factor (TGF)-β in mouse pancreatic stellate cells (mPSCs). However, it is unknown whether Grem1 mediates TGF-β's pro-fibrogenic activity in CP. This study tests the hypothesis that Grem1 is a downstream mediator of TGF-β for pancreatic fibrosis. **Methods:** PSCs, the key cells that produce extracellular matrix during the development of pancreatic fibrosis in CP, were used as an *in vitro* model for this study. Primary human PSCs (hPSCs) were isolated from a resected human pancreas and primary mPSCs were isolated from C57BL/6 mice. hPSCs and mPSCs were treated with TGF-β1 (1 ng/ml) for 24 hours for mRNA analysis, for 48 hours for protein expression. Grem1 and fibronectin (FN, an extracellular matrix molecule as a measurement of fibrosis) expression were determined by quantitative real-time (q)PCR and immunofluorescence analysis. To knockdown Grem1 expression, mPSCs were transfected with *Grem1* siRNAs (20 nM) for 5 hours prior to TGF-β1 treatment. To block TGF-β1/Smad3 signaling, mPSCs were pretreated with SB431542 (TGF-β1 receptor II inhibitor, 3 μM), or SIS3 (Smad3 inhibitor, 10 μM) for 45 minutes prior to TGF-β1 or Grem1 (500 ng/ml) treatment. **Results:** TGF-β1 induced Grem1 expression in hPSCs (2.0-fold vs. vehicle control, $p<0.001$) and in mPSCs (1.5 fold vs. vehicle control, $p<0.05$). TGF-β1 induced FN expression in mPSCs (1.6 fold vs. vehicle control, $p<0.05$). *Grem1* siRNAs blocked TGF-β-induced Greml1 and FN expression. Pretreatment with TGF-β1 receptor II inhibitor (SB431542) or Smad3 inhibitor (SIS3) inhibited TGF-β1-induced Grem1 and FN expression but had no effect on Grem1-induced FN expression. **Conclusions**: These results suggest that Grem1 mediates TGF-β's pro-fibrogenic activity in the pancreas and Grem1 acts downstream of TGF-β1 receptor and Smad3 signaling. Taken together, our findings provide novel insights for targeting Grem1 to inhibit pancreatic fibrosis during CP progression.

Store-operated calcium entry activates calcium-activated chloride channels in rotavirus-infected cells

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Rotavirus (RV) causes acute gastroenteritis in children worldwide and results in severe secretory diarrhea from overstimulation of apical chloride (Cl^-) channels in intestinal epithelial cells. Although bacterial toxins are well studied, less is known about how RV causes diarrhea through elevation of cytosolic calcium ($[Ca^{2+}]$cyt), activation of plasma membrane (PM) Ca^{2+} channels, and activation of Ca^{2+}-activated chloride channels (CaCCs). RV nonstructural protein 4 (NSP4) functions as a viroporin that conducts Ca^{2+} from the endoplasmic reticulum (ER) into the cytosol, increasing $[Ca^{2+}]$cyt. Depletion of ER Ca^{2+} activates stromal interaction molecule 1 (STIM1), an ER Ca^{2+} sensor, which activates Orai1, a PM Ca^{2+} channel, for ER Ca^{2+} repletion called store-operated Ca^{2+} entry (SOCE). SOCE elevates $[Ca^{2+}]$cyt and can activate CaCCs such as anoctamin1 (Ano1), which could contribute to RV diarrhea. However, whether SOCE is coupled to CaCC activation during RV infection is unknown. Therefore, we characterized Ca^{2+} and Cl^- channel expression in intestinal epithelial cells and performed live cell fluorescent imaging to determine their roles during RV infection.

Using qRT-PCR, we first determined the expression of ion channels in intestinal cell lines and human intestinal enteroids. We detected high levels of STIM1 and Orai1 as well as expression of Ano1. We then used HEK293 cells and Caco-2BBe intestinal epithelial cells stably expressing the fluorescent Ca^{2+} biosensor GCaMP5G to show that pharmacological inhibitors of SOCE decrease basal Ca^{2+} and block Ca^{2+} entry into RV-infected cells. Furthermore, these treatments decreased CaCC activation in HEK293 cells stably expressing mouse Ano1 and halide-sensitive YFP, a fluorescent Cl^- channel biosensor. Overall, these results support that Ca^{2+} entry through SOCE channels is important for Ano1 activation and ultimately Cl^- secretion during RV infection. Determining the molecular mechanisms that regulate Ca^{2+} entry and CaCC activation during infection is critical for developing life-saving anti-diarrheal drugs.

The role of Gfi1 transcription factor in colorectal cancer progression and metastasis

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Background: Colorectal cancer (CRC) is the third most common and the third leading cause of cancer death in the United States. Over 90% of colorectal cancer-related deaths are associated to the ability of cancer cells to spread beyond the large intestine to distant locations. Growth Factor-Independent 1 (GFI1) is a zinc finger transcriptional repressor implicated in the differentiation of secretory precursors into goblet and Paneth cells in the intestinal epithelium. Gfi1 plays a significant role in the development of human malignancies, including leukemia, lung cancer and prostate cancer. However, it is unclear whether Gfi1 has a functional role in the initiation and progression of colorectal cancer. Our preliminary analysis of CRC mRNA expression highlights that Gfi1 expression is reduced in colorectal carcinoma compared to benign adenomas and noncancerous tissue, suggesting that Gfi1 may be involved in limiting tumor progression. Therefore, we would like to investigate the role of Gfi1 in the colorectal cancer progression and metastasis.

Methods: We analyzed the expression of Gfi1 using immunohistochemical staining in human colon cancer tissues. In order to determine the role of Gfi1 *in vitro* and *in vivo*, we established doxycycline-inducible Gfi1 in human colorectal cancer cells. Cell cycle analysis and cell viability assays were used to examine Gfi1-expressing cells and control cells. Subcutaneous xenograft mouse model and CRC metastatic mouse model by spleen implantation of a doxycycline-inducible Gfi1 human colorectal cancer cells were performed in immunocompromised mice. Gfi1 was induced by the administration of doxycycline via feeding mice doxycycline in drinking water and chow.

Results: Immunohistochemical staining indicates that Gfi1 expression is significantly decreased in malignant colonic epithelium compared with adjacent normal colonic epithelium. Cell cycle analysis using flow cytomertry and MTT assay indicate that re-expression of Gfi1 does not dramatically affect cell cycle and cell proliferation in human colon cancer cell lines. Consistent with *in vitro* results, subcutaneous xenograft model shows Gfi1-expressing cancer cells have similar growth rate with control cells. Interestingly, in the CRC metastatic model, we found that re-expression of Gfi1 in CRC cells promotes tumor metastasis to liver upon intrasplenic injection. Therefore, we conclude that Gfi1 may play a role in the distant metastasis but not in cell growth in CRC. In the future, we will examine metastatic liver tumors from CRC using immunohistochemical staining to confirm whether metastatic CRC tumors have the increased expression of Gfi1 compared to primary tumor. Future studies for identifying the molecular function of Gfi1 in CRC may improve our current understanding of tumor metastasis and benefit to therapeutic strategies.

Gender-specific effects of arsenic exposure in hepatic glucose and lipid metabolism

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Inorganic arsenic (iAs) is the top chemical on the ATSDR priority list of hazardous substances. Its role in carcinogenesis has been studied extensively, whereas its role in metabolic disorders has not[1,2]. Type 2 diabetes (T2D) reaches pandemic levels worldwide and is recently found to be correlated with arsenic exposure, meriting mechanistic studies in animals to address cause-effect relationships[3–10]. We found that arsenic exposure dramatically increases the susceptibility to diet-induced metabolic disorders in mice. Interestingly, the effect of iAs on metabolism and gene expression shows sexual dimorphism, with male mice more susceptible to glucose intolerance and female mice more susceptible to changes in hepatic lipid accumulation. We further identified that iAs induces differential expression of several metabolic genes in liver. Our findings suggest that arsenic may disrupt epigenomic functions of sex hormone nuclear receptors, which could underlie its gender-specific effects in liver metabolism.

Modeling *NGLY1* deficiency using human intestinal organoids

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BACKGROUND: N-glycanase 1 (*NGLY1*) is a cytosolic enzyme which is responsible for the cleavage of asparagine-linked glycans from glycoproteins. With export of misfolded proteins from the ER, it is postulated that *NGLY1* mediated de-glycoslylation is an important step in the series of modifications responsible for targeting these proteins for degradation by the 26S proteasome. With expression in multiple organ systems, patients mutant in *NGLY1* present with a phenotype within the spectrum of congenital disorders of glycosylation (CGD), characterized by severe neurological and developmental deficits, hypolacrima/alacrima, liver abnormalities and constipation. OBJECTIVE: Determine whether human intestinal organoids (HIOs) can be used to model the clinical phenotype and enzymatic activity of *NGLY1*, both *in vitro* and *in vivo*. METHODS: HIOs were derived from both embryonic stem cells (ESCs; line H9) and induced pluripotent stem cell lines (iPSCs) generated from individuals who are either heterozygous or compound heterozygous for mutations in *NGLY1*. These HIOs were analyzed both *in vitro* and after *in vivo* transplantation under the renal capsule of immunodeficient mice. RESULTS: No differences were observed in the growth characteristics or efficiency of HIO formation of *NGLY1*-deficient iPSC lines compared to *NGLY1*-heterozygous iPSCs or control ESCs. Immunohistochemistry and qPCR were used to assess the expression levels of transcription factors and epithelial cell lineage markers associated with differentiation and maturation of the intestine. In transplanted HIOs, a trend towards increased goblet cell numbers is observed in the epithelium of *NGLY1*-deficient HIOs. Future investigations will focus on incorporation of patient-derived enteric neurons into the HIOs and functional analysis *in vitro* and after *in vivo* transplantation. Immunohistochemistry and qPCR will be used to assess the ERAD pathway and markers of ER stress to correlate this with specific cell lineages.

Reconstructing the origin and stages of progression to esophageal adenocarcinoma from patient-matched stem cells

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Barrett's esophagus is a precancerous condition of the distal esophagus in the form of an intestinal metaplasia. While Barrett's is associated with a 30- to 100-fold increased risk for esophageal adenocarcinoma, the sequence of genomic changes that accompany the assumed 10-20 year march from Barrett's, low-grade and high-grade dysplasia, and finally adenocarcinoma remain unknown (Sharma, 2009; Reid et al., 2010). The model we proposed for the origin of Barrett's suggested that it initiates from population of cells at the normal gastroesophageal junction that are developmentally distinct from both the esophageal and gastric epithelia and emerges initially without driver mutations (Wang et al., 2011). We have recently examined a cohort of pure Barrett's cases via cloning patient-matched stem cells of esophagus, Barrett's, and gastric cardia and confirmed the unique origin of Barrett's and the absence of mutations in fully one-third of these cases (Yamamoto et al., 2016). We further found that most of these Barrett's cases had stem cells with the same recurrent deletions seen in esophageal adenocarcinoma though lacking the corresponding amplifications of proto-oncogenes and receptor tyrosine kinases in all but the two most advanced cases. We anticipate that similar clonal approaches to esophageal adenocarcinoma and patient-matched dysplasia and Barrett's offer a unique opportunity to reconstruct the multi-decade events and transitions that drive the evolution of a lethal cancer.

Gut dysbiosis is involved in the development of hypertension

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Hypertension affects nearly one out of three adults in the U.S. and is strongly associated with obesity, diabetes mellitus, cardiovascular disease, and obstructive sleep apnea (OSA). The importance of a healthy gut microbiota, and detriment of a dysbiotic microbiota, on host physiology is becoming increasingly evident. Recent studies suggest that gut dysbiosis can have adverse effects beyond the gastrointestinal tract, and has been linked to metabolic disorders and cardiovascular diseases, such as obesity, diabetes mellitus and atherosclerosis. **We hypothesized that alterations to the gut microbiota are involved in the development of hypertension.** To test this hypothesis we used two models of hypertension; (1) a surgical model of OSA that develops hypertension and (2) a genetic model of hypertension, the spontaneously hypertensive rat (SHR). In the surgical model of OSA we observed a significant elevation in blood pressure after 1 (19mmHg; n=10-13, $p<0.05$) and 2 weeks (21mmHg; n=10-11, $p<0.05$) of apneas, as compared to sham rats (underwent surgery without apneas). Transplant of cecal contents from hypertensive OSA rats, but not sham rats, into normotensive rats resulted in hypertension (+32mmHg; n=4, $p<0.05$), demonstrating a causal role of the gut microbiota in OSA-induced hypertension. Composition of the gut microbiota was assessed by 16S rRNA gene sequencing. Compared to normotensive rats, the relative abundance of multiple butyrate producing taxa was significantly reduced in hypertensive OSA rats, including Eubacterium, Clostridria and Ruminococcaceae (n=4-7, $p<0.05$). Similarly, Phylogenetic Investigation of Communities (PICRUSt) analysis, used to predict functional gene family abundances using 16S rRNA data, revealed that the hypertensive microbiota was associated with a significant downregulation of several steps involved in butyrate metabolism, including acetate CoA transferase (n=4-7, $p<0.05$). Butyrate plays a key role in maintaining gut barrier integrity and regulating immune responses. In an attempt to increase butyrate production in the gut, 10^9 CFU of the butyrate producing bacteria *Clostridium butyricum* was administered by gavage every three days during apneas. Treatment with *C. butyricum* prevented OSA-induced hypertension. In the genetic model of hypertension comparison of the SHR microbiota to the normotensive WKY parent strain revealed gut dysbiosis, including a significant decrease in the relative abundance of the order Clostridiales, known to include numerous butyrate producers (57 vs. 21% of total sequences; n=4-7, $p<0.05$). When cecal contents from SHR or WKY rats were gavaged into WKY rats, those that received SHR cecal contents exhibited significant elevations in blood pressure (+18mmHg; n=4-7, $p<0.05$). Interestingly, treatment with tributyrin (butyrate conjugated to glycerol; 4g/kg daily by gavage) prevented the development of hypertension in SHR rats (n=4-8; $p<0.05$). These data demonstrate a causal role for gut dysbiosis in the development of hypertension in two distinct models. Impaired microbiota butyrate production appears to play a key role in this process.

Uptake and circulatory characteristics of the plant-based small RNA MIR2911

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Inconsistent detection of plant-based microRNAs (miRNAs) in consumer circulation has thwarted the use of dietary based delivery systems for therapeutic RNAs. Here we demonstrate vegetables contain high levels of a bioavailable plant specific small RNA termed MIR2911. Mice consuming diets rich in spinach, cauliflower, cabbage, or broccoli displayed enhanced serum levels of MIR2911. Assayed by differential centrifugation, size-exclusion chromatography and proteinase K treatment, the MIR2911 from plant extracts appeared to be part of a protein complex. Dietary uptake was more efficient when the plant-derived MIR2911 was used compared to the synthetic RNA. The circulating MIR2911 was not associated with exosomes and fractionated as a soluble complex that was insensitive to proteinase K treatment, suggesting the plant MIR2911 was stabilized by modifications conferred by the host. These results indicate plant-based modifications of specific small RNAs orchestrate digestive stability and consumer uptake and invite revisiting plant-based miRNA delivery approaches.

Alterations in adherent tumor mucin protein and glycans by probiotic *lactobacillus reuteri* promotes tumor chemotherapy uptake

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Background: Colorectal cancer (CRC) is the second leading cause of cancer related deaths in the United States. Although CRC is commonly treated with the chemotherapeutic agent Fluorouracil (5-FU), only 10-15% of advanced CRC tumors respond. Mucins, a hallmark of carcinomas derived from epithelia, have long been implicated in the pathogenesis of cancer including CRC. The cancer mucin complex created by adherent mucins (MUC1, 3, and 4) and secreted mucins (MUC5AC) has been hypothesized to limit the uptake of the chemotherapeutic agents, thus promoting chemoresistance. Several members of the gut microbiota are capable of altering mucin production and may represent potential treatment to increase the chemocherapeutic susceptibility in resistant cancers. We hypothesized that exposure of tumors to probiotic human gut microbe *Lactobacillus reuteri* would alter tumor-associated mucins and promote chemotherapy uptake. **Methods & Results**: HT29, T84 and HT29-MTX-E12 intestinal cell lines were incubated with *L. reuteri* ATTC 6475 conditioned media (*CM*). Analysis of MUC expression by qRT-PCR revealed that *L. reuteri* CM was capable of decreasing MUC1, MUC4 and MUC5AC expression. *L. reuteri* decreased total adherent mucin proteins, as determined by immunofluorescence (IF), alcian blue, and biotinylation. Incubation of *L. reuteri* CM with purified HT29-MTX mucins revealed that *L. reuteri* was capable of cleaving mucin oligosaccharides N-acetyl-glucosamine, galactose, mannose and sialic acid. These data suggest that *L. reuteri* is able to alter tumor-associated mucin protein and glycans. To assess whether bacterial-specific changes in mucin expression correlated with increased cancer cell susceptibility to drug treatment, HT29, T84 and HT29-MTX-E12 cells were incubated with *CM* for 3 hours followed by the chemotherapeutic agent 5-FU. *L. reuteri CM* increased metabolic activity as determined by resazurin and cell cycle analysis. Magpix multiplex assay revealed that *L. reuteri CM* promoted ERK, CREB, and JNK phosphorylation. No changes were observed in p7056, STAT3, STAT5, p38 or NF-κB. Furthermore, *L. reuteri CM* enhanced susceptibility to 5-FU treatment resulting in decreased cancer cell viability as determined by resazurin, trypan blue, and Annexin V staining. This effect was independent of lactate. The *L. reuteri* secreted factor was found to be a >10 kDa, heat stable protein. To establish the ability of *L. reuteri* to promote tumor 5-FU susceptibility *in vivo*, C57BL/6J-*Apc*^{Min}/J mouse tumors were excised and *ex-vivo* treated with *L. reuteri CM* followed by 5-FU. Addition of CM resulted in enhanced susceptibility of male and female intestinal tumors to 5-FU treatment. *Apc*^{Min} mice colonized with *L. reuteri* bacteria had significantly decreased adherent Muc1, Muc4, and Muc5ac tumor masses and tumor associated Tn glycans (Galβ1-3GalNAc-), consistent with cell line data. **Conclusions**: Together this data suggests that bacterial-induced modifications of cancer-driven mucus in combination with traditional chemotherapeutic agents may provide new therapeutic strategies for the treatment of chemoresistant cancer.

Complex and defined bacteriotherapy can inhibit acute colitis in mice

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Introduction: Inflammatory bowel disease (IBD) is thought to develop secondary to an uncontrolled immune response against the gut microbiome that is transmitted by the intestinal mucosa. Therefore, the disruption of the healthy microbiome (dysbiosis) may be an important element in disease development. It has been hypothesized that the restoration of normal microbiota structure via fecal microbiota transplantation (FMT) may be therapeutic for IBD. However, the fecal microbiome is a highly complex and dynamic community, leading to substantial difficulties in deciphering the potentially key therapeutic attributes of FMT. Therefore, the identification of stable bacterial communities that carry the therapeutic effects of FMT could provide a more consistent and testable bacteriotherapy for IBD. Importantly, a bacterial preparation with 10 species has been shown to effectively treat *C. difficile* colitis. Key species in this combination belonged to the *Bacteroides* genus. Our goal was to compare the anti-colitic effects of delivery of a triple-*Bacteroides* combination and FMT in a murine model of acute colitis.

Methods: Experimental colitis was induced in 8 to12-week old C57BL/6 mice using 3% dextran sulfate sodium (DSS) for 5 days. The mice were simultaneously treated by oral gavage for 9 days with a triple-*Bacteroides* combination, FMT using stool from healthy (non-DSS exposed) donor mice (FMT group), or autologous FMT (control group). Weight loss was monitored as a marker of the severity of colitis. Animals were euthanized if more than 25% weight loss occurred. Histologic examination of colonic specimens, metagenomic studies on fecal and mucosal microbiomes are currently being conducted.

Results: Survival was lower in the control group (40%, 6 of 15) than in the FMT (73%, 11 of 15) and triple-*Bacteroides* (100% survival) groups. Mice in the control group lost 17.4% of their body weight on average, as compared to 9.1% in the FMT group. Mice receiving triple-*Bacteroides* therapy lost 3.5% of their body weight, significantly less than both the control and FMT groups. Histopathologic and metagenomic analyses are pending.

Conclusion: FMT from healthy donor mice and a triple-*Bacteroides* combination of bacteria can reduce weight loss during acute colitis in mice. The triple-*Bacteroides* therapy was significantly more effective than FMT in this respect. We suspect that weight loss inhibition is a result of decreased colitis activity in association with a significantly modified microbiome, which was induced by the complex (FMT) and defined (triple-*Bacteroides*) bacteriotherapy. Our findings may have readily applicable relevance for human IBD treatment.

Histamine-generating *Lactobacillus reuteri* in the intestinal microbiome attenuates inflammation-associated colonic carcinogenesis in a histamine-deficient mouse model

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Colorectal cancer (CRC) is one of the most common cancers and leading causes of mortality. Patients with inflammatory bowel disease have a greater risk of CRC than the general population. Supplementation of gut microbiota with probiotic *Lactobacillus* strains has been effective at ameliorating intestinal inflammation and CRC in rodent models, but the underlying mechanisms are not clear. Previously we showed that *L. reuteri* ATCC PTA 6475 harboring an *hdc* gene cluster which is responsible for the synthesis and secretion of histamine from L-histidine suppresses TNF production in activated THP-1 cells via histamine production. This strain also attenuates trinitrobenzene sulfonic acid-induced colitis *in vivo*, and the *hdcA* gene essential for histamine production is required for the anti-inflammatory effects. By using an azoxymethane/dextran sodium sulfate-induced inflammation associated CRC model, we demonstrate that *L. reuteri* 6475 administration which increased bacterial *hdc* gene abundance in the gut microbiome significantly decreased the number and size of colon tumors and uptake of [18F]fluorodeoxyglucose in the colon by positron emission tomography in histamine-deficient mice. Administration of *L. reuteri* 6475 down-regulated KC, IL-22, IL-6, TNF, and IL-1α gene expression in colonic mucosa, reduced KC, IL-22 and IL-6 production in plasma, and decreased $CD11b^{+}Gr\text{-}1^{+}$ immature myeloid cells in the spleen. Meanwhile, *hdcA* gene inactivation lacked such effects, indicating a significant role of the bacterial histamine for suppression of colorectal tumorigenesis. These findings link luminal conversion of dietary components (amino acid metabolism) by gut microbiome and probiotic-mediated suppression of CRC, and may result in opportunities for therapeutic microbiology.

Recycled locally bioavailable COXIBs for the treatment of inflammation-associated diseases in the colon

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Background: COXIBs is a subclass of nonsteroidal anti-inflammatory drugs (NSAIDs) designed to selectively inhibit cyclooxygenase (COX-2). Clinical trials showed that COXIBs could reduce the incidence of colon cancer in familial adenomatous polyposis patients. However, the promise of using COXIBs in chemoprevention was shattered due to severe side effects including unstable angina, myocardial infarction, and cardiac thrombus. Drug companies even have to withdraw the drug or the chemopreventive indication from the market due to these serious side effects. The aim of this study is to design recycled locally bioavailable (r-LB) drugs to localize the drug in the colon to solve this problem. Our central hypothesis is that highly effective r-LB COXIBs can be obtained through structural modification and local delivery to achieve high colonic concentration without or with minimal systemic exposure. We will modify the structure of celecoxib to fit the molecules for enterohepatic recycling to achieve r-LB.

Methods: Celecoxib was used as a reference compound to design r-LB drugs. Chemical synthesis approach was used to modify the structure of celecoxib. Human recombinant COX-1/2 were used to screening the activity and selectivity of the synthesized derivatives. Raw cell and a TNBS-induced colonic perfusion model were used to confirm the activity of the leading compounds. Rat and human microsomes and S9 fractions were used to determine the metabolism rats of the leading compound. LC-MS was used to quantify the relevant compounds.

Results: Totally 13 compounds were synthesized with a phenolic moiety, which facilitate the metabolism and enterohepatic recycling, in the structure of celecoxib. One leading compound (**7a1**) was identified by using enzymatic and cell line assays. Metabolism studies showed that the glucuronidation rates of 7a1 with rat and human liver microsomes were 4 or 5 folds higher than that with rat or human colon microsome, indicating **7a1** was slowly metabolized in the colon but rapidly metabolized in the liver. The TNBS-induced colonic perfusion model showed that 1) after 1 hour of perfusion, all of the absorbed 7a1 was excreted from bile as glucuronided form, which indicate an efficient enterohepatic recycling; 2) inflammation in the colon was inhibited by 7a1; 3) systemic exposure of 7a1 was significantly lower than that of celecoxib.

Conclusion: For these newly synthesized compounds, the extensive first-pass metabolism in liver resulted in a poor oral bioavailability and extremely low blood concentrations in the systemic circulation, reducing or avoiding the exposure of the cardiovascular systems. Compared with celecoxib, the r-LB COX-2 inhibitors are more promising to be developed as safe agents in colorectal cancer

Rifampicin treatment reduces parenteral nutrition associated liver disease in neonatal piglets

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Purpose: Preterm infants with complications due to gastrointestinal immaturity can receive parenteral nutrition (PN) for periods greater than two weeks. These infants are at risk for a progressive increase in cholestatic liver disease marked by elevated serum bilirubin and bile acid levels and in severe cases steatosis and liver damage (PNALD). The elevated levels of bile acids in the liver due to cholestasis can precipitate this liver injury. Modalities to minimize bile acids in the liver may be beneficial for preventing liver damage. Treatment with the antibiotic rifampicin has been used in adults to treat cholestatic pruritus. Treatment with rifampicin has been shown to increase clearance of bile acids through activation of the nuclear hormone receptor, pregnane x receptor (PXR). PXR target genes are enzymes involved in Phase I and Phase II drug detoxification, which lead to increased 6-alpha hydroxylation of bile acids and their subsequent glucuronidation increasing overall hydrophilicity and clearance. Our aim was to test whether rifampicin treatment can suppress the development of PNALD in neonatal PN-fed piglets

Methods: Newborn pigs (4-d-old) were implanted with jugular catheters and received PN for 14 d. Pigs received daily PN consisting of 240 mL/kg fluid, 240 kcal/kg, 25 g/kg carbohydrate, 14 g/kg protein and 10 g/kg lipid; the parental lipid used was Intralipid. Pigs (n=8/group) were infused intravenously with either saline (IL) or 600 mg/kg Rifadin IV (RIF) each day for 14 days.

Results: Treatment with RIF led to a 3-fold increase in the PXR target Phase I enzyme Cyp3a29 gene expression compared to IL. RIF increased protein but not mRNA expression of the Phase II glucuronidation gene, Ugt1a6. Pigs in the IL vs. RIF group had higher levels of serum direct bilirubin (2.62 ± 0.33 mg/dL vs.1.33 ± 0.23 mg/dL) and total bile acids (34.05 ± 6.69 µM vs.17.06 ± 2.50 µM). However, the liver and gall bladder bile acid pool did not differ between groups. RIF treatment did not adversely affect the liver, as liver weight was significantly lower in the RIF group compared to the IL group (65.99 ± 3.70 g/kg vs.55.02 ± 2.21 g/kg). A marker of liver injury, serum ALT levels were not different between IL (15.75 ± 1.92 U/L) and RIF (14.75 ± 1.53 U/L) groups and within the normal range.

Conclusions: Treatment with rifampicin was effective in suppressing serum direct bilirubin and bile acid levels. The clearance of bile acids did not appear to occur via increased biliary secretion into the gall bladder, but likely through increased clearance in the urine. Rifampicin did not appear to be hepatotoxic to neonatal pigs at the dosage level used. We conclude that short term rifampicin treatment can reduce cholestasis associated with PNALD.

Eric2 regulates histamine production machinery via an ion-transport dependent mechanism in *Lactobacillus reuteri*

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Background: Certain strains of *Lactobacillus reuteri* can reduce inflammation and tissue damage in mouse models of colitis. This anti-inflammatory capacity stems in part from *L. reuteri*'s ability to produce and secrete histamine, which can act on specific receptors present on mammalian cells. In the bacteria, histamine is generated by histidine decarboxylase (HdcA), and released from the cell by a histidine/histamine exchanger (HdcP). This process consumes protons, resulting in an increasingly alkaline intracellular pH and an inside-negative membrane potential. These factors can suppress the activities of HdcA and HdcP. However, *hdcA* is not constitutively expressed, and it is unclear which signals may trigger its upregulation. Additionally, it is unknown how *L. reuteri* balances its internal ion environment during histamine production, especially in the presence of a complex external environment like the GI tract. A proton/chloride antiporter, EriC2, was recently identified in *L. reuteri* as a potential regulator of intracellular pH and membrane potential, and thus histamine production. We hypothesize that EriC2 maintains intracellular pH via proton influx and membrane potential via chloride efflux at levels that allow histamine production by *L. reuteri*. **Methods & Results:** To investigate how EriC2 ion transport might alter expression and function of histamine production machinery in *L. reuteri*, single-strand recombineering was used to generate protein knockout, proton transport deficient, and transport null EriC2 strains. These mutations are stable, and do not significantly alter the growth of the resulting strains compared to wild-type (WT) *L. reuteri*. Intracellular pH was assessed in each strain with the pH-sensitive fluorophore, pHrodo Green AM. Loss of ion transport via EriC2 increases intracellular pH, supporting its potential role in maintaining the intracellular proton pool. RT-qPCR showed that *hdcA* gene expression is increased in EriC2 transport mutants relative to the WT strain. T84 secretory colonic epithelial cells were co-cultured with WT and EriC2 mutant *L. reuteri* strains. Host gene expression was examined via RT-qPCR, and differential expression of histamine receptors was observed in the presence of mutant *L. reuteri* strains. **Conclusion:** Together these data suggest that EriC2 ion transport mutants differentially regulate histamine production machinery in *L. reuteri,* and can lead to changes in host physiology. The beneficial effects of *L. reuteri*-derived histamine may depend on a regulatory system mediated by ion transport in the GI tract. A mechanistic understanding of histamine production by the gut microbiome may enable the development of diet or probiotic-based strategies to suppress intestinal inflammation.

The natural history of pediatric ulcerative colitis in the era of biologic therapy

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Background: The clinical outcomes of pediatric ulcerative colitis (UC) are not well known. Most of the population-based studies were conducted outside of the United States and prior to the introduction of biologic (anti-tumor necrosis factor α) agents in the standard treatment of pediatric UC. We aimed to describe the natural history of pediatric UC in the era biologic therapies.

Methods: We conducted a retrospective review of 152 pediatric patients at Texas Children's Hospital with a new diagnosis of UC between January 2003 and December 2009. The patient records were followed through July 2014. Localization of disease at diagnosis, use of steroids, immunomodulator therapy or biologic agents, presence of extraintestinal manifestations, and need for surgery at a minimum five year follow up were noted. A follow-up phone call was made to all patients who were lost to follow-up or transitioned to adult care within five years of diagnosis to assess these clinical outcomes. Only patients with a minimum of 5 years of follow up or need for surgery within the first 5 years of diagnosis were included in the final analysis.

Results: We identified 106 pediatric UC patients with a minimum of 5 years of follow up or colectomy within the first 5 years of diagnosis. Mean age at diagnosis was 10.7±4.1 years, with an average length of follow up of 6.5±1.9 years. Eight percent (8%) presented at diagnosis with ulcerative proctitis (E1), 12% had left sided disease (E2), 48% had extensive UC (E3), 18% had an incomplete scope at the time of diagnosis, and 13% did not have initial colonoscopic data available. Biologic medications were used in 36% of patients (97% infliximab, 10% adalimumab); 27% were treated with immunomodulators (70% 6-mercaptopurine, 35% azathioprine, 13% methotrexate); 94% received 5-aminosalicylic acid therapy; and 90% were exposed to steroid therapy throughout the follow up period. Extra-intestinal manifestations occurred in 26 patients (25%). In respect to all patients, 4% had arthritis, 11% arthralgia, 10% had primary sclerosing cholangitis, 2% had autoimmune hepatitis, and 1% had aphthous stomatitis. Cumulative rate of colectomy was 20% at 5 years, with 8% progressing to colectomy within 1 year of diagnosis.

Conclusion: This is the largest pediatric cohort of UC patients with 5 year clinical outcomes. Compared to previous studies from the same geographic region in the pre-biologic era, pediatric UC patients in our cohort presented with more extensive disease and required a higher rate of colectomy; however, these rates are similar to other worldwide studies in the pre-biologic era. This work emphasizes the need for novel preventative and therapeutic measures to combat pediatric UC.

Manipulation of the microbiome improves functional recovery after ischemic stroke

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Background and Purpose: It is widely known that as organism's age, circulating inflammatory markers increase. The pro-inflammatory milieu makes the organisms less capable of coping with various stressors such as stroke. We have confirmed reports that as animals age, there is also a corresponding change that occurs in the microbiome, the commensal gut microbes. The change occurs most notably in the two major phyla of the microbiome, the *Firmicutes* and *Bacteroidetes*. During early stages of life, a low *Firmicute* to *Bacteroidetes* ratio is present. As organisms age, we have seen that the ratio increases. The same ratio that is present in aged organisms has been linked to many diseased states such as obesity, hypertension, and diabetes which are all direct risk factors for stroke and can both increase the incidence of ischemic stroke and dramatically reduce recovery from it. We sought to explore whether manipulating the microbiome to resemble the young compositions could improve functional recovery in aged organisms.

Methods: Fecal transplants were prepared from young and aged donors and were administered to recipient animals following concentrated doses of Streptomycin, which suppressed endogenous microbial compositions allowing for successful colonization of the gut with the newly transplanted microbiomes. A murine MCAO was used as the stroke model. Functional recovery was assessed via neurological deficit scoring, the hang wire test, open field test, and the Y-maze test to assess the animals' cognitive impairments.

Results: Both aged and young organisms that hosted "aged" (high *Firmicutes/Bacteroidetes*) microbiome compositions pre-stroke presented with worsened functional recovery as assessed through behavioral tests and presented with increased mortality rates when compared with animals with "young" (low *Firmicutes/Bacteroidetes*) microbial compositions. Aged organisms' functional recovery was markedly increased ($P < 0.05$) through transplantation of "young" microbiomes prior to stroke when compared to aged control animals with the normal "aged" microbiomes.

Conclusion: Successful manipulation of the microbiome in young and aged organisms was proven possible and has shown a trend of improved functional recovery in both aged and young organisms when their microbiome has a high relative abundance of *Bacteroidetes* and low abundance of *Firmicutes*. Further work will be done to confirm more specific microbial populations responsible for the differences in recovery.

Quantitative real-time monitoring of glutathione

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Glutathione (GSH) is the most abundant non-protein thiol in mammalian cells. Together with its oxidized partner (GSSG), GSH maintains the cellular redox homeostasis, regulates protein functions through S-glutathionylation, and acts as a signaling molecule to directly activate gene expression. All these important functions are regulated by the intracellular concentration and distribution of GSH. Currently, very few method could provide reliable information about the dynamics of GSH concentration changes and crosstalk between GSH concentration differential in different cellular compartments. Previously, we developed the first ratiometric fluorescent probe (ThiolQuant Green, TQG) for quantitative imaging of intracellular GSH by introducing reversible Michael Addition. However, the slow reaction kinetics between GSH and TQG has limited its application in live monitoring of GSH dynamics. Through quantum mechanical calculation and experimental modification, we significantly improved reaction kinetics and optical profile of our probe. The new probe, designated as ThiolQuant Green Real-Time (TQG-RT), has a time resolution below 40s, which provides the first ever molecular tool for monitoring transient changes of GSH within cells upon various stimulations. In a pilot experiment, we observed minute-scale GSH fluctuations after H2O2, GSH-ester and EGF treatment in TQG-RT loaded HeLa cells. We were also able to apply our probe in hard-to-transfect cells such as primary cells and macrophages. We could even achieve in vivo GSH mapping in C. elegans. In summary, we developed the first fluorescent probe that enable quantitative real-time imaging of GSH in living cells and animals. We envision that our GSH probes will enable unprecedented opportunities to study GSH dynamics and revolutionize our understanding of the physiological and pathological roles of GSH in cells and organisms.

Enteral obeticholic acid Promotes intestinal growth in total parenteral nutrition-fed neonatal pigs

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Intestinal atrophy is an adverse outcome associated with prolonged total parenteral nutrition (PN) partly due to disruption of normal enterohepatic circulation of bile acids. Previously we showed that enteral treatment with chenodeoxycholic acid (CDCA), a dual agonist for the nuclear receptor, farnesoid X receptor (FXR) and G protein-coupled receptor TGR-5, induced intestinal mucosal growth in a parenteral nutrition associated liver disease (PNALD) piglet model. We hypothesized that the intestinal trophic CDCA effects were mainly mediated by TGR5 receptor-mediated glucagon-like peptide 2 (GLP-2) released from enteroendocrine cells. However, CDCA may also exert trophic effects via FXR signaling in intestine. The aim of the current study was to compare the physiological effects of a selective and potent FXR agonist, obeticholic acid (OCA) vs CDCA on intestinal growth in TPN-fed pigs. Term, newborn pigs were assigned to receive complete TPN (PN), PN + enteral CDCA (30 mg/kg), or PN +enteral OCA (0.5, 5, 15 mg/kg) daily for 19 d. The daily parenteral lipid was Intralipid given at 10 g/kg. Intestinal growth and crypt cell proliferation (in vivo BrdU labeling) were measured. We found that both CDCA and OCA treatments significantly increased small intestinal weight, compared to PN pigs, but OCA15 was higher than CDCA (146% vs. 118%). The OCA-induced increase in jejunal and Ileal weight was dose-dependent, yet the trophic effects of OCA and CDCA were greater in the ileum than jejunum. Ileal villus height and crypt depth were increased by OCA and CDCA. The percentage of BrdU positive crypt cells in PN, CDCA, OCA0.5, OCA5 and OCA15 groups were 31%, 40%, 40%, 46% and 46% respectively, suggesting OCA and CDCA increased crypt cell proliferation. Portal plasma GLP-1 and -2 concentrations were significantly increased in pigs treated with CDCA, but not OCA, compared to PN pigs suggesting differential intestinal activation of TGR5 signaling. OCA, but not CDCA, treatment dose-dependently increased ileal transcriptional expression of FXR target genes, small heterodimer partner (SHP), ileal lipid binding protein (ILBP), and fibroblast growth factor 19 (FGF19). The expression of fibroblast growth factor receptor 4 (FGFR4) and β-Klotho mRNA were relative abundant in ileal tissue in all groups. We conclude that the intestinal trophic effects of OCA are greater than CDCA in TPN-fed pigs. The trophic effects of CDCA appear to occur via TGR5-mediated GLP-2 secretion rather than FXR signaling. We show novel evidence that OCA exerts trophic effects in the neonatal intestine and this appears to act mainly via FXR-dependent mechanisms.

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Fecal microbiota and risk of colorectal tubular adenoma

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Background: Most colorectal cancer arises from colorectal adenomas. Gut microbiota is important in maintaining intestinal homeostasis. Dysbiosis, in particular more abundant *Fusobacterium*, has been associated with increased risk of colorectal adenoma and cancer. However, there has been no comprehensive evaluation of fecal microbiome in association with colorectal adenoma.

Methods: We collected fecal samples before bowel preparation from seven men with tubular colorectal adenomas (cases) and seven men and one woman with normal appearing mucosa with no polyps or history of polyps (controls) in the endoscopy suite at the Michael E. DeBakey VA Medical Center between January 2015 and April 2015. Cases and controls were frequency-matched according to age, sex, ethnicity and body mass index. We extracted microbial DNA from fecal samples and amplified and sequenced the 16S V4 region on Illumina MiSeq platform. We analyzed sequencing data using UPARSE and SILVA database for operational taxonomic unit (OTU) classification. We calculated alpha and beta-diversity indices and conducted Weighted UniFrac principal coordinates analysis (PCoA). The difference between cases and controls was compared using the Mann-Whitney test for alpha-diversity and relative abundance or PERMANOVA for beta-diversity.

Results: Proteobacteria, Bacteroidetes, and Firmicutes are the major phyla accounting for > 90% of microbiota. Cases and controls had no significant differences in richness or evenness of fecal microbiota (P = 0.08 for observed OTUs). However, we found increased abundance of the phylum Actinobacteria in cases compared with controls (1.39% vs. 0.16%, false discovery adjusted P value = 0.007). Mean relative abundance of *Fusobacteria* was 0.03% in cases and 0% in controls. Weighted UniFrac PCoA indicated that the composition of the microbiota was not different between cases and controls (P value = 0.46). There were no significant differences in relative abundance at the genus level between cases and controls.

Conclusions: In this pilot case-control study, we found that Actinobacteria was more abundant in fecal samples of cases with tubular adenoma than among controls with no polyps. Our ongoing study with greater statistical power is expected to confirm this finding, provide more information on the abundance of *Fusobacteria* by using a targeted approach, and define other dysbiotic conditions that may relate to the presence of adenoma.

Detection of human norovirus in intestinal biopsies from immunosuppressed transplant patients

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Human noroviruses (HuNoV) are an important cause of acute gastroenteritis worldwide. HuNoV infections cause histopathological changes in human intestinal tissues, however, the presence of HuNoV antigens in these tissues has not been described. HuNoV results in chronic infections in solid organ and hematopoietic stem cell transplant patients. Since this patient population represents a group where biopsies are routinely collected, we analyzed tissue samples to look for histopathological changes in conjunction with detection of HuNoV antigens. The objective of this study was to characterize NoV illness in immunocompromised persons and determine the cell types that may be permissive for HuNoV replication. We found that infection in immunocompromised patients leads to histopathological changes such as disorganization of the intestinal epithelium and infiltration of immune cells in the lamina propria. Biopsies also show gastric metaplasia in the duodenum and edema in all the intestinal sections. The HuNoV major capsid protein-VP1 was detected in areas of biopsies that showed histopathological defects in all the segments of small intestine. VP1 was detected in enterocytes, macrophages, T cells and dendritic cells. Staining for non-structural proteins RdRp and VPg was used as a marker for HuNoV replication. RdRp and VPg in conjunction with VP1 were detected in the duodenal and jejunal enterocytes. Some staining for RdRp and VPg was seen in macrophages but was also associated with a marker for epithelial cell phagocytosis. This study provides insight into the cellular tropism of HuNoV *in vivo* and identifies enterocytes as a cell type permissible for HuNoV replication. These results establish a foundation for understanding pathogenesis of HuNoV infection and will guide the efforts to identify a physiologically important cell culture system to grow HuNoV.

Two-step forward genetic screen in mice identifies the Ral pathway as a potential new drug target in hepatocellular carcinoma

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Backgrounds and Aims: Hepatocellular carcinoma (HCC) is a deadly cancer and its mortality rate ranks third among all cancer types worldwide. To comprehensively understand its genetic drivers, recent innovative next-generation sequence technology was applied to HCC genomes. Over 500 cases of HCC have been sequenced but they reveal the existence of thousands of infrequently mutated genes. This intra- and inter-tumor heterogeneity, combined with large number of passenger mutations, has made it difficult to identify the true drivers of HCC. To overcome this and better understand the evolutionary forces driving HCC, we perform two-step *in vivo* forward genetic screens. **Methods:** First, hepatocyte-specific whole-genome transposon mutagenesis was performed in mice to generate an unbiased catalogue of HCC candidate cancer genes (CCGs). Next, to further validate CCGs in this catalogue in a high-throughput manner, 750 shRNAs targeting 250 CCGs were selected and arrayed into 15 pools of 50 shRNAs each. These pooled shRNA libraries were then introduced into immortalized mouse fetal liver cells (IFLCs) and the cells monitored for their tumor-forming ability following injection into nude mice. **Results:** Transposon mutagenesis significantly accelerated HCC formation in mice and total 480 tumors were collected. Sequence of their transposon insertions sites identified 1917 statistically significant CCGs and 3 potent trunk drivers, Rian, Hras and Kras, highlighting the importance of Ras signaling in hepatocarcinogenesis. Metabolic genes were significantly enriched in these CCGs, suggesting strong link between metabolic dysregulation and HCC development. In the pooled shRNA screen, while only 4 tumors were developed following 84 injections of IFLCs transduced with negative control (NC) shRNA, 71 tumors were observed following 398 injections of IFLCs transduced with pooled CCG shRNAs, which is a highly significant difference. Eleven pools individually showed significantly higher tumor formation rates than NC shRNA. Sequence of 61 tumors in these 11 pools identified 27 TSGs targeted by 30 highly enriched shRNAs. Three of twenty-seven TSGs, including *Aldh2*, *Ralgapa2* and *Kif1b*, were targeted by two shRNAs. Nine of them showed a negatively correlation between their expression and patient poor survival in human HCCs. Thirteen of them negatively regulated cell proliferation in human HCC cell lines *in vitro*. Among these 13 TSGs, top four anti-proliferative genes, *Acaa2*, *Hbs1l*, *Ralgapa2*, and *Ubr2*, upon their individual knockdown, significantly accelerated *in vivo* tumor growth in multiple human HCC cell lines. Tumor suppressive role of *Ralgapa2* was mediated through inhibition of Rala and Ralb, revealing an oncogenic role of Ral pathway in HCC. Lastly, we show that dual inhibition of Ras downstream pathways, Ral and Raf, by RBC8 and Sorafenib, synergistically suppressed *in vitro* HCC cell proliferation and *in vivo* tumor growth. **Conclusion:** Two-step forward genetic screens in mice provide a rich resource for identifying new drivers of HCC and potential new therapeutic targets.

Unmasking hidden norovirus epitopes to improve diagnostics

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Noroviruses are the major cause of acute epidemic nonbacterial gastroenteritis worldwide. They are highly communicable and have a low infectious dose. The lack of sensitivity of rapid diagnostic assays makes it difficult to control or prevent noroviruses infection. Previously, we developed a group of MAbs by immunization of mice with virus-like particles (VLPs) derived from Norwalk virus and Snow Mountain virus. We further utilized some of these MAbs to develop sandwich methods to target antigens or epitopes that are shared across multiple norovirus strains and detect norovirus infection. However, some of MAbs (such as NV3 or NV37) did not show promising results when used to coat wells to capture noroviruses, although these same MAbs interacted well with both GI and GII viruses initially bound to wells. These results suggested the epitopes recognized by these MAbs may be hidden in intact virus particles. To unmask the hidden epitope(s) and improve the ability to use these MAbs to capture virus with broad reactivity and perhaps increased sensitivity, we tested whether pre-treatment of samples using modified methods (copper sulfate or citrate buffer) reported to inactive animal noroviruses could enhance the ELISA virus detection signal. We found that copper sulfate did not change the ability of NV3 or NV37 by themselves to capture virus by ELISA. Instead, copper sulfate (1M-12.5mM) treatment increased the detection signal for clinical stool samples (n=8) using a combination of MAb with a single chain antibody (A9) [NV23/A9or of NS22/A9], but not NV23/polyclonal antibody (PAb) combinations. Copper sulfate (1M) further increased the detection sensitivity with lower sample concentration using the NV23/A9 combination. This result suggests copper sulfate facilitates antibody/antigen recognition and enhances the reaction signal. We also found that citrate buffer pre-treatment (50mM sodium citrate, pH 6, at 37^0C) increased signals in sandwich ELISAs. Citrate buffer dramatically increased the ability of NV3 and NV37 to capture the majority of GI and GII group viruses. Citrate buffer also helped both NV23/A9 and NV23/PAb combinations to increase the detection signal in tested clinical samples (n=15). Finally, citrate buffer increased the signal using MAb NS14/A9 for sandwich detection, with even GI group samples yielding a positive reaction. Moreover, citrate buffer increased the detection sensitivity using lower concentrations of stool sample. These results demonstrate that citrate buffer facilitates antibody/viral antigen interactions and allows MAbs recognition of norovirus capsid antigen targeting a broader range of epitopes. These data indicate that the treatments described above likely alter the capsid structure to allow exposure of buried epitopes and subsequent virus detection.

YAP1 mediated CDK4/6 activation confers radiation resistance in esophageal cancer

- Rationale for the combination of YAP1 and CDK4/6 inhibitors in EC

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Background: Esophageal carcinoma (EC) is a lethal disease with high incidence globally and often exist therapy resistance. Alterations (either overexpression or amplification) of YAP1 and CDK4/6 were found frequently in esophageal cancer. Deregulation of these pathways may represent key elements for resistance in esophageal cancer. **Methods:** Expression of YAP1 and CDK4/6 were examined in esophageal tumor tissues as well as cell lines using immunohistochemistry and immunoblotting. Inducible YAP1 overexpression in EC cells by lentivirus system was performed to test YAP1 mediated CDK4/6 expression and activation and association with radiation resistance. YAP inhibitor CA3 and CDK4/6 inhibitor Lee001 were used to test their antitumor activities in vitro and in vivo. Cell proliferation assay (MTS), Flow cytometry and immunofluorecence and tumor sphere formation assay were performed to test cycle distribution, cancer stem cell (CSC) population maintenance and over growth and therapy resistance. **Results:** We demonstrate that overexpressed YAP1 is positively associated with CDK4/6 expression in multiple cell systems. Overexpression YAP1 by lentivirus system in EC cells up-regulates expression of CDK4/6 at level of its transcription, while CA3 was able to decrease activation of CDK4/6 and phosphorylation RB and cell cycle progression induced by YAP1. Interestingly, radiation resistant cells are enriched CSC population and demonstrated increased level of both YAP1 and CDK4/6, while blocking the YAP and CDK4/6 pathway by using their inhibitor CA3 or Lee001 reduced EC cell survival and CSCs properties especially in resistant cells. Most importantly, we found combination of Lee001 and CA3 performed an extremely efficient anticancer effect to EC cells with high YAP1 and CDK4/6 as well as radiation resistant cells. **Conclusions:** Our data provide evidence that YAP1 mediated CDK4/6 up-regulation plays an important role in conferring radiation resistance in esophageal cancer cells. Targeting both YAP1 and CDK4/6 may provide novel therapeutic strategies in EC.
Keywords: esophageal cancer, stemness pathways, Cancer stem cells, radiation resistance

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The authors have no conflict of interest to declare.

Impact of oral feeding *Lactobacillus reuteri* DSM17938 on microbial composition of feces and CD62L$^+$T cells in intestinal mucosa of healthy breast-fed mouse pups

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BACKGROUND: *Lactobacillus reuteri* DSM17938 (LR17938) has been shown to have anti-inflammatory effects in animal models of intestinal inflammation. Early administration of LR17938 in infancy may also improve gut health by reducing pathogen colonization and facilitating immune surveillance. L-selectin (CD62L) is normally highly expressed in naïve T cells, which utilize CD62L expression to facilitate immune surveillance by programming T cells. We hypothesize that early administration of LR17938 will change microbial composition and increase resident CD62L$^+$ cells in the gut.
OBJECTIVES: To analyze the effect of early oral feeding LR17938 on gut microbiota and mucosal CD62L-expressing T cells in healthy dam-fed mouse pups.
METHODS: C57BL/6J (WT, n=6) mice were fed LR17938 (10^7 cfu/day, daily) (WTL, n=5) by gavage at 8 days of life (DOL) for 2 weeks. Cecum/rectum contents were collected at DOL 22. The composition of the stool microbiota was analyzed using high-throughput sequencing analysis of PCR-amplified 16s rRNA genes. Bacterial diversity, species composition, and abundance were assessed using the QIIME. Immune cells isolated from the mesenteric lymph nodes (MLN) and intestines (INT) of each mouse were stained with T cell markers of CD4, CD8, and CD62L and analyzed by flow cytometry.
RESULTS: Principal coordinates analysis indicated that oral feeding of LR17938 is associated with a shift in microbial community composition and an increase in the relative abundance of the phylum *Firmicutes* (37.7% by LR17938 compared to 17.9% without LR17938, $p<0.05$) and the genus *Parabacteroides* (44.7% by LR17938 compared to 12.9% without LR17938, $p<0.001$). *Parabacteroides* species have been reported to produce bacteriocins which protect against the invasion of pathogens. *Anaeroplasma* was undetectable at DOL8 and 15, but its relative abundance was 12.8% on DOL22, which was decreased by LR17938 (0.3%, $p<0.001$). *Bifidobacterium* from breast milk generally represents an important commensal, being among the first microbial colonizers of the gut. In our study, *Bifidobacterium* was only detectable on DOL8 (0.3%), and it was undetectable on DOL15 or 22. *Lactobacillus* was 33.7% on DOL8 and decreased to 3.9% on DOL15, and 0.1% on DOL22. However, lactobacilli were increased to 12.5% on DOL22 after feeding LR17938. We tested the % of CD4$^+$, CD8$^+$ T cells and CD62L expressing-naïve T cells in the MLN and INT of mice. We found that LR17938 increased the % of CD62L$^+$CD8$^+$ cytotoxic T cells in the INT ($p<0.05$) and MLN ($p<0.01$).
CONCLUSIONS: Early oral administration of LR17938 to healthy dam-fed mice resulted in microbiota profiles that would be predicted to be beneficial to gut health. The increased % of CD62L$^+$CD8$^+$ T cells by LR17938 indicating that LR17938 may promote immune surveillance by increasing the number of cytotoxic T cells in healthy individuals.

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SPDEF shifts the transcriptional targets of activated β-catenin to enforce tumor quiescence

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Background: Colorectal cancer (CRC) carcinogenesis is driven by a series of genetic and epigenetic changes that results in the oncogenic transformation of normal colonic mucosa. Canonical Wnt/β-catenin signaling pathway activation, with resultant high β-catenin transcriptional activity, is frequently implicated human CRC (~ 90% of CRC); however, there are currently no treatments targeting this pathway. We have previously reported that SAM Pointed Domain Ets transcription Factor (SPDEF) is a colonic tumor suppressor that negatively regulates canonical Wnt/β-catenin signaling. In agreement with the tumor repressor role of SPDEF, the absence of SPDEF enhances intestinal tumor formation, while re-expression of SPDEF inhibits colon adenocarcinoma proliferation in both genetic ($Apc^{min/+}$) and chemically colitis-associated (AOM/DSS) CRC models. **However, the molecular mechanism by which SPDEF mediates colorectal tumor repression is still largely unknown.** Here we aim to elucidate the molecular mechanism that SPDEF-mediated repression of canonical Wnt/β-catenin signaling in CRCs.

Material and Methods: To achieve our goal, we directly analyzed the effects of SPDEF expression in β-catenin-driven intestinal tumors *in vivo* using a new inducible mouse model (*Lgr5*^{CreERT2}; *β-catenin*^{exon3}; *Rosa26*$^{rtta\text{-}ires\text{-}EGFP}$; *TRE-Spdef*) and human colon cancer xenografts. Moreover, wild type or truncated SPDEF mutants were used for β-catenin transcriptional activity assay, co-immunoprecipitation, and chromatin immunoprecipitation in human colon cancer cell lines.

Results: In this study, we find that the transcription factor SAM Pointed Domain containing ETS transcription Factor (SPDEF) is sufficient to inhibit β-catenin-driven intestinal tumorigenesis and shrink established tumors in both transgenic mice and xenografted human colon cancer cells. SPDEF inhibits canonical β-catenin transcriptional activity through protein-protein interaction, independent of its DNA binding capacity. We find that SPDEF disrupts the binding between β-catenin and its DNA binding partners TCF1 and TCF3, but not LEF1 and TCF4, selectively displacing β-catenin from the promoter/enhancer regions of cell cycle genes without affecting Lgr5+ intestinal stem cell signature genes. Consistent with this observation, re-expression of SPDEF enforces a quiescent state on β-catenin-driven cancer stem cells *in vivo*. **Taken together, for the first time, we unveil a novel mechanism in which SPDEF shifts the transcriptional targets of activated β-catenin to regulate the active and quiescent switch in cancer stem cells.** These findings provide insights into the mechanisms that can regulate cancer stem cell quiescence, and offer a novel angle for targeting canonical Wnt/β-catenin transcriptional machinery as a therapeutic strategy.

Colonization with *Bifidobacterium dentium* alters colonic mucus and serotonin production in a germ-free mouse model

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In humans, bifidobacteria are the predominant genus of the infant intestinal microbiota, comprising up to 80% of the bacterial community. *Bifidobacterium* species are detectable within days after birth, and in some children this *Bifidobacterium* predominant profile is observed as early as the first week. *Bifidobacterium* abundance declines after the introduction of solid foods, and this decline persists into adulthood where *Bifidobacterium* spp. make up less than 10% of the adult gut microbiota. In addition to changes in abundance between infants and adults, the species composition of the *Bifidobacterium* population also varies significantly. *Bifidobacterium* species are applied for probiotic prevention and treatment of pathologies typical of newborns such as necrotizing enterocolitis and studies show a correlation between enteric pathologies and decreased populations of *Bifidobacterium* spp. Early colonization with select species contributes to proper immune development, confers resistance to colonization of potential pathogenic microorganisms, and improves intestinal motility. However, these protective abilities appear to be species specific, and we currently lack detailed knowledge regarding how certain infant-type *Bifidobacterium* species alter the intestinal niche. *Bifidobacterium dentium* is one such organism which is known to be present in the infant gut microbiota, but effects of colonization with this organism remain largely undescribed. We hypothesized that colonization with *B. dentium* would increase colonic mucus production and serotonin concentrations. In order to address this hypothesis, adult germ-free mice were mono-associated with *B. dentium* over the course of two weeks. Colonization was tracked via collection of fecal pellets, and *B. dentium* was observed to colonize the murine intestine at a density of approximately 1x10e8 CFUs/g feces. Histological investigation revealed that short-term colonization with *B. dentium* increased goblet cell numbers in the colon of *B. dentium*-fed mice relative to germ-free mice. Alcian blue and mucin-specific staining in conjunction with qRT-PCR demonstrated an accompanying increase in colonic mucus production. Additionally, luminal contents were assayed for serotonin via ELISA and we observed an increase in ileal but not colonic serotonin concentrations. *B. dentium*-mediated mucus and serotonin production may be beneficial for pathogen resistance and gut motility, making this species a potential candidate for use as a probiotic therapeutic agent in infants.

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Fecal Indole Correlates With Loss of Microbiome Diversity in Hematopoietic Stem Cell Transplant (HSCT) Recipients With and Without Intestinal Graft Versus Host Disease (iGVHD)

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Background: Antimicrobial and immunosuppressive therapy during HSCT results in loss of microbial diversity and injury to the intestinal epithelium which are risk factors for iGVHD. Indole, produced by the metabolism of phenylalanine by *Enterobacteriaceae* and some anaerobes, is a major quorum sensing signal that inhibits pathogenic bacterial injury and strengthens the intestinal epithelium mucosal barrier. **Methods**: Fecal indole concentrations were measured in stools from healthy volunteers (HV) without a history of antibiotic exposure, use of immunosuppressants or recent travel (n=18), HSCT recipients at the time of conditioning (n=21) and patients with biopsy proven iGVHD (n=10) using a colorimetric assay. Fecal bacterial DNA from 13 HSCT and 6 iGVHD individuals was isolated and 16S rDNAV4 PCR sequencing performed using an IlluminaMiSeq 2x250 platform. **Results**: Comparison of indole concentrations amongst the 3 groups showed a significant difference between HV & HSCT (3498 vs. 812 µM; p=0.0001), HSCT & iGVHD (812 vs. 233µM; p=0.02), and HV & iGVHD (3498 vs 233 µM; p=0.0001). Fecal microbiome comparative analysis showed a lower number of operational taxonomic units in patients with iGVHD compared to HSCT at the time of conditioning with dominance of *Lactobacillus, Streptococcus, Citrobacter* and *Enterococcus* in patients with GVHD. Whereas *Bacteroides, Escherichia, Shigella* were the major species identified in patients without GVHD. **Conclusion**: When compared to HV and to HSCT recipients at the time of conditioning, fecal indole levels of patients with iGVHD were decreased. Low indole concentrations in iGVHD correlated with the loss of microbial diversity, particularly with the loss of *Escherichia-Shigella* and *Bacteroides* species. Fecal indole may be of use in the early identification of patients at risk for HSCT related iGVHD. Prospective studies that evaluate the microbiome shifts in diversity, indole and other tryptophan metabolites in patients at risk for HSCT related iGVHD are in progress.

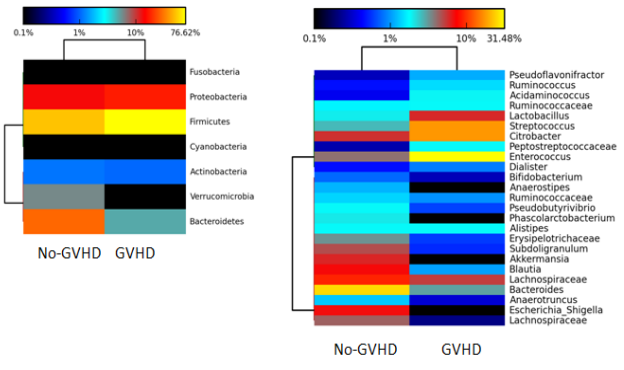

Figure 1. Phylogenetic microbial diversity in patients with iGVHD and in patients at the time of conditioning for HSCT

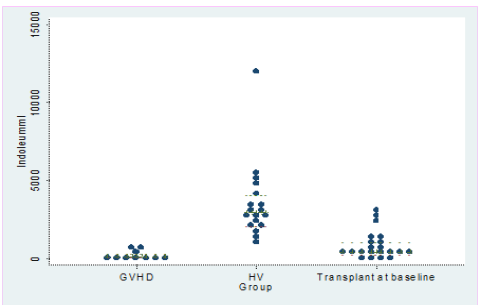

Figure 2. Dot plot graph representing the fecal indole concentrations measured in three groups of subjects (iGVHD, HV and Transplant patients at baseline). +++ Represents median values and ---- Error bars.

P2X3 purinergic receptor overexpression is associated with poor recurrence-free survival in patients with hepatocellular carcinoma

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Background: Hepatocellular carcinoma (HCC) is the second leading cause of cancer deaths worldwide. Recent studies suggest that extracellular ATP-mediated activation of P2 purinergic receptors induce hepatocyte proliferation *in vitro* and P2 purinergic receptors are overexpressed in certain cancer tissues. However, the pathophysiologic relevance of purinergic signaling in HCC remains unknown. The purpose of this study was to examine the role of P2 purinergic signaling in the pathogenesis of HCC and characterize extracellular nucleotide effects on HCC cell proliferation.

Hypothesis: *Dysregulation of purinergic signaling facilitates aberrant cell proliferation underlying hepatocellular carcinogenesis.*

Methods: Two independent HCC patient cohorts (n=42, n=188), publicly available human datasets, mouse liver tumors (Mst1/2$^{-/-}$) and human derived HCC cell lines (Huh7, Hep3B, SNU387, PLC/PRF/5) were analyzed for P2 purinergic receptor (15 isoforms) expression. Nucleotide treated HCC cell lines and primary human hepatocytes were evaluated for effects on proliferation and cell cycle progression by Western blotting, qRT-PCR, MTT assay and BrdU incorporation. Pharmacological and genetic manipulation of HCC cell lines was used to asses a role for P2X3.

Results: Our studies suggest that multiple P2 purinergic receptor isoforms are overexpressed (≥2-fold) in liver tumors, as compared to uninvolved liver, and dysregulation of P2 purinergic receptor expression is apparent in HCC cell lines, as compared to human primary hepatocytes. High P2X3 purinergic receptor expression is associated with poor recurrence-free survival (RFS), while high P2Y13 expression is associated with improved RFS. Extracellular nucleotide treatment alone is sufficient to induce cell cycle progression, via activation of JNK signaling. Extracellular ATP-mediated activation of P2X3 receptors or P2X3 overexpression alone promotes proliferation in HCC cells. Interestingly, ATPγS mediated calcium signaling induced a distinct downregulation of cyclin D1 expression in Huh7 cells, which was associated with poor prognosis in HCC patients. Mouse tumors exhibit dysregulated expression of multiple P2 purinergic receptor isoforms as compared to WT while nucleotide treatment of Huh7 cells induced hippo target genes; cyclin E, amphiregulin, connective tissue growth factor (CTGF), Sox9 and survivin – all implicated in HCC pathogenesis.

Conclusions: Our analysis of HCC patients, Mst1/2$^{-/-}$ livers and HCC cells *in vitro* identifies a novel role for dysregulation of P2 purinergic signaling in the induction of a hyper-proliferative HCC phenotype and identifies P2X3 purinergic receptors as potential new targets for therapy.

Regulation of histamine production by *Lactobacillus reuteri* by other bacterial metabolites

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Amino acid metabolism by the gut microbiome is critical for human health. The decarboxylation of L-histidine to histamine by *Lactobacillus reuteri* reduces the production of the pro-inflammatory molecule, TNF. This work examines histamine and histidine metabolism by *L. reuteri*, as well as the effects of microbial histidine metabolism on the host. Supernatants from bacterial cultures with $^{13}C_6{}^{15}N_3$ L-histidine were processed and NMR was used to identify novel bacterial metabolites. We have determined that the addition of carnosine (β-alanyl-L-histidine) in the media significantly increases histamine production. We have also determined that media concentrations of acetate and folate have effects on viability, histamine production and TNF production by THP-1 cells treated with bacterial supernatants. Currently we are working to determine the mechanism by which carnosine, folate, and acetate affect histamine production. Our plans include the oral administration of $^{13}C_6{}^{15}N_3$ L-histidine to mice and co-culture of *L. reuteri* with human ileal enteroids. These studies will determine the effects of *L. reuteri* and histidine/histamine metabolism on the human intestinal epithelium. Together the data will facilitate understanding of bacterial metabolites produced by this probiotic and their effects on the mammalian intestine.

Replication of GII.3 human norovirus in human intestinal enteroids

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Human norovirus (HuNoV) is the leading cause of gastroenteritis worldwide. Although HuNoV was discovered over 40 years ago, our understanding of HuNoV is still limited due to the lack of a robust *in vitro* cultivation system. Recently novel *ex vivo* cultures termed human intestinal enteroids (HIEs), which proliferate from stem cells from crypts of human intestinal tissue, have been developed. We have evaluated whether HuNoV will replicate in these novel minigut cultures using jejunal HIEs (jHIEs).

Stool filtrates containing GII.3 HuNoV TCH04-577 strain were inoculated onto monolayers of jHIE for 1 hour at 37^{o}C, washed to remove unbound virus and cultured for 3-7 days. To mimic the natural intestinal environment, human bile was added to the medium prior, during and following inoculation. The cells and medium were harvested, and virus replication was evaluated from extracted RNA by qRT-PCR. Increases of viral genome copies were observed at 6 hours post infection (hpi) and reached a plateau at 24 hpi, where copy numbers were over one log higher than that at 0 hpi. This increase was only observed when infection was carried out in the presence of bile. Infection of multiple lines of jHIEs is reproducible and virus replication can increase up to 2.1 logs over 7 days. Several experiments were performed to characterize the active component in bile. Heat treatment of the bile or sonication did not change the enhancing effect of the bile, indicating that the active component is not a protein or a lipid micelle. Other experiments showed that bile acids, especially conjugated bile acids, are one component of bile that allow HuNoV infection.

These results demonstrate that jHIEs support *in vitro* GII.3 HuNoV replication, which is only achievable when the native intestinal milieu is mimicked. Ongoing work is evaluating other factors that may further optimize HuNoV replication.

Alterations in the gut microbiota can elicit hypertension in rats

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Gut dysbiosis has been linked to pathological conditions outside of the gastrointestinal system. Our lab has recently demonstrated that gut dysbiosis is responsible for hypertension induced by obstructive sleep apnea (Durgan, *Hypertension*, 2015). The purpose of this study was to determine if the gut microbiota has a role in other types of hypertension. We tested the hypothesis that hypertension could be induced in a normotensive strain of rats (WKY) or attenuated in a hypertensive strain of rats [spontaneously hypertensive rats (SHR)] by exchanging the gut microbiota between the two strains.

Cecal contents from 20 wk SHR rats were pooled; similarly, cecal contents from 9 wk WKY rats were pooled. Each of the pooled samples was diluted and the supernatants from each of the pooled samples were aliquoted and frozen to use for inoculating recipient rats. Three week old recipient WKY and SHR rats were gavaged daily for 10 days with broad spectrum antibiotics to reduce the native microbiota load. Two days after the last antibiotic administration, the cecal supernatant was gavaged into the recipient rats daily for four consecutive days and weekly thereafter. Cecal supernatant from the SHR donors was gavaged into WKY and SHR rats. In a similar manner, cecal supernatant from the WKY donor pool was gavaged into WKY and SHR rats (n=6 to 7/group). Group designations are indicated in the following example: “WKY g-SHR” represents WKY rats gavaged with cecal contents from SHR rats. Fecal pellets were collected weekly for identification of the microbiota by sequencing the 16S ribosomal RNA gene. Systolic blood pressure (SBP) was measured weekly using tail-cuff plethysmography.

Prior to cecal gavages, SBP (~143 mmHg) was similar in SHR and WKY rats. At 12 weeks of age, SBP was 182 ± 8 and 156 ± 8 mmHg in WKY g-SHR and WKY g-WKY, respectively ($p=0.02$). Although the SBP for SHR g-WKY tended to be 12-17 mmHg less than SHR g-SHR between the ages of 10 and 16 weeks, the differences were not significant. The microbiome of the WKY rats was significantly different depending on the strain of the gavage donor. The Firmicutes : Bacteroidetes ratio increased in WKY rats gavaged with supernatant from SHR rats; however, this change did not reach statistical significance. At the genera level there were a number of highly significant differences in the WKY microbiota which were dependent on the strain of the donor gavage.

Although altering the gut microbiota in SHR had no significant effects on SBP, we did find that inoculating WKY rats with SHR microbiota significantly increased SBP. We show that (1) the gut microbiota was significantly altered by, and was dependent upon, the donor strain of the gavage; and (2) gavaging SHR cecal contents into WKY rats was capable of increasing SBP in WKY rats. We conclude that the contents of the gut can directly affect systolic blood pressure.

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The Effect of Flavonoids Luteolin and Quercetin on Colon Cancer Cells in Vitro

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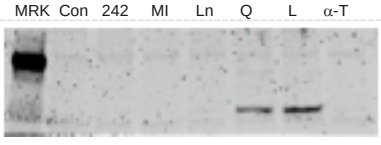
Colon cancer is a major public health concern in both developed and developing countries. Several plant-derived anti-cancer agents including taxol, vinblastine, vincristine, the campothecin derivatives, topotecan, irinotecan and etoposide are in clinical use. Flavonoids are present in the human diet and comprise many polyphenolic secondary metabolites with broad- spectrum pharmacological activities and have been touted for their antioxidant properties and for prevention and treatment of hypertension, inflammatory diseases and cancer. Cancer chemoprevention, by the use of natural, dietary or synthetic agents has been shown to suppress carcinogenic progression and has become an appealing strategy to combat the dogma associated with increasing cases of cancers worldwide [Tsao, A. S. et al.; PMID 15195789 (2004)]. A positive correlation between flavonoids-rich diet (from vegetables and fruits) and lower risk of colon cancers leads to a question that whether flavonoids mediate the cancer preventive effects interacting with the cellular proteins affecting on cellular signaling pathways and/or inducing the differential expression of the genes. Both luteolin (L) & quercetin (Q) are flavonoids found in a wide variety of other dietary fibers [e.g., burdock (daisy family)] and may directly contribute to the anticancer effects of quality dietary fiber. Eminium regelii is a tuberous herb (Araceae family) that is particularly rich in flavonoids content that has been advocated for gastrointestinal cancer prevention, but the MOA has not been well studied. **STUDY GOAL:** to determine if the phenolic acids L and/or Q have true therapeutic potential. **METHODS**: The activity of L & Q was tested in comparison to other candidates (e.g., (E)-methyl isoeugenol) in vitro for their stress effects on PARP, Capsase-3, apoptosis, Capsase-9, p53 and SAPK/JNK using the HCT-15. **RESULTS**: *Summarized in Figure 1.* Both, L & Q showed significant phosphorylation and activation of p53. Furthermore, experiments also showed that L & Q upregulated stress-activated protein kinases (SAPK/JNK) and increased PARP cleavage was observed after L&Q exposure with formation of an 89 kDa catalytic domain fragment as an indication of apoptosis induction. When cells were exposed to L & Q, several other apoptosis signaling pathways were affected. L & Q appeared capable of inducing the caspase cleavage 3 and 9. **DISCUSSION**: (1) activation of p53 by L&Q may compromise colon cancer cells and might prove synergistic with other cytotoxic drugs, and (2) L & Q activated SAPK/JNK, while the other control drugs showed no activation, which might be valuable in cases where p53 mutations are a factor. The findings suggest that a true therapeutic potential exists for L & Q beyond alternative medicine uses. Although the chemical nature and L & Q are similar in structure, their MOA on specific signaling pathways could be different. Additional assays should be done using esophageal, pancreatic and hepatic cancer cell lines. Other promising anti-cancer agents include flavopiridol, roscovitine, combretastatin A-4, betulinic acid and silvestrol and should be similarly tested and tests should be done in the IBD setting too because the similarly observed proliferative responses [James SP; PMID 1717381 (1991)].



The **p53** plays a major role in DNA damage. Activation of p53 can lead to either cell cycle arrest and DNA repair or apoptosis. L and Q induced p53 phosphorylation.

FimH mediates extraintestinal pathogenic *E. coli* invasion and translocation

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Bacterial translocation across the intestinal epithelium of immunocompromised patients is a major medical problem. Extraintestinal Pathogenic *E. coli* (ExPEC) are the number one isolated Gram-negative bacteria in gut-derived bacteremia and sepsis. Unlike *E. coli* associated with diarrheal disease, ExPEC are part of the intestinal microflora and colonize the gut of a substantial fraction of the healthy population. Even though several virulence factors and their respective roles in ExPEC pathogenesis have been described, these studies have primarily focused on the urinary tract and have not been directly linked to translocation out of the gastrointestinal tract. Using Caco-2 cells and a novel *ex vivo* monolayer model generated from human intestinal enteriods, we demonstrate that ExPEC strain CP9 binds to and invades intestinal epithelium. This process is in part dependent on the mannose binding type 1 fimbriae adhesin FimH. An ExPEC strain lacking *fimH* displayed reduced binding and invasion and was compromised in its ability to translocate through intestinal monolayers formed on Transwell inserts, including those isolated from untransformed cells differentiated in human intestinal enteroids. A similar phenotype was also observed in a murine model of chemotherapy-induced translocation. However, ExPEC stains lacking the salmochelin siderophore receptor, *iroN*, or the cytotoxic necrotizing factor 1 toxin, *cnf-1*, invaded and translocated as well as wild-type ExPEC indicating the specificity of this process. Collectively, this study indicates that FimH is important for ExPEC translocation which can potentially serve as a therapeutic target to prevent this process and highlights an additional application for enteriod engineering.

Mouse models of early-life protein-energy undernutrition reveal striking bile acid dysregulation, coagulopathy, steatosis, intestinal dysmotility, and microbial dysbiosis

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Background: Protein-energy undernutrition contributes to half of all childhood deaths and perpetuates a "vicious cycle" of hepatic, intestinal, and metabolic co-morbidities that make it difficult for severely undernourished children to assimilate nutrients and grow at optimal rates. Why these co-morbidities occur and how they may be prevented is unknown. We sought to establish three mouse models of early-life undernutrition to facilitate discovery of mechanisms underlying multiple aspects of this vicious cycle.

Materials & Methods: Undernutrition was established in C57BL/6 mice by three methods. First, in timed separation, newborns were isolated for 12 h/d from lactating dams. Second, mothers were fed low-protein low-fat Regional Basic Diet (RBD) chow. Samples were obtained from these suckling pup models at 2 wk. Third, pups of RBD mothers were weaned to RBD chow, and continued on this diet until 6 wk. Metabolomic and bile acid profiles were generated using mass spectrometry; livers were examined by routine histology and plasma chemistry; motility was assessed by measuring transit of gavaged dye through the intestine; and microbial community composition and function were determined using next-generation metagenomic sequencing.

Results: Both pup models of undernutrition yielded 30% decreased body weight and 10% decreased length at 2 wk. Likewise, RBD 6-wk males (but not females) were underweight and stunted. Bile acid pools were strikingly altered in all models, including reductions in hepatic and fecal α/β-muricholic acid and taurodeoxycholic acid to <1% control levels. All three models exhibited profound coagulopathy, with plasma international normalized ratio (INR) >12.4 and partial thromboplastin time (PTT) >100. Steatosis was observed in RBD 6-wk male livers. Small bowel transit was delayed in both groups of undernourished pups, while 6-wk males had striking fecal retention; this dysmotility was linked to low plasma serotonin. Undernutrition induced profound microbial dysbiosis, with enrichment of *Akkermansia muciniphila* and decreased *Alistipes* and *Bacteroides* spp. with loss of microbial N-linked glycan-metabolizing genes.

Conclusions: Early undernutrition leads to bile acid dysregulation, coagulopathy, steatosis, intestinal dysmotility, and microbial dysbiosis. These models are now serving as powerful tools to discover how bile acid levels, coagulation, and long-term metabolic changes are regulated by hepatocyte nuclear hormone receptors during undernutrition, and how serotonin levels, intestinal motility, and nutrient assimilation from the undernourished diet are regulated by gut microbes.

Granulomatous changes in upper gastrointestinal tract biopsies of pediatric ulcerative colitis patients.

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Background: Given considerable overlap in symptomatology and histology, differentiating ulcerative colitis (UC) and Crohn disease (CD) can be clinically challenging especially in children. The presence of granulomatous inflammation has traditionally been attributed to CD. However, isolated cases of crypt-associated giant cells and granulomas have been observed in colonic biopsies of patients with UC. This phenomenon has not yet been described in the upper gastrointestinal (GI) tract in association with UC. We present seven cases with clinical course, imaging, and endoscopy supportive of pediatric UC, where granulomatous changes along with giant cells were detected on upper GI histopathology.

Methods: We identified 7 pediatric patients with histological presence of upper GI granulomatous lesions, whose inflammatory bowel disease was consistent with UC on the basis of symptoms, physical exam, laboratory findings, imaging and endoscopy. The cases were diagnosed between 2011 and 2015 at Texas Children's Hospital.

Results: Upon review of the duodenal and/or stomach biopsies obtained, giant cells and/or granulomas in near vicinity of gland destruction were seen. All cases were associated with profuse bloody diarrhea and moderate to severe pancolitis. Small bowel imaging did not reveal involvement in any of the patients. Standard therapies including steroids, mesalamine, 6-mercaptopurine and infliximab were utilized with subsequent improvement in the majority of the cases. One patient, however, required total colectomy within 2 weeks of diagnosis.

Conclusions: This is the first report on pediatric UC-associated granulomatous inflammation in upper GI tract biopsies. All cases involved moderate to severe pancolitis. We speculate that these lesions may be extra-colonic manifestations of intense UC where the granulomatous inflammation is associated with upper GI cryptolysis. Alternatively, the upper GI granulomatous changes may indicate a unique phenotype of UC in these pediatric patients since extraintestinal giant cell-associated disease, such as giant cell arteritis/myocarditis, has been reported in association with adult UC. These atypical histologic findings expand the diagnostic considerations that have to be incorporated during the differentiation between UC and CD in the pediatric age group.

Dysregulated arginine metabolism is linked to necrotizing enterocolitis in the premature piglet

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Necrotizing enterocolitis (NEC) is a disease affecting premature infants and is the leading cause of premature death due to intestinal disease. The etiology of NEC is not established but poor intestinal blood flow and inflammation are key factors; and NEC occurs after the onset of enteral feeds. Arginine is a conditionally essential amino acid that serves as the sole precursor of nitric oxide (NO). Thus, arginine availability is critical to blood flow regulation as well as innate immune responses and cell proliferation. Arginine becomes essential during high nutritional demand such as rapid growth or disease states, when the organs involved in synthesis cannot meet demands. The arginine demands of prematurity seem especially important since reduced plasma arginine is a feature of NEC. Citrulline is a non-protein amino acid that is mainly synthesized by intestinal enterocytes from ornithine; and citrulline is the endogenous precursor of arginine in tissues such as gut and kidney. Plasma citrulline is considered a 'biomarker' of gut mass and function but it is unknown whether intestinal citrulline synthesis is sufficient to meet the arginine needs of prematurity, and if arginine availability is linked to the risk of NEC.

We hypothesize that preterm infants have limited capacity for citrulline-arginine-NO synthesis and this contributes to the increased risk for NEC. We tested this in our preterm pig model of NEC that closely reflects human NEC by first measuring the expression of citrulline-arginine-NO genes in distal ileum of premature piglets (90% gestational age) with (n=9) and without (n=16) NEC. Expression of ornithine aminotransferase, an enzyme involved in citrulline production, was ~40% lower in NEC pigs ($P < 0.05$). Furthermore, the expression of enzymes that catabolize arginine were variable during NEC. Arginase I was increased ~100%, whereas arginase II was decreased ~60% in NEC vs. healthy pigs ($P < 0.05$). Arginase II activity is exclusive to the intestinal epithelial cells, and it is likely that reduced expression during NEC was due to intestinal atrophy. In contrast, arginase I is expressed in macrophages suggesting that the active inflammatory response during NEC may consume available arginine for ornithine synthesis, rather than NO production. Endothelial nitric oxide synthase expression was unchanged.

We recently completed a study investigating the in vivo rates of whole body citrulline, arginine and NO kinetics in preterm piglets (n=14) implanted with umbilical and jugular catheters; and 6 hr prior to enteral feeds, pigs received a primed:constant IV infusion of [$^{13}C_6$]arginine, [^{15}N-ureido]citrulline, [$^{15}N^{18}O_3$]nitrate, [$^{2}H_5$]phenylalanine and [$^{2}H_2$]tyrosine. The tracer infusion continued for 12 hr after the initiation of enteral feeds and the citrulline and nitrate infusion continued until death from NEC or 48hr. Enteral formula feeding robustly induced the development of NEC in 92% of piglets. Plasma nitric oxide surged prior to and after the first enteral feeding in all animals; and survivors (n=3) trended towards a greater NO response ($P > 0.05$). Plasma arginine, citrulline and ornithine values will be presented in response to the first feed. Rates of citrulline, arginine and nitrate appearance are forthcoming, as are the rates of citrulline conversion to arginine, arginine to citrulline (NO production), and phenylalanine into tyrosine (protein turnover). We postulate that dysregulated citrulline-arginine metabolism occurs in the development of NEC, and that intestinal macrophage-mediated arginine consumption for ornithine synthesis may limit local NO availability for vasoregulation of blood flow and tissue oxygenation.

Characterization of oligomer/conformation specific monoclonal antibodies to the rotavirus nsp4 coiled-coil domain

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Rotavirus (RV) is the most common cause of life-threatening infantile gastroenteritis. RV nonstructural protein 4 (NSP4) is first described viral enterotoxin, and is a virulence factor that plays a role in the pathophysiology of RV-induced diarrhea. NSP4 is secreted from virus-infected cells and binds to non-infected intestinal cells where it can elicit a phospholipase C-dependent signaling cascade that elevates intracellular Ca^{2+} and subsequent chloride secretion. NSP4 is also thought to activate the enteric nervous system. Changes in cytoplasmic Ca^{2+} levels are sensed by a myriad of Ca^{2+} binding proteins that undergo conformational changes upon Ca^{2+} binding. In the absence of a crystal structure of full-length NSP4, crystallographic studies of the NSP4 coiled-coil domain (CCD, residues 95 to 146) have provided valuable structural information on the possible oligomeric states of NSP4. Our recent results indicate that the NSP4 CCD exhibits structural plasticity and can adopt both Ca^{2+}-bound tetrameric and Ca^{2+}-free pentameric states and undergo a reversible transition between these states in response to variation in pH. We are seeking to understand the functional differences between the distinct oligomer-specific conformations of the NSP4 CCD using monoclonal antibodies (mAbs). These studies aim to distinguish different functional oligomeric forms of NSP4 in RV-infected cells and determine if they interact with different viral or host proteins. NSP4 WT-CCD can transition between tetramer and pentamer states; however, the Ca^{2+} -binding site mutant NSP4 E/Q-CCD forms a stable pentameric structure. We immunized mice with this pentameric mutant and derived clonal hybridoma cells, which were tested for specific antigenic reactivity by using ELISA, western blot, and immunofluorescence (IF). Five mAbs that detected different NSP4 localization patterns in RV-infected cells were selected and further characterized. A single mAb (1096) detects only the pentameric NSP4 E/Q-CCD mutant strongly by western blot and detects a subset of NSP4 by IF. Further characterization of 1096 mAb using bilayer interferometry kinetic analysis confirms that this mAb 1096 specifically binds to a pentameric form of NSP4 CCD. These preliminary results suggest that mAb 1096 detects pentameric NSP4 in RV-infected cells. Our results indicate that these antibodies may be useful reagents to distinguish different oligomeric forms of NSP4 and to delineate the mechanisms by which different structures of NSP4 regulate RV replication and morphogenesis.

Human intestinal epithelial response to human rotavirus virus infection using enteroids

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Introduction: A limitation in translational research in the gastrointestinal tract has been the absence of models that recapitulate the diverse nature of the epithelium. Human intestinal enteroids (HIEs) contain the normal complement of intestinal epithelial cell types (stem, enterocyte, goblet, enteroendocrine, and Paneth cells). We have utilized HIE cultures as pre-clinical models to study the response of the epithelium to common viral pathogens such as human rotavirus (HRV), which kills ~500,000 children annually by causing dehydrating gastroenteritis. Studies on HRVs have been limited because they are difficult to culture in transformed cell lines and do not infect small animals.

Methods and Materials: We established HIEs derived from small intestinal tissue from healthy control subjects and showed that they support HRV infection and demonstrated previously unappreciated pathophysiologic and molecular responses to infection.

Results and Discussion: Undifferentiated HIEs, consisting primarily of immature enterocytes, Paneth and stem cells, were less susceptible to infection compared to fully differentiated HIEs that consist predominately of mature enterocytes, confirming in vivo findings that the villus enterocyte is the primary target of HRV infection and replication. Enteroendocrine cells also were infected suggesting signaling that may be related to pathogenesis. Infection altered cellular metabolism pathways and upregulated innate immune responses, primarily the type 3 interferon system. The HIEs also exhibit host range restriction with HRVs infecting 45% of cells within differentiated enteroids but animal rotavirus infecting <15% of cells in the same enteroids.

Conclusions: These findings establish HIEs as new models to understand the intestinal epithelial response to gastrointestinal infections such as HRV. HIEs can be used to address new questions about human hostpathogen interactions such as innate immune responses, stem cell activity, cell-cell communication within the epithelium, transport protein changes that account for diarrhea, and to identify and test new drug therapies to prevent/treat diarrheal disease.

Development of *Lactobacillus reuteri* as a biotherapeutic delivery system

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Lactic acid bacteria (LAB) are an attractive option for the development of therapeutic delivery systems (TDSs) due to their remarkable safety profile and ability to survive in the intestine. The probiotic LAB *Lactobacillus reuteri* 6475 (*LR* 6475) is a promising candidate for several reasons. This strain has anti-inflammatory activity and it is considered safe as it has never caused human disease. Additionally, we and others have developed genetic tools that allow for precision editing of its genome.

As proof of concept, we are engineering *LR* 6475-derivative strains that efficiently deliver or stimulate production of regenerating islet-derived protein 3-alpha (Reg3α) or interleukin-22 (IL-22). The Reg3α protein has antimicrobial activity against gram-positive bacteria including *Listeria monocytogenes* and vancomycin resistant enterococci, which is one of the leading causes of nosocomial diseases in the United States. IL-22 stimulates the production of Reg3α in the gastrointestinal tract and protects intestinal stem cells from immune-mediated tissue damage. The development of therapeutic delivery systems for Reg3α or IL-22 could potentially be used not only for the treatment of infectious diseases, but also for diseases where the integrity of the intestinal barrier is crucial, such as Graft Versus Host Disease (GVHD). Currently there are neither specific nor efficacious treatments for GVHD.

Thus far we have generated a strain containing a construct that allows the inducible expression of Reg3α with 95% of the total protein produced being soluble and more than 70% of the protein being secreted into the extracellular medium. We have also constructed two strains that secrete human IL-22, one using inducible expression and the other with high level constitutive expression of IL-22. The bioactivity of the secreted IL-22 has been confirmed *in vitro* through the induction of IL-10 and phosphorylation of STAT3 in colonic cells, and *in vivo* by stimulating the production of Reg3γ in animals gavaged with IL-22-secreting *LR*. We are currently working on strategies to optimize and increase Reg3α production by using *LR* antimicrobial peptide mutants, as well as evaluating the therapeutic effect of our TDSs using *in vivo* models of VRE colonization. We are also designing tools to achieve precise control over heterologous protein expression, including the development of a promoter and ribosomal binding site library and techniques to efficiently integrate genes into its genome.

Texas Medical Center Digestive Diseases Center
7th Annual Frontiers in Digestive Diseases Symposium:
Frontiers in Regenerative Medicine in Digestive and Liver Diseases

The unique inflammatory bowel disease phenotype of pediatric primary sclerosing cholangitis

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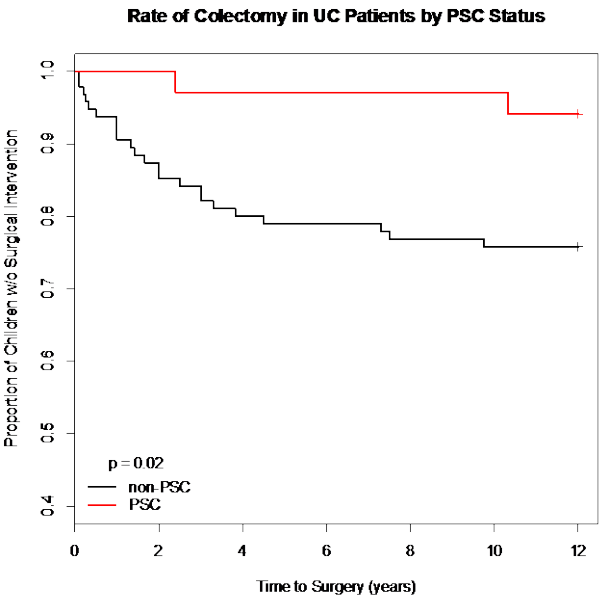
Background: Primary sclerosing cholangitis (PSC) is a progressive cholestatic liver disease characterized by inflammation and fibrosis of intra- and/or extra-hepatic bile ducts. PSC is strongly associated with inflammatory bowel diseases (IBD), and in adult patients with a milder form of ulcerative colitis (UC). The IBD phenotype of PSC in children, however, has been less well characterized. **Methods:** We performed a retrospective, single-center study to examine PSC-IBD in patients seen at Texas Children's Hospital (TCH) between January 2000 and June 2015. The data extracted from patient charts included IBD phenotype (Montreal Classification), clinical outcomes, medication history, and IBD-based hospital admissions. PSC-UC phenotype was compared to a cohort of non-PSC-UC patients from the same center. **Results:** Fifty-two (52) patients were identified with PSC. Thirty-nine (75%) had a co-diagnosis of IBD. Thirty-four (87.2%) of the IBD patients had UC (PSC-UC) and five (12.8%) had Crohn's Disease. We compared PSC-UC to 95 UC subjects without PSC (non-PSC-UC). Gender and ethnic distribution (table 1), as well as median age of UC diagnosis (11.72, 12.0, p=0.65) did not differ between PSC-UC and non-PSC-UC. PSC was diagnosed before, simultaneously, or after the diagnosis of UC in 11.8%, 58.8%, and 29.4% of patients, respectively. Among patients who had a full colonoscopic evaluation (27 PSC-UC, 50 non-PSC-UC), pancolitis was more common in PSC-UC than in non-PSC-UC (96.3% vs. 64%, p=0.0009). While no significant difference was found between PSC-UC and non-PSC-UC patients in thiopurine treatment (p=0.8372), PSC-UC patients were more commonly treated with Azathioprine (55.9% vs. 18.9%, p=0.0001) and less commonly treated with 6-MP (11.7% vs. 51.6%, p=0.0001) than non-PSC-UC patients. PSC-UC patients were less commonly treated with infliximab (8.8% vs. 37.9%, p=0.0011) and required fewer IBD related hospital admission (32.4% vs. 63.2%; p=0.0025) than non-PSC-UC patients. Disease progression to colectomy was also significantly less common (5.8% vs. 24.2%; p=0.02) in PSC-UC than in non-PSC-UC patients. Median time from diagnosis of UC to colectomy was 6.37 years and 2.5 years in PSC-UC and non-PSC-UC patients (p=0.0739), respectively. **Conclusion:** Pediatric PSC-IBD has a relatively mild form of pancolitic UC compared with non-PSC-IBD. Our findings are likely to provide useful information towards the etiology, management and future treatment of pediatric PSC and related IBD.



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PPARδ mediates its oncogenic function in gastric cancer through interaction and activation of Hippo coactivator YAP1

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Background: Despite the established functions of PPARδ in lipid metabolism, inflammation, and tumorigenesis, the mechanism underlying its role in gastric cancer (GC) development is unclear. We investigated the novel mechanism and functions of PPARδ in GC development. **Methods:** Immunohistochemistry and immunoblotting were used to detect the expression of SOX9 and YAP1 in tumor tissues of PPARδ-transgenic mouse and human tissues and GC cell lines. Immunoprecipitation and two yeast hybridization were used to identify the interaction between YAP1 and PPARδ full length and their domains, Luciferase activity and Chromatin-IP (ChIPs) were performed to identify the transcription and binding at the promoter of SOX9. Serial functional studies-proliferation, Invasion and tumor sphere assay were used to demonstrate the importance of PPARδ/YAP1 axis in GC malignancy. **Results:** we demonstrate SOX9, a target of the Hippo effector YAP1, is extensively upregulated in gastric tumors of villin-PPARδ transgenic mice and in majority of human gastric cancer tissues. SOX9 expression and transcription was dramatically induced by stably PPARδ cDNA expression in human gastric cancer cell lines. PPARδ induces SOX9 transcription through direct interaction with and activation of Hippo effector YAP1. We further demonstrate that PPARδ interacts with the YAP1 *C*-terminal transactivation domain and both TEAD and PPARE binding sites are required for SOX9 induction. Moreover, Genetic knock out or pharmacological inhibition of YAP1 abolishes PPARδ-mediated oncogenic functions and SOX9 induction. Importantly, the expression of PPARδ, YAP1 and SOX9 is correlated in around 500 cases of human GC tumor samples and high expression of them is associated with patients' poor survival respectively. **Conclusion:** These findings elucidate a novel mechanism for PPARδ promotion of gastric tumorigenesis by converging Hippo coactivator YAP1 and the PPARδ/YAP1/SOX9 axis is a novel therapeutic target in gastric cancer.
Keywords: Hippo coactivator YAP1, PPARδ, SOX9, Gastric cancer

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The authors have no conflict of interest to declare.

Synbiotic targeting of *Clostridium difficile*

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As a genus, Lactobacillus spp. are intrinsically resistant to antibiotics making this a potentially resilient treatment option for *Clostridium difficile* infection (CDI). Meta-analysis of reported 16S rDNA sequence data from CDI patients across North America (including our own pediatric and adult Houston cohorts) confirmed a progressive antibiotic-associated expansion of *Lactobacilliaceae* - especially in recurrence - yet this was not associated with disease protection. Preliminary analysis was suggestive that probiotic *Lactobacillus* spp. may not form part of such blooms, but sets precedence for targeted synbiotic therapy in CDI.

We identified *L. reuteri* as a safe and intrinsically antibiotic-resistant strain with greater cytotoxicity towards multiple *C. difficile* isolates than vancomycin and fidaxomicin when delivered with glycerol, either *in vitro* or in a human microbiota bioreactor model. Preferential targeting of *C. difficile* – but not other commensal spp. - was mediated by the glycerol fermentation product reuterin (3-hydroxypropionaldehyde), resulting in toxic adducts forming on vital protein targets for *C. difficile* survival *e.g.* SIpA. Mutant strains unable to synthesize reuterin did not inhibit *C. difficile* growth, and synbiotic therapy required the horizontally acquired pdu-cbi-hem-cob cluster and glycerol. *In vivo* killing of *C. difficile* was demonstrated in a mouse CDI model, and clinical efficacy is currently being optimized using a novel nanoparticle biofilm delivery system which increases efficacy against *C. difficile* when compared to planktonic cells.

In summary, because gut commensals appear to have evolved natural resistance to reuterin bioactivity, synbiotic targeting of *C. difficile* may form the basis of prototypic therapy in CDI.

Maternal BMI is significantly correlated with endotoxin core IgM antibodies in both maternal and neonatal serum

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Obesity, diabetes and inflammation have long been associated, but the cause of the inflammation is unknown. One hypothesis is that bacterial products, such as lipopolysaccharides (LPS) are translocated across the gut in association with a high-fat diet which drives inflammation, a condition termed metabolic endotoxemia (ME). Data have shown that an increase in serum LPS and reduced levels of endotoxin-core IgM (endoCAb IgM) antibodies are associated with both obesity and diabetes. In this study we sought to determine if changes in serum LPS and endoCAb IgM are associated with maternal obesity and if the fetus is exposed to these markers of ME. We also tested whether markers of inflammation (TNFα and IL-6) were increased with maternal obesity and if these markers were observed in the neonate. Commercially available ELISA assays were utilized to measure LPS and endoCAb IgM levels in matched maternal and neonatal (cord blood) serum samples (N=146). The Milliplex system was used to measure TNFα and IL-6 through the DDC core. While we did not find any significant change in LPS levels in either maternal or neonatal samples, we found that maternal endoCAb IgM is significantly decreased in obese women compared to normal weight. When stratifying the data based on prepregnancy BMI into the categories of normal weight, overweight and obese we find that both maternal and neonatal endoCAb levels negatively correlate with BMI category. There is also a significant increase in TNFα in maternal serum in obese women compared to normal weight women. We also found that maternal and neonatal TNFα and IL-6 are positively correlated. While we do not find significant changes in LPS levels in the mother or neonate, the finding of altered endotoxin IgM levels is of interest. Because IgM antibodies do not cross the placenta, the endoCAb IgM detected in neonatal serum is likely due to the fetal immune response to circulating LPS levels. Correlation of maternal and neonatal TNFα and IL-6 may similarly point to fetal exposure to markers of maternal ME during gestation.

Analysis of autophagy related genes in patients with *Helicobacter pylori* infection

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Background: Recent studies have indicated that infection with *Helicobacter pylori* (*Hp*) induces autophagy in gastric epithelial cells. However, prolonged exposure to *Hp* also reduces autophagy by preventing maturation of the autolysosome. The role of the autophagy related genes (ATGs) alteration in *Hp* infection is not yet fully understood.
Aim: To investigate alterations of ATGs in *Hp* infection.
Method: We analyzed ATGs expression in *Hp* infected human gastric mucosa compared with uninfected gastric mucosa. We also studied single nucleotide polymorphisms (SNPs) of ATGs to identify the influence on susceptibility of *Hp* infection. Gastric mucosal biopsy specimens were obtained from Bhutanese patients with mild dyspeptic symptoms. *Hp* status was assessed by the combination of rapid urease test, culture and histology. Total RNA was extracted and microarray and quantitative RT-PCR were performed. Genomic DNA was also extracted for SNP genotyping analysis.
Results: Microarray analysis using 8 specimens (4 *Hp*-positive and 4 negative) found that 16/226 (7%) of ATGs were up-regulated and 9/226 (4%) were down-regulated in *Hp* infected gastric mucosa. In down-regulated genes, the core components of the autophagy machinery (*ATG16L1*, *ATG5*, *ATG4D* and *ATG9A*) were included. Quantitative RT-PCR was then performed on 136 patients (86 *Hp*-positive and 50 negative) and down-regulation of *ATG16L1* and *ATG5* expression were confirmed ($P < 0.001$ and $P = 0.049$, respectively). *ATG16L1* mRNA levels were decreased in a step-like manner by *Hp* density. There was no association between *ATG16L1* or *ATG5* mRNA expression and clinical or histologic features such as age, sex, *vacA* genotype, gastritis score (inflammation, activity, atrophy). Finally, SNPs analyses using 283 patients (206 *Hp*-positive and 77 negative) showed that *Hp* infection rates were significantly lower in patients bearing rs2241880 (*ATG16L1*) GG genotype compared to AA/AG genotypes (58.1% vs 74.7%, $P = 0.049$). After adjusted by age and sex, the odds ratios for *Hp* susceptibility of GG genotype were 0.40 (95%CI: 0.18-0.91) relative to the AA/AG genotypes. No association was found between rs510432 (*ATG5*) genotypes and *Hp* susceptibility.
Conclusion: Examination of ATG expression profiling in human gastric mucosa with *Hp* infection showed that down-regulation of core autophagy machinery genes may depress autophagy functions and possibly assist *Hp* to reside inside gastric epithelial cells. Our data suggest that *ATG16L1* SNP may be a genotypic marker to predict genetic susceptibility to *Hp* infection.

Improved detection of hepatocellular carcinoma using a longitudinal α-fetoprotein screening algorithm

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Background and Aims: Hepatocellular carcinoma (HCC) has limited treatment options when diagnosed at advanced stages; therefore early detection is critical to reduce mortality. Very early stage HCC is asymptomatic and can only be detected through surveillance programs. Current practice guidelines recommend ultrasonography surveillance for HCC in patients with cirrhosis. There is disagreement about the value of incorporating α-Fetoprotein (AFP) into HCC surveillance. However most previous studies evaluating AFP in HCC screening have focused on comparing each AFP measurement to a standard threshold for all patients. We aim to improve the sensitivity of AFP in HCC surveillance using an algorithm that incorporates screening history to define patient-specific thresholds for a positive screen. Methods: The Hepatitis C Antiviral Long-term Treatment against Cirrhosis (HALT-C) Trial enrolled 1050 patients with chronic hepatitis C and either advanced fibrosis or cirrhosis and prospectively followed them every 3-6 months. All patients had radiological imaging to exclude HCC prior to enrollment. The de-identified data from the trial was analyzed. AFP was assayed at each visit and ultrasonography was performed every 6-12 months. A panel adjudicated the diagnosis of all HCC cases. The parametric empirical Bayes (PEB) screening algorithm, which incorporates the patients screening history, was compared to the standard single threshold (ST) approach for interpreting AFP results. Results: During a median follow-up of 80 months, 88 patients (48/427 with cirrhosis and 40/621 with advanced fibrosis) were diagnosed with HCC. The PEB algorithm improved the sensitivity of AFP for detecting all HCC from 60.4% to 77.1% (p-value<0.0005) in patients with cirrhosis and from 72.5% to 87.5% (p-value=0.0015) in patients with advanced fibrosis, when the false positive rate among all screenings was set at 10%. PEB algorithm detected HCC 1.7-1.9 years earlier in the cirrhosis group and 1.4-1.7 years earlier in the advanced fibrosis group, compared to ST approach. We examined the timing of a positive screening more closely since a positive screening more than two years prior to clinical diagnosis is unlikely to lead to visualization of the tumor by imaging and more likely reflects AFP as a risk factor. When we focused on the period within 24 months prior to HCC diagnosis, we found that the sensitivity of the PEB algorithm was between 10.6-14.3% higher in patients with cirrhosis and between 5.1-10.5% higher in those with advanced fibrosis, compared to the ST method when the false positive rate among all screenings was set at 10%. Conclusions: The PEB algorithm increases the sensitivity of AFP testing and detects HCC earlier among hepatitis C patients with advanced fibrosis or cirrhosis compared to the standard threshold approaches currently used. The PEB algorithm has improved performance for two key reasons. Firstly, it adjusts for the high baseline AFP values in patients with persistently elevated but stable AFP, thereby decreasing the number of false positive screens. Secondly, for patients with low baseline AFP values, the PEB algorithm identifies increases in AFP values even though they remain below the fixed threshold thus increasing the sensitivity. These data should prompt a re-evaluation of how AFP is used in combination with ultrasound in HCC surveillance.

Prostate stem cell antigen gene polymorphisms as a predictive factor for *H. pylori* infection associated intestinal metaplasia

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Background: *Helicobacter pylori* (*H. pylori*) is associated with atrophic gastritis and gastric cancer. The infection causes histological changes in gastric mucosa including inflammation, atrophy and intestinal metaplasia (IM). However, the outcome among infected patients varies widely and in part is affected by host factors identified by genetic polymorphisms. Recently, the *prostate stem cell antigen* (*PSCA*, rs2294008) TT genotype has been identified as a possible important genetic risk factor for gastric cancer. IM are also precursor lesions for gastric cancer. Here, we compared *PSCA* polymorphisms and histological grading of gastritis among *H. pylori* infected Bhutanese (i.e., a high gastric cancer region).

Methods: 339 Bhutanese without past treatment of *H. pylori* infection, potent acid inhibitor and other exclusions were enrolled after written informed consent was obtained. Gastroscopy with gastric biopsy specimens from the antrum and corpus were evaluated using the updated Sydney system. The subjects were classified into 3 single nucleotide polymorphism of *PSCA* by PCR-RFLP (rs2294008 T/T, T/C and C/C). *H. pylori* was diagnosed using among rapid urease test, culture, histology and serological examination.

Results: The prevalence of *H. pylori* infection was 74% (age: 38.7 ± 14, male:female = 150:189). Among *H. pylori* infected the frequency of the *PSCA* rs2294008 T/T genotype was 12.0%. *PSCA* gene polymorphisms correlated with IM scores of the *H. pylori* positive subjects. In the antrum, the proportion with IM in TT genotype was significantly higher than with CT or CC genotypes (TT genotype:30%, CT genotype:10% and CC genotype 8.4%); In the corpus the pattern was similar (TT genotype:10%, CT genotype:1.1% and CC genotype 0%). In addition, the IM score of TT genotype was significantly greater than those of CT and TT genotypes in both the antrum and corpus among *H. pylori* positive subjects (antrum: vs CC $p < 0.01$ and CT $p = 0.01$, corpus: vs CC $p < 0.01$ and CT $p = 0.02$).

Conclusion: *PSCA* polymorphisms were associated with an increased prevalence of IM in *H. pylori* infected Bhutanese consistent with those with TT genotype of *PSCA* being at increased risk for gastric cancer.

Intestinal stem cells from IBD patients: insights into etiology and therapeutics

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Inflammatory bowel disease (IBD), including Crohn's and ulcerative colitis, traditionally have been considered and treated as diseases of the immune system. However, aggressive immunosurppression therapies have not reduced the high rates of surgical intervention required to manage these diseases, suggesting the possibility that other factors initiate or drive IBD. Recently, genetic association studies have implicated genes expressed in the intestinal epithelium as risk factors for these diseases, leading to the hypothesis that IBD is a consequence of defects in the intestinal epithelial "barrier" to intestinal microbiota. We have now developed powerful technologies for cloning stem cells from the human intestine and colon in their most immature form (Wang et al., 2015). Our analyses have shown that these highly clonogenic cells are exquisitely regio-specific for differentiation to 3-D intestinal epithelia and can be expanded to unlimited numbers. We have now applied this technology for the analysis of multiple cases of pediatric Crohn's disease and have discovered that ileal and colonic stem cells from these patients have acquired a hyperinflammatory gene signature that is stable to months of cultivation in sterile conditions. This epigenetic signature not only reveals key targets for the treatment of Crohn's but also the possibility of using autologous stem cells lacking this inflammatory signature for regenerative medicine resolutions to the chronic lesions in these patients. We are now expanding these studies to adult-onset Crohn's as well as ulcerative colitis in a broader program of pharmacologic and regenerative therapies for IBD.

Natural influence: investigating the impact of microbes on host health using *C. elegans*

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Associated bacteria can broadly influence our health in a myriad of ways. As a consequence, studies on microbiome have emerged as a new frontier in human health research and elucidated functional roles of many microbes. A challenge for the field moving forward is identifying microbiota-derived products themselves and characterizing the host pathways that they influence. Our lab has therefore sought to develop a high throughput, whole animal experimental system to characterize these underlying molecular mechanisms.

My research uses genetically tractable and microbially 'tuned' nematode *C. elegans* to study host-bacteria interaction under an ecological context. In their natural habitats of rotting fruits and vegetation, worms encounter and adapt to thousands of bacterial species, that together constitute both their diet and microbiome. We characterized these natural microbiomes by 16S rRNA sequencing and found that Proteobacteria-rich communities support worm proliferation while 'hostile' Bacteroidetes-rich microbiomes do not. By feeding previously 'germ-free' worms natural bacteria isolated from these microbial communities, we identified proteobacteria strains (e.g., *Providencia* and *Rhizobium*) that promote animal growth and progeny production, while strains like *Chryseobacterium* and *Spingobacterium* can dramatically delay worm growth and reproduction. In parallel, colonization levels in worm intestine by different natural bacteria was greater and occurred earlier in life with several pathogenic bacteria compared with more growth-promoting probiotic bacteria. However, consistent with host adaptation to its microbiome, a wild worm strain isolated from the same apple as the detrimental *Chryseobacteria* exhibits better growth and lower levels of colonization. Using this system, we therefore have a foundation where we can now employ a combination of genetics, comparative genomics and biochemical approaches to further dissect the molecular pathways for both the microbial influence and host adaptations that govern organism health in growth, reproduction and life span.

The effects of apoptosis inhibition on pulmonary alveolar type 2 epithelial cell alterations in experimental hepatopulmonary syndrome after common bile duct ligation

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Introduction: Abnormal oxygenation due to lung ventilation-perfusion mismatch drives the development of hepatopulmonary syndrome (HPS). In addition to alterations in microvascular perfusion, dysfunction in type II alveolar epithelial cells (AT2), which play a critical role in maintaining alveolar ventilation through surfactant protein (SPs) production, develops in experimental HPS. Specifically, AT2 cell apoptosis and decreased SP production associated with increased circulating bile acid levels and bile acid nuclear receptor (FXRα) activation, reduce alveolar airspace and impair ventilation. However, the relative effects of FXRα activation on AT2 cell apoptosis and SP expression and whether apoptosis inhibition influences AT2 cells and the development of HPS are unknown.

Aim: To evaluate the effects of apoptosis inhibition on FXRα activation in AT2 cells and in experimental HPS.

Methods: A murine AT2 cell line (MLE-12) was treated with a FXRα agonist (GW4064, 1-10μM) in the presence or absence of a pan caspase inhibitor (Z-VAD-FMK). SD rats underwent common bile duct ligation (CBDL) or sham operation and were treated with Z-VAD-FMK (1.0mg/kg/day, IP, 3 weeks). Lung and cell apoptosis was determined by TUNEL staining or cleaved caspase-3 levels. Co-staining of TUNEL and SP-C was performed to assess lung AT2 cell apoptosis. SP levels were assessed by westerns or qRT-PCR. Lung morphology (alveolar mean chord length, Lm) and gas exchange (PO_2) were measured to evaluate HPS.

Results: GW4064 treatment in MLE-12 cells caused a dose dependent increase in cleaved caspase-3 protein expression and reduction of SP-C and SP-B mRNAs. Z-VAD-FMK pretreatment attenuated GW4064 induced cleaved caspase-3 protein increases (76.0±6.1% reduction, $p<0.05$), while cellular SP mRNA decreases were unchanged. Relative to controls, CBDL animals developed selective pulmonary AT2 cell apoptosis reflected by the presence of double positive staining for TUNEL and SP-C and by increases in cleaved caspase-3 protein production. Apoptosis inhibition using Z-VAD-FMK decreased the numbers of TUNEL positive cells (82.8±3.8% reduction, $p<0.05$) and abolished lung SP protein reduction (SP-C, 1.6±0.2; SP-A, 1.7±0.2; fold *vs* CBDL, $p<0.05$). These events were accompanied by significant improvements in Lm and PO_2 (mmHg, CBDL 90.2±0.9 *vs* Z-VAD-FMK 97.3±0.9, $p<0.05$).

Conclusion: Pulmonary AT2 cell apoptosis and cellular SP reduction are two distinct pathways that contribute to alveolar alterations in experimental HPS. Caspase inhibitors attenuate the development of experimental HPS *via* limiting AT2 cell apoptosis, which provides a potential therapeutic intervention for clinical application.

In vivo Gene Delivery to Lymphocytes Using Novel Biodegradable Nanoparticles

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Introduction: Immunotherapy has gained increasing attention in cancer therapy recently based on the unprecedented success in clinical trials. As one of the immunotherapies, anti-CD19 chimeric antigen receptor (CAR)-modified T cells demonstrated remarkable effectiveness in refractive acute lymphoblastic leukemia (ALL) in a Phase I trial. Despite the tremendous success of CAR-T cell therapy, the main drawback of this treatment is the highly expensive *ex vivo* procedures to produce genetically modified CAR-T cells. We rationalize that direct *in vivo* production of CAR-T cells can significantly reduce the treatment cost and eliminate the potential side-effects involved in the *ex vivo* procedures. Herein, we report a novel star-shaped polymer (SP) based non-viral gene delivery system that can deliver genes to primary lymphocytes *in vivo* through intravenous administration.
Methods: We used plasmids encoding firefly luciferase pCMV-Luc2 and enhanced green fluorescent protein (EGFP) as model systems to test the gene transfection efficiency and specificity of SP formed nanoparticles (NPs) in the immune cells *in vivo*. Histochemsitry was performed to evaluate *in vivo* toxicity. Gene expression in lymphocytes *in vivo* was confirmed by bioluminescence live imaging, by immunohistochemistry (IHC), and by flow cytometry through staining lymphocyte surface markers.
Results: (1) Systemic intravenous administration of pCMV-Luc2-SP NPs showed a transient but strong luciferase expression in major lymph nodes *in vivo*, which was confirmed by whole body luminescence imaging. We observed little histological damage in the major organs in mice treated with the NPs.
(2) Systemic intravenous administration of pCMV-GFP-SP NPs led to effective transfection in various primary lymphocytes in lymph nodes. Flow cytometry followed by staining surface markers of different lymphocyte subsets demonstrated that 20% of CD4+ T lymphocytes, 19% of CD8+ T lymphocytes and 15% of total lymphocytes were GFP positive 24 h after administration. The percentage of cells expressing GFP decreased over a 4-day period based on flow cytometry measurements.
(3) Antigen presenting cells (APCs) and B lymphocytes were also transfected by SP NPs 24 h after administration with lower percentage than T lymphocytes. Flow cytometry showed that more than 10% of APCs were GFP positive 24 h after administration. Staining of surface marker further confirmed that more than 17% of B lymphocytes were GFP positive.
Conclusion: In this study, we successfully developed a method to deliver genes into lymphocytes directly *in vivo*. In addition, the SP polymer is biodegradable and induces minimal toxicity. In future studies, we will use SP NPs to directly deliver plasmids encoding CAR to CD8+ T lymphocytes and test its therapeutic efficacy compared to current CAR-T cell therapy.

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Galectin-3 Mediates Tumor-stromal interaction by activating Pancreatic Stellate Cell and promoting Cytokine/Chemokine production

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Background: Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive and desmosplastic tumor with poorer survival in PDAC patients. Proinflammatory cytokines/chemokines play an essential role in tumor microenvironments associated with its invasive and aggressive phenotype. Galectin-3, a member of a family of β-galactoside–specific lectins, has been found to be involved in tumor progression and tissue fibrosis in many organs. However, the precise role of Gal-3 mediates abundant tumor-stromal interaction and cytokine production is unknown. **Methods:** The effects of Gal-3 on secretion of cytokines/chemokines from HPSC were assessed by cytokine array. HPSC proliferation and invasion were assessed by the MTS and Matrigel invasion assays. Co-culture and Conditional medium from PDAC tumor cells with genetic alterations of Gal-3 levels were used to study the interaction between tumor cell and stromal cells. IL-8 production in both tumor cells and stromal cells was confirmed by ELISA. Transcriptional activities of NF-κB and IL-8 were determined by transient transfection. An IKB mutant construct (IKBM), Mutation of the IL-8 promoter at the NF-κB site, and an inhibitor of NF-κB (Bay11) were used to determine the signaling pathways evoked by Gal-3 in HPSC cells. **Results**: Gal-3 overexpression is in both tumor cells and stromal cells in tumor tissues of human and genetic mouse model. Secretion of cytokines such as IL-8, GM-CSF, CXCL1, CCL2 and IL6 were significantly increased in HPSC cells treated with recombinant Gal-3 which is concomitant with activation of HPSC. Conditioned medium or co-culture from Panc-1 cells in which gal3 was down-regulated inhibited the growth and invasion capacity of HPSC cells compared with the vector controls. RGal-3 dramatically induced production and secretion of IL-8 in a dose dependent manner in HPSCs. Mechanistically, Gal-3 stimulates IL-8 secretion at the level of transcription and through NF-kB signaling in HPSC. Gal-3 stimulated NF-κB transcriptional activity, and the NF-κB inhibitor Bay11 blocked IL-8 transcriptional activity and its induction by Gal-3. Furthermore, co-transfection of NF-κB mutant construct IKBM and IL-8 promoter construct (-1481) completely block the induction of IL-8 activity by Gal-3. After mutation of the NF-κB site in the IL-8 promoter, rGal-3 lost its ability to induce IL-8 transcriptional activity. GEO data and our own patients' cohort analysis of pancreatic tumor samples demonstrated that the level of Gal-3, IL-8 and other cytokines is significantly higher in tumor tissues than those of normal and pancreatitis. **Conclusion:** Collectively, these data suggest that Gal-3 secreted from tumor cells activate HPSC cells and induce many inflammatory cytokines including IL-8 production via NF-κB signaling. Thus, Gal-3 may play a critical role in mediating tumor-stromal interaction and remodeling tumor associated microenvironments.

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Phospholipid spatiotemporal dynamics mediate bile acid-stimulation of EGFR-dependent signaling and pro-inflammatory response.

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Background: Bile acids have been implicated in pro-inflammatory responses in the gastrointestinal (GI) tract, which contribute to numerous diseases in the digestive system including cholestasis, inflammatory bowel disease and cancer. Among many cellular pathways targeted by bile acids, cell surface signaling pathways dependent upon epidermal growth factor receptor (EGFR) are stimulated by bile acids. EGFR is a key element in regulating pro-inflammatory responses in cells. Aberrant EGFR activities have been linked to elevated inflammation and cancer in the GI tract. Thus, it is critical to understand how bile acids stimulate EGFR. However, the molecular mechanism(s) is still poorly understood.

Recent studies show that multiple EGFR molecules oligomerize and form nanometer-sized signaling platforms with various lipids in the plasma membrane (PM). Signaling lipids suggested contributing to EGFR oligomerization and signaling include phosphatidic acid (PA), phosphatidylserine (PS), phosphoinositol 4,5-bisphosphate (PIP_2), phosphoinositol 3,4,5-trisphosphate (PIP_3) and cholesterol. Although these lipids comprise only small portion of the PM, they play critical roles in cell signaling because most EGFR downstream effectors have lipid-binding domains and require specific binding to one or multiple signaling lipids for their phosphorylation and activation. Thus, the ability to sort different lipids determines the stability of these nano-proteolipid signaling platforms, also called nanoclusters with diameters between 10 and 20nm, and consequentially regulates signaling efficacy.

Bile acids have been well established to associate with lipids. Traditionally, bile acids at concentrations above their critical micelle concentrations (CMCs) solubilize lipids. Thus, bile acid – lipid interactions have been mainly used to explain bile acid toxicity at high doses. New studies found that, at doses well below their CMCs, biological detergents including bile acids induce spatial segregation of various lipids and enhance heterogeneity of the PM without changing the overall integrity of the membrane.

Results: Using a novel quantitative imaging technique, electron microscopy (EM) – spatial mapping, we found that deoxycholic acid (DCA) markedly enhances the spatial segregation of PA (formation of nanoclusters with diameter at ~20nm) in colorectal adenocarcinoma Caco2 cell basalateral PM with an EC50 of ~0.6μM. The DCA effect on PA level and lateral clustering in the PM is also very rapid, starting to show effectiveness within 30 second and peaks at 5-10 minutes. PA is a key structural component of EGFR signaling nano-proteolipid nanoclusters and tightly regulates EGFR-dependent signaling. As expected, DCA also significantly enhanced EGFR nanoclustering and signaling with similar efficiency (EC50 of ~0.3μM for EGFR nanoclustering; EC50 of ~0.2μM for EGFR-MAPK signaling). Depletion of PA biosynthesis effectively abrogated DCA-stimulation of EGFR-dependent signaling. Similar lipid-mediated mechanism has been found in non-GI cells as well as human intestinal enteroids.

Conclusions: Our data strongly suggest that bile acids at extreme low doses typically found in the plasma of fasting healthy human associate with and alter the spatial distribution of multiple signaling lipids in the PM, which leads to changes in the lipid composition of EGFR signaling nanoclusters and stimulation of EGFR-mediated signal transmission.

The Plasticity of the Intestinal Stem Cell Compartment and its Induction Following Rotavirus Infection

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The intestinal epithelium exhibits a remarkable ability to self-renew due to the continuous proliferation and differentiation of intestinal stem cell (ISC) populations. LGR5-expressing cells are thought to actively cycle, whereas HOPX-expressing cells remain quiescent. Intestinal epithelial injury has mainly been studied using radiation models in which the stem cell itself is injured. Little is known about ISC responses that result from damage to differentiated cells. Using human intestinal enteroid (HIE) model in the context of a prevalent human intestinal pathogen, rotavirus (RV), we show that damage to differentiated cell types can promote ISC induction.

HIEs were generated from human jejunal tissue and grown into 3D structures with support of growth factor WNT. Differentiation was induced by withdrawing WNT and assessed via morphology, ISC marker expression, and proliferation and differentiation status. To examine the ISC response to pathogenic insult, differentiated HIEs were infected with human RV. qRT-PCR was used to assess expression of LGR5, HOPX, and WNT target genes. To hone in on the ISC niche, we utilized FACS for CD44+ cells to isolate and specifically examine the proliferating compartment.

We determined that undifferentiated and differentiated HIEs have distinct morphology, proliferation and differentiation status. Differentiation also results in a dramatic alteration of ISC marker expression, where LGR5 expression decreases and HOPX expression increases. Moreover, upon WNT addition or RV exposure, this differentiation phenotype is reversed: with an induction of LGR5 and other WNT target genes and a downregulation of HOPX. More importantly, through FACS, we show that LGR5 induction is specific for CD44+ population and, therefore, the ISC compartment. These experiments identify a plastic ISC niche in HIEs, which can be induced during epithelial damage such as RV infection. This research uses clinically relevant, human pathophysiology models to delineate ISC responses that are not currently well understood.

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