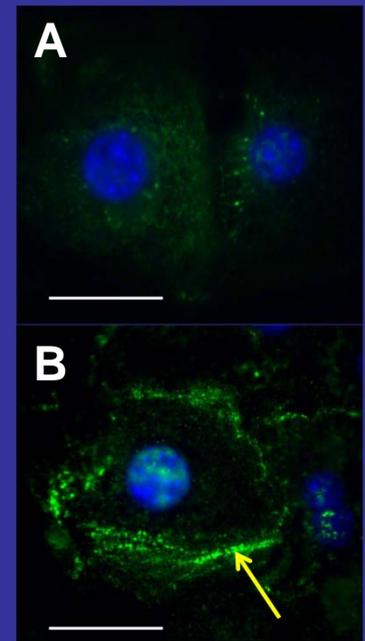
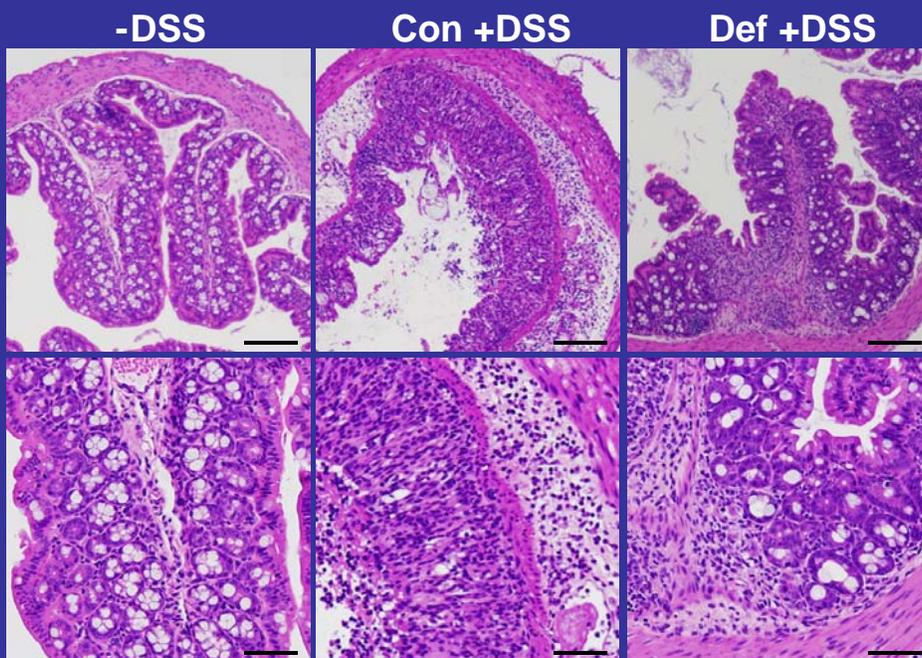


**Texas Medical Center Digestive Diseases Center
3rd Annual “Frontiers in Digestive Diseases” Symposium:
Epigenetics in GI Health and Disease**



**Monday, March 7, 2011
Hickey Auditorium, UT MD Anderson Cancer Center
Houston, Texas**

**Texas Medical Center Digestive Diseases Center
3rd Annual “Frontiers in Digestive Diseases” Symposium:
Epigenetics in GI Health and Disease**

**Texas Medical Center Digestive Diseases Center
3rd Annual Frontiers in Digestive Diseases:
*Epigenetics in GI Health and Disease***

**Monday, March 7, 2011
Hickey Auditorium, University of Texas MD Anderson Cancer Center
Main Building, Floor 11 (R11.1400)
Houston, Texas**

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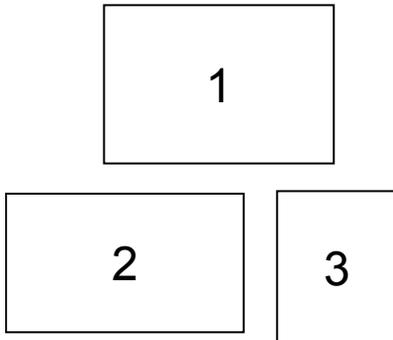
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On the cover:



1. Genetically identical 100-day-old *A^{Vy}/a* sisters, differing only in the level of methylation in the *A^{Vy}* region. Although we are accustomed to the notion that all phenotypic variation is genetically based, epigenetic variation clearly can have a profound influence on phenotype. From Waterland RA. Epigenetic mechanisms and gastrointestinal development. *J Pediatr.* 2006. 149:S137-42.
2. P-22. Mei, Y. Epidermal growth factor (EGF)-induced p-eNOS Ser1177 expression in primary mouse hepatocytes. Detection of p-eNOS Ser 1177 expression (green) in (A) untreated or (B) following one hour EGF treatment. Nuclei (blue). Scale bar =30µM.
3. P-2. Benight, N. H&E stained sections indicate that non-DSS treated animals have normal histological architecture and cytology, and are not inflamed; mice given 3% DSS (+DSS) had severe and diffuse destruction of the epithelial layer, neutrophil infiltration in both epithelium and lamina propria along with edema, affecting most of the tissue. Mice given DSS+MTA (100mg/kg BW) had focal inflammation in both the epithelium and lamina propria, but significantly less severe tissue damage. Scale bar = 100 uM.

**Texas Medical Center Digestive Diseases Center
3rd Annual “Frontiers in Digestive Diseases” Symposium:
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A G E N D A

Monday, March 7, 2010

**Hickey Auditorium, University of Texas MD Anderson Cancer Center
Main Building, Floor 11 (R11.1400)
Houston, Texas**

- 8:00am **Coffee and Continental Breakfast**
- 8:30 - 8:45am **Welcome: Douglas Burrin, Ph.D.**, Professor, Pediatric-Nutrition & GI, BCM

Theme: Role of Epigenetic Mechanisms in GI and Liver Disease.
- 8:45 – 9:30am **Guest Speaker: Jean-Pierre Issa, M.D.**, Professor, Department of Leukemia, Chief, Section of Translational Research, UT MD Anderson Cancer Center
DNA Methylation as a Mechanism of Aging and Inflammation Effects on GI Malignancies
- 9:30 – 9:50am **Hongzhen Hu, Ph.D.**, Assistant Professor, Integrative Biology & Pharmacology, UTHSC
Transient Receptor Potential Channels in the Gut
- 9:50 – 10:10am **Richard Kellermayer, M.D.**, Assistant Professor, Pediatric-GI, BCM
Epigenetics and the Developmental Origins of Inflammatory Bowel Diseases
- 10:15 – 10:30am **Morning Coffee Break**
- 10:30 – 10:50am **Yongde Luo, Ph.D.**, Assistant Professor, Center for Cancer and Stem Cell Biology, Institute of Biosciences and Technology, Texas A&M Health Science Center
Regulation of Cellular and Metabolic Homeostasis by Endocrine FGFs
- 10:50 – 11:10am **Shujuan Pan, Ph.D.**, Assistant Professor, Dept Pathology, BCM
Genetic Modifiers of Liver Disease Associated With Alpha1-Antitrypsin Deficiency
- 11:10 – 11:30am **Heidi Karpen, M.D.**, Assistant Professor, Pediatric-Neonatology, BCM
Interrelated Roles of Phytosterols and Inflammation in TPN-Associated Cholestasis
- 11:30 – 1:30pm **Lunch & Poster Viewing**
Poster Judging: Richard Kellermayer, Marc Rhoads, Claudia Kozinetz, Lopa Mishra, and Rob Shulman
- 1:30 – 2:15pm **Guest Speaker: Anil K. Rustgi, M.D.**, T. Grier Miller Professor of Medicine, Chief, Division of Gastroenterology, University of Pennsylvania, School of Medicine.
Genetics, Epigenetics and the Tumor Microenvironment
- 2:15pm **Closing Remarks:** Mark Gilger, BCM (*Announcement of poster award winners*)

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7.	Nancy Benight	Methylthioadenosine is rapidly absorbed and taken up by the gastrointestinal epithelium in mice
8.	Douglas Burrin	Supplementing glutamate to partial enteral nutrition slows gastric emptying rate in preterm pigs
9.	Samson Cantu	P2Y2 purinergic receptor knockout mice exhibit increased susceptibility to liver injury in a mouse model of biliary fibrosis
10.	Yanna Cao	Anti-fibrogenic effects of bone morphogenetic protein 2 in pancreas
11.	Yanna Cao	Dysregulation of autophagy in acute pancreatitis
12.	Ji Chu	Dual functions of p21-activated kinase in regulating myosin light chain
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14.	Joseph Dasso	Epigenetic regulation of insulin-like growth factor-1 by a methyl donor-rich diet in a transgenerational model of intrauterine growth restriction
15.	Ali A Hashmi	Gastric variceal hemorrhage in a patient with a history of a splenic abscess
16.	Peera Hemarajata	A putative folate transporter in <i>Lactobacillus reuteri</i> is potentially involved in immunomodulation
17.	Paula M. Hertel ☆	Upregulation of hepatic OPN occurs in rotavirus-induced murine biliary atresia and requires replicating virus, But is not necessary for development of biliary atresia.
18.	Ryan W. Himes ☆	Tlr2 is necessary for hepatic steatosis and metabolic syndrome in a murine model of diet-induced obesity
19.	Charles Hotte	Massive lower gastrointestinal bleeding after abdominal paracentesis treated with endoscopic therapy
20.	Jason Hou	The incidence and prevalence of inflammatory bowel disease among veterans: A national cohort study

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23.	Li Jiao	Plasma soluble receptor for advanced glycation end-products and risk of colon polyps in men
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25.	Ying Li	β2SP interacts with Smad3 and regulates TGF-β signaling in liver tumorigenesis
26.	Yuying Liu ☆	<i>Lactobacillus reuteri</i> DSM 17938 reduces inflammation in necrotizing enterocolitis and increases regulatory T cells in intestines and mesenteric lymph nodes in neonatal rats
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29.	Yuko Mori-Akiyama ☆	SOX9 acts as a tumor suppressor by direct regulation of <i>Igfbp4</i> , <i>CEACAM1</i> , and <i>Vegfa</i> in colorectal cancer cells
30.	Dorottya Nagy-Szakal	Dietary cellulose supplementation during childhood induces transient trophic, anticolitic and microbiomic effects in the large intestine of mice
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33.	Muralidhar H Premkumar ☆☆☆	Intestinal cell specific contributions of nitric oxide to the pathogenesis of necrotizing enterocolitis
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37.	Shumei Song [☆]	Reata, E3 ligase inhibitor, potently inhibits Hepatocellular carcinoma cell growth through PRAJA/Smad3/ β - Spectrin and Keap1/Nrf2 signaling
38.	Jennifer K. Spinler	Regulatory control of reuterin production varies between probiotic <i>Lactobacillus reuteri</i> strains and is dependent upon Pocr
39.	Vivek Vijayamadhavan	P2X7 purinergic receptor-mediated early activation of JNK signaling is essential for endotoxin-induced acute liver injury in mice
40.	Donna L. White [☆]	Higher total serum testosterone is associated with advanced hepatic fibrosis and inflammatory activity risk in chronically HCV-infected male veterans
41.	Zhixing Yao	TGF- β pathway regulates transcription of telomerase (TERT) in hepatocellular cancer formation.

☆ Denotes past Pilot/Feasibility awardee

☆☆ Denotes 2011 Pilot/Feasibility awardee

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**Fasting Insulin and Gamma Glutamyl Transferase (GGT) Predict
Nonalcoholic Steatohepatitis (NASH) in Hispanic Children**

Abrams SH, Fairly LA, Smith EO, Smith CW.

Department of Pediatrics, Baylor College of Medicine

Background: Nonalcoholic steatohepatitis (NASH) affects 3% of all children and is most prevalent in Hispanic children. Currently, the only method of diagnosis is a liver biopsy which is both costly and invasive.

Methods: To test the hypothesis that biomarkers exist for Hispanic children with NASH, 3 study groups were recruited: (1) obese children with a liver-biopsy demonstrating NASH in the 60 days prior to study visit, (2) obese children with no liver disease, and (3) lean children with no liver disease. Each child had blood drawn and serology was performed for lipid panel, hepatic panel, insulin, glucose, and uric acid. ELISA was performed for high-sensitivity c-reactive protein (hs-CRP), caspase generated cytokeratin-18 fragments (CK-18), fetuin-A, and adiponectin. Area under the curve ROC analysis was performed to differentiate Hispanic children with NASH from those without NASH.

Results: 57 Hispanic subjects were recruited with a mean age of 12.1 ± 2.1 years. The mean BMI z-score and BMI percentile for age/sex were the same for the NASH and obese control groups. Adiponectin was significantly lower, while Fetuin-A, HOMA-IR, and CK-18 levels were significantly higher in NASH subjects when compared with obese and lean controls. ROC analysis for fasting insulin + GGT demonstrated that a cut-off of 40.5 U/L+mg/dl predicted NASH vs. not-NASH with an AUC of 93.4%.

Conclusion: Cytokeratin-18 fragments are significantly higher in obese Hispanic children with NASH compared to obese and lean controls. Fasting insulin + GGT may be an effective clinical tool for the pediatrician when managing the obese Hispanic child with elevated aminotransferases.

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Methylthioadenosine is rapidly absorbed and taken up by the gastrointestinal epithelium in mice

Nancy M Benight¹⁻², Barbara Stoll², Juan C Marini², Douglas G Burrin²

¹Translational Biology Molecular Medicine Program, Baylor College of Medicine ²Pediatrics
Baylor College of Medicine/Children’s Nutrition Research Center

Background: Methylthioadenosine (MTA) is a salvage pathway precursor for methionine (Met) and adenine. Animal models of liver injury and multiple sclerosis indicate that MTA has anti-inflammatory properties, possibly through changes in histone methylation. Our previous work showed that oral delivery of MTA is protective against DSS induced colitis in mice as evidenced by reduced disease scores, histological injury, and tissue inflammation. The oral bioavailability of MTA in animals and humans is unknown. To further investigate how MTA exerts its anti-inflammatory action in the colonic mucosa, we tested whether MTA is transported into and directly affects the inflammatory response in colonic epithelial cells and if this occurs via changes in histone methylation.

Methods: We determined the bioavailability of MTA in the gastrointestinal tract (GIT) by giving 8 wk-old C57Bl/6 mice a single oral gavage of ¹⁴C-MTA. Mice were euthanized at 5, 10, 30 and 90 min and the radioactive ¹⁴C-MTA in the small intestine, stomach, colon, liver, kidney and blood were measured by liquid scintillation counting. The anti-inflammatory effects of MTA were tested in sub-confluent HT-29 cells treated with a TLR agonist (LPS or flagellin) in the presence (+/-) of MTA, Met or adenosine. After 24 h incubation, we measured MTA and Met concentrations in media and cells by HPLC and IL-8 secretion using ELISA.

Results: Radioactivity (¹⁴C) was detected in all measured regions including blood and colon as early as 5 min post-dosing. The peak radioactivity occurred earliest in the blood and most proximal regions and progressed distally along the GIT. A total of 25% of the tracer dose was recovered in the organs measured. Cell studies indicate that while 50% of the MTA provided to cells in the media disappears, there is no increase in MTA in the cells after 24 h. MTA suppressed both LPS and flagellin induced increases in IL-8 while adenosine and Met had no effect.

Conclusions: Our current results indicate that MTA is rapidly absorbed into the circulation and taken up in all regions of the GIT. Further, we show that MTA directly exerts anti-inflammatory actions on colonic HT-29 cells via suppression of TLR signaling.

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Supplementing glutamate to partial enteral nutrition slows gastric emptying rate in preterm pigs

Caroline Bauchart-Thevret, PhD¹, Barbara Stoll, PhD¹, Nancy Benight¹, David Lazar², Oluyinka Olutoye, MD/PhD² and Douglas Burrin, PhD¹.

¹USDA Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, and ²Pediatric Surgery, Baylor College of Medicine

Background: Premature infants frequently present with gastroduodenal motor dysfunction, which is manifest clinically as feeding intolerance resulting from slow gastric emptying. Glutamate (GLU) is the major excitatory neurotransmitter in the body and multiple GLU receptors and transporters have been found in the gut and enteric nervous system. Emerging evidence suggest that GLU may play a functional role in promoting gastric emptying and digestion. However, the importance of dietary GLU on gastric motor function in the developing gut is unknown.

Objective: To determine whether supplementing GLU to partial enteral nutrition can stimulate gastric emptying in preterm pigs.

Design/Methods: 10-d preterm, parenterally-fed pigs received partial enteral nutrition (25%) as 4 orogastric feeds every 6 h as milk-based formula supplemented with monosodium glutamate (MSG) at 0, 2, 4 and 6 times the basal GLU intake (117 mg/kg per feed)(n=5-8 pigs/group) for 7 d. Whole-body respiratory calorimetry and ¹³C-octanoic acid breath test were performed on d 3, 5, 7 and 9 of life.

Results: Body weight gain, stomach and intestine weights and arterial plasma GLU and glutamine concentrations were not different between the MSG groups. However, GLU and aspartate concentrations were 3-4 times higher in the portal vs. arterial plasma in all treatment groups, suggesting a significant net portal absorption. In addition, portal GLU concentration was significantly higher while portal arginine concentration was significantly lower in pigs fed the MSG 4 and 6 doses. There was no treatment effect on VO₂ uptake, VCO₂ production, respiratory exchange ratio and heat production. At d 9 (Table), we found lower (P<0.05) breath ¹³CO₂ enrichments and ¹³CO₂ production, % of ¹³CO₂ recovery/h and cumulative % recovery of ¹³C-octanoic acid in MSG-4 and MSG-6 vs. MSG-0 groups. The average lag time (T_{lag}) and gastric half emptying time (T_{1/2}) between all treatment groups were 121 and 188 min, respectively. Regression analysis showed that T_{lag} and T_{1/2} were increased (P<0.05) by MSG dose.

Gastric Emptying Results (d 9)					
	MSG-0	MSG-2	MSG-4	MSG-6	SEM
T _{lag} , min	107	116	123	131	5
T _{1/2} , min	172	168	188	223	12
Cum % ¹³ C ₂ recovery	38.7	38.5	31.4	34.9	2.2

Conclusions: Our results suggest that adding glutamate to partial enteral nutrition slows the gastric emptying rate but does not affect intestinal mucosal growth in premature pigs.

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**P2Y2 purinergic receptor knockout mice exhibit increased
susceptibility to liver injury in a mouse model of biliary fibrosis**

Samson Cantu, Arunmani Mani, Bryan Tackett, Moreshwar Desai, Sundararajah Thevananther

Pediatrics/Gastroenterology, Hepatology & Nutrition.

Background: Extracellular ATP via the activation of P2 purinergic receptors influence multiple hepatic functions. Cellular stress and injury can induce ATP release and contribute to elevated extracellular ATP levels at the sites of injury *in vivo*. However, the functional significance of P2Y2 purinergic receptor activation during chronic liver injury remains unexplored. The purpose of this study was to test the **hypothesis** that extracellular ATP and P2Y2 purinergic receptor-mediated signaling protects against hepatobiliary injury and biliary fibrosis.

Methods: Adult male wild type (WT) and P2Y2 *-/-* (KO) mice were fed chow or 3, 5-diethoxycarbonyl-1, 4-dihydrocollidine (DDC; 0.1%), for 1-3 weeks. Serum, liver sections, and RNA were analyzed to assess the extent of cholestasis, biliary fibrosis and liver injury. Statistical analysis was performed using unpaired Student's t-test and a *P* value of <0.05 was considered significant.

Results: KO mice sustained exaggerated liver injury in response to DDC feeding (1 week), as evidenced by the several fold increase in serum ALT (7.7-fold), AST (6.2-fold), ALP (1.8-fold), and direct bilirubin (13.5-fold), as compared to WT (1.0). Correspondingly, TUNEL analysis demonstrated increased apoptosis in the KO livers at 1 week (1.4-fold). Moreover, KO livers exhibited exaggerated increase in pro-inflammatory TNF- α (2.6), MCP-1 (1.9), and osteopontin (2.3) mRNA expression, as compared to WT (1.0). H&E, Mason-Trichrome, and CK-19 immunohistochemical analysis of liver sections of DDC-fed mice (1 and 3 weeks) confirmed higher neutrophil infiltration, periductular fibrosis and exaggerated ductular reaction (1 week) in the KO livers, as compared to WT.

Conclusions: Our findings suggest that P2Y2 purinergic receptor KO livers have higher susceptibility to hepatobiliary injury in a well-established xenobiotic-induced mouse model of biliary fibrosis. Elevated markers of cholestatic liver injury, inflammation, and biliary fibrosis in KO mice is highly suggestive of hepatoprotective roles of extracellular ATP and P2Y2 purinergic receptors during chronic liver injury, with implications for the development of targeted therapies for hepatobiliary disorders.

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Anti-fibrogenic effects of bone morphogenetic protein 2 in pancreas

Y Cao^{1*}, W Yang^{1*}, C Duan^{1*}, MR Hellmich^{2,1}, and TC Ko^{1,2}

¹Department of Surgery, UTHSC-Houston; ²UTMB, Galveston. *Equal contribution

Introduction: Chronic pancreatitis (CP) is a progressive disease characterized by inflammation, fibrosis, and loss of exocrine and endocrine functions through repeated episodes of acute pancreatitis. Activation of pancreatic stellate cells (PSCs) recently have been recognized as a key step in the development of pancreatic fibrosis in CP. Activated PSCs are characterized by expression of α -smooth-muscle actin (α -SMA) and produce large amounts of extracellular matrix proteins. Transforming Growth Factor- β (TGF- β) is a potent activator of PSCs and inhibition of TGF- β signaling blocks PSC activation and subsequent pancreatic fibrosis. Bone morphogenetic proteins (BMPs) are members of TGF- β superfamily and can antagonize TGF- β function. On the other hand, TGF- β has been shown to induce gremlin, a BMP antagonist, in renal epithelial cells. Previously, we have shown that BMP2 is activated in CAE-induced acute pancreatitis and we hypothesize that BMP2 antagonizes TGF- β -induced activation of PSCs while TGF- β induces gremlin to override the inhibitory effects of BMPs. **Methods:** PSCs were isolated from female Swiss Webster mice and used within 1 to 3 passages. PSCs were activated by treatment with TGF- β 1 (1 or 3 ng/ml) for 48-72 h in the presence or absence of pretreatment with BMP2 for 30 mins. The expression of α -SMA and gremlin were detected by immunofluorescence assays. **Results:** TGF- β increased α -SMA expression in PSCs in a dose-dependent fashion (4.4 fold of vehicle control at 1 ng/ml and 7.8 fold at 3 ng/ml). BMP2 inhibited TGF- β -induced α -SMA expression to near basal level. TGF- β increased gremlin expression (1.6 fold at 1ng/ml) compared with vehicle control. **Conclusion:** Activation of BMP2 during acute pancreatitis may serve as a protective mechanism to prevent or limit PSC activation. Overriding this inhibitory effect of BMP2 may be required for PSC activation during development of pancreatic fibrosis and may be mediated by TGF- β induction of gremlin.

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Dysregulation of autophagy in acute pancreatitis

Y Cao^{1*}, M Tyler^{1*}, W Yang^{1*}, J Aronson², V Popov², C Chao², MR Hellmich², and TC Ko^{1,2}

¹Department of Surgery, UTHSC-Houston; ²UTMB, Galveston

Introduction: Autophagy is a homeostatic mechanism in which a cell ‘recycles’ cellular material in response to dynamic changes in its energy pools. Recently, it has been shown that dysregulated autophagy contributes to AP. Autophagy is controlled by sequential expression of proteins such Beclin-1 and LC3-II. Beclin-1 is expressed during phagophore formation which precedes LC3-II expression during autophagosome formation. Previously, we have shown that BMP signaling is activated in cerulein (CR)-induced AP, and treatment with noggin, a BMP antagonist, attenuates CR-induced AP (Yang W et al. APA Annual meeting 2009). The purpose of this study is to test the hypothesis that BMP regulates autophagy in CR-induced AP.

Methods: C57BL/6 mice were randomized into two groups (n=7/group): (1) 9 hourly injections of CR (50 µg/kg, ip); (2) pretreatment with noggin, (0.5 µg/kg, ip) followed by CR (50 µg/kg, 9 hourly ip injections). Mice were euthanized 1 hr after last CR injection. Pancreas tissue was harvested to examine for the presence of autophagic vacuolization by electron microscopy and for autophagy markers by immunoblotting.

Results: Noggin pretreatment attenuated CR-induced autophagic vacuolization, and lowered LC3-II levels by 72.5% compared to CR treatment alone ($p<0.05$). Interestingly, in the presence of noggin, CR resulted in a 2.5-fold increase in Beclin-1 levels compared to CR treatment alone ($p<0.05$).

Conclusion and Discussion: These results suggest that noggin blocks autophagy at the transition from phagophore to autophagosome which is associated with attenuated AP. Targeting BMP signaling pathway may be a novel strategy to block dysregulated autophagy seen in AP.

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Dual functions of p21-activated kinase in regulating myosin light chain

Ji Chu *, Kislitsyna, Karina, Charles S. Cox Jr., Karen Uray

Department of Pediatric Surgery, University of Texas Medical School at Houston

Intestinal edema and subsequent decreased intestinal contractile activity often occur under various pathologic circumstances. In an *in vivo* intestinal edema rodent model, our laboratory showed a significantly decrease in intestinal contractile activity and corresponding decreases in both myosin light chain (MLC) and myosin light chain phosphatase targeting subunit (MYPT1) phosphorylation in edematous tissue. P21-activated kinase (PAK) activity is also increased in edematous tissue. Moreover, intestinal tissue contractility was rescued by inhibition of PAK activity *in vivo*. To investigate the role of PAK in edema-induced intestinal contractile dysfunction, a human primary intestinal smooth muscle cell (hISMC) model was developed. hISMCs were subjected to either basal cyclical stretch (CCS) or an increasing cyclical stretch (ECS), mimicking pathologic changes in edematous tissues. ECS induced significant decreases in phosphorylation of both: MYPT1 and smooth muscle myosin light chain (MLC) mimicking the *in vivo* intestinal edema findings. The role of PAK in regulating MLC phosphorylation has been investigated by transfecting hISMCs with two constitutively active PAKs (caPAK) or dominant negative PAK (dnPAK). In ECS group, transfection with caPAK induced further decrease in MYPT1 and MLC phosphorylation while dnPAK produced the opposite effect. In contrast, in CCS group, caPAK transfection increased MLC phosphorylation and dnPAK decreases MLC phosphorylation. The PAK activator (BPIPP) and the PAK inhibitor (IPA-3) induced similar results as transfection with caPAK and dnPAK, respectively. We conclude from this data that under physiologic environment, PAK is responsible for maintaining MLC phosphorylation; however, increased PAK activity under pathologic conditions switches PAK signaling to a pathway inhibiting MLC phosphorylation through decreased MYPT1 phosphorylation.

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The role of autophagic membranes in rotavirus morphogenesis

Sue E. Crawford, Budi Utama, Joseph M. Hyser and Mary K. Estes

Department of Molecular Virology and Microbiology, Baylor College of Medicine

Background: Rotavirus (RV) is the leading cause of severe diarrhea among infants and young children. A unique feature of RV replication involves the RV nonstructural protein 4 (NSP4). NSP4 is synthesized as an endoplasmic reticulum (ER)-specific transmembrane glycoprotein. The C-terminus of NSP4 extends into the cytoplasm and serves as an intracellular receptor for double-layered particles (DLPs) that are assembled in viroplasms, sites of virus replication. This interaction triggers the budding of DLPs into the lumen of the ER acquiring a transient envelope. The membrane envelope is lost and the outer capsid proteins are assembled onto particles resulting in infectious particles. Currently, the membranes through which the DLPs bud are thought to be ER membranes. We have recently shown NSP4 colocalizes with the autophagy marker LC3 in membranes, forming puncta that merge and cap viroplasms. This result suggested that these membranes may be autophagy membranes.

Autophagy is a cellular response that functions to dispose of excess or defective proteins and organelles by the formation of double-membrane vesicles. Other RNA viruses such as poliovirus and rhinovirus subvert autophagy membranes for viral replication.

Aim: To evaluate the role of autophagy membranes in RV morphogenesis, I examined whether (1) RV infection induces the formation of autophagy membranes, (2) inhibition of autophagy membrane formation reduced the yield of RV progeny, and (3) NSP4 viroporin mediated elevation of intracellular Ca^{2+} is mechanistically related to autophagy membrane formation.

Results: The induction of autophagy membranes can be determined by detection of the membrane-inserted form of LC3, LC3 II and formation of LC3-positive puncta. LC3 II was detected and NSP4/LC3 puncta were observed as early as 4 hours post infection indicating that RV induces the formation of autophagy membranes. To determine whether autophagy membranes play a role in RV morphogenesis, the yield of RV was assessed in cells deficient in proteins required for the induction of autophagy or in cells treated with an inhibitor of autophagy, 3-methyladenine (3MA). The yield of RV in Atg 3 or Atg 5 deficient cells or in cells treated with 3MA was reduced by one to two logs. Wild type NSP4, but not a NSP4 viroporin mutant that fails to increase cytoplasmic calcium, triggered the induction of autophagy membranes.

Conclusions: These data are paradigm shifting and suggest RV morphogenesis occurs at autophagy membranes and not through the ER membrane, as previously thought.

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Epigenetic regulation of insulin-like growth factor-1 by a methyl donor-rich diet in a transgenerational model of intrauterine growth restriction

Joseph Dasso, Min Hu, Cindy Shope, Lori Showalter, Robert Lane, Kjersti Aagaard-Tillery

Baylor College of Medicine; Houston, TX and University of Utah, Division of Neonatology

Decades of data on the developmental origins of health and disease underlie the observation that intrauterine growth restriction (IUGR) gives rise to later in life risk of dysregulated lipid metabolism, glucose homeostasis, insulin resistance, and atherosclerotic disease. These effects can be observed across generations and are thought to occur in association with modified epigenomic regulatory mechanisms. Animal models of IUGR from our laboratory and others reveal that these epigenomic changes include modified gene-specific DNA methylation and histone acetylation in the IUGR animal, such as with *Igf1*. Moreover, we have previously demonstrated that diet supplementation of essential nutrients (ENS) can prevent adult metabolic disorders in a transgenerational uteroplacental insufficiency rat model of IUGR. However, it is not clear to what extent associated epigenetic changes in *Igf1* are inherited across generations and whether these changes can be prevented by dietary interventions. We hypothesized that ENS supplementation would prevent transgenerational effects of adult metabolic disease in rats resulting from maternal IUGR in association with altered epigenetic regulation of *Igf1*. To specifically interrogate differential epigenetic regulation of *Igf1* in response to dietary intervention, we examined the DNA methylation status of the second promoter (P2) of the *Igf1* gene in fetal liver tissue by bisulfite modification and sequencing. We found that maternal IUGR reduced DNA methylation of the *Igf1* promoter 2 in male F2 offspring at day 21 of life relative to sham-operated control animals. In contrast, dietary supplementation with one carbon metabolites maintained methylation of the *Igf1* promoter 2 in males at day 21 [Mean % methylation of CpG sites was 0.11% in Sham-Regular diet vs. 0.03% in IUGR-regular diet groups ($p=0.025$) and 0.04% in Sham-ENS diet vs 0.09% in IUGR-ENS diet groups ($p=0.31$)]. We conclude that prevention of the adverse transgenerational effects of maternal IUGR on metabolism by giving a methyl donor-rich diet occurs (in part) in association with maintaining DNA methylation of *Igf1*.

**Texas Medical Center Digestive Diseases Center
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Epigenetics in GI Health and Disease**

Gastric variceal hemorrhage in a patient with a history of a splenic abscess

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Case Presentation: A 33 year old African American female with a history of previously treated splenic abscess of unclear etiology, gallstone pancreatitis, and cholecystectomy presented with epigastric pain and hematemesis. She denied any alcohol or nonsteroidal anti-inflammatory use. On admission, she was hypotensive and tachycardic. On exam she had diffuse abdominal tenderness to palpation. Her labs were significant for hemoglobin of 7 gm/dl. Her white blood cell count, platelet count, comprehensive metabolic profile, coagulation labs, hepatitis serologies, amylase and lipase were within normal limits. Esophagogastroduodenoscopy showed large gastric varices in the cardia with stigmata of recent bleed. Abdominal ultrasonography revealed splenomegaly, splenic vein thrombosis and a patent portal vein. An abdominal CT showed resolution of the previous splenic abscess and noted multiple hypodensities in the pancreas. An endoscopic ultrasound showed cysts in the pancreas and a diffusely abnormal pancreatic parenchyma with scattered hyperechoic foci possibly representing calcifications. A fine needle aspiration of the mid-body of the pancreas was nondiagnostic. Given a high risk of rebleeding from gastric variceal hemorrhage a splenectomy was performed. She tolerated the surgery well without any complications.

Discussion: Left sided portal hypertension (LSPH), a rare cause of gastrointestinal bleeding, usually occurs as a result of isolated obstruction of the splenic vein. To date, approximately 450 cases of LSPH have been reported in all. The splenic vein runs behind the tail and body of the pancreas. Because of this anatomy, acute and chronic pancreatitis and pancreatic neoplasms are the most common causes of splenic vein thrombosis. Single episodes of pancreatitis may lead to splenic vein thrombosis and the risk does not correlate with the severity of pancreatitis. Patients are usually asymptomatic, but in symptomatic cases, the first clinical manifestation is a bleed from the varices. Management should be directed at the underlying cause. Endoscopic management of varices with sclerotherapy is effective but rebleeding occurs in more than 25 percent of patients within one year. Splenectomy is curative for gastric varices from splenic vein thrombosis. Although our patient had recurrent pancreatitis, abdominal imaging did not reveal the splenic vein occlusion until her most recent CT after treatment of her splenic abscess, and therefore in her case it is possible that the splenic abscess was the etiology of splenic vein thrombosis. It is important for physicians to maintain a high degree of awareness for a silent splenic vein occlusion in patients with histories of pancreatitis because of the potential risk of bleeding from gastric variceal hemorrhage.

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**A putative folate transporter in *Lactobacillus reuteri* is potentially
involved in immunomodulation**

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Baylor College of Medicine

Culture supernatant from human-derived probiotic *L. reuteri* ATCC PTA 6475 suppresses tumor necrosis factor (TNF) production by human myeloid (THP-1) cells. *L. reuteri* 6475 also produces long-chain folylpolyglutamates, which may be associated with TNF suppression. Transcriptomic data showed that a gene encoding a hypothetical protein, LR0977, was strongly upregulated (>20-fold) in wild-type 6475 during growth in stationary phase (when TNF inhibition is most potent) compared to log phase. Moreover, in strain 6475 containing a mutation in the folylpolyglutamate synthase 1 (fpgS1) gene, LR0977 was dramatically downregulated compared to wild type, suggesting a link between these two genes. Culture supernatant from LR0977 insertional mutant lost the ability to suppress TNF production in THP1 cells and yielded diminished protective effect in a trinitrobenzene sulfonic acid (TNBS)-induced acute colitis mouse model compared to that of wild type. However, factors associated with the cell membrane of the mutant demonstrated moderate TNF suppression in THP-1 cells, compared to a complete loss of inhibition with the cell-free supernatant. This finding suggested that the release of immunomodulatory factors may be inhibited due to the absence of LR0977. From these findings, we hypothesize that LR0977 is involved in the transport of immunomodulatory compounds in 6475. In silico analysis of the LR0977 protein sequence predicted the presence of a signal peptide near the amino terminus and four internal transmembrane helical domains. The Protein Function Prediction (PFP) tool also suggested that this protein might be involved in folic acid transport. The ability to suppress TNF production by THP-1 cells was also partially restored by complementation with a full length LR0977 gene. These findings suggest that LR0977 may be involved in the transport of folate compounds in 6475 and may play a role in immunomodulation. Ongoing studies will identify the cellular pathways affected by a mutation in LR0977 using DNA microarrays. Nuclear magnetic resonance (NMR) spectroscopy analysis of cell-surface associated factors and secretory fractions will compare metabolomic profiles of wild type and the LR0977 mutant in order to further understand the function of LR0977. This study is expected to characterize a novel protein that may be involved in immunomodulation in the host and provide a better understanding of mechanisms of probiosis in *L. reuteri* 6475.

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Upregulation of hepatic OPN occurs in rotavirus-induced murine biliary atresia and requires replicating virus, but is not necessary for development of biliary atresia

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BACKGROUND: Biliary atresia (BA) is a progressive, irreversible pediatric liver disease in which obliteration of bile ducts in the neonatal period leads to obstruction of biliary drainage and progressive scarring (fibrosis) of the liver. Osteopontin (OPN), a glycoprotein with inflammatory and pro-fibrogenic activity, is believed to have important roles for disease pathogenesis in several models disease, including fatty liver disease. The current studies were performed in an established mouse model of biliary atresia, in which inoculation of neonatal pups with rotavirus (RV) leads to inflammation and obstruction of bile ducts and features that very closely resemble human biliary atresia.

HYPOTHESIS: We hypothesized that live but not inactivated RV causes antigenemia (presence of RV antigen in the blood), upregulation of hepatic OPN expression, and induction of BA and fibrosis, and that BA does not develop in OPN-deficient mice (i.e. OPN is necessary for BA).

RESULTS: RV antigenemia developed both in mice inoculated with live (prolonged antigenemia) or with inactivated(transient antigenemia) virus. OPN was expressed in *all* intra- and extrahepatic bile ducts in healthy mice and in mice with BA. Only live RV-treated mice, however, had upregulation of hepatic OPN, BA, and hepatic fibrosis. OPN-deficient mice, similarly to WT mice, developed BA and fibrosis.

CONCLUSIONS: We report, for the first time, that RV antigenemia develops both with live and inactivated RV, but that replicating rotavirus is necessary for development of BA. We also show that OPN is expressed constitutively in the bile ducts of healthy mice, a finding that has been reported in humans but not clearly shown in mice previously. Finally, despite its roles in inflammation and fibrogenesis, which both occur in BA, OPN is not necessary for development of BA in the mouse model. Further work is required to dissect mechanisms of inflammation and fibrosis in human BA and in the mouse model.

2010 DDC Pilot/Feasibility Awardee

**Texas Medical Center Digestive Diseases Center
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**Tlr2 is necessary for hepatic steatosis and metabolic syndrome
in a murine model of diet-induced obesity**

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Purpose/Background: Toll-like receptors (TLR’s) are widely expressed and highly conserved transmembrane proteins that bridge diet and molecular inflammation, the harbinger of metabolic syndrome. Among the TLR’s, *Tlr2* may be particularly relevant to nonalcoholic fatty liver disease, the hepatic manifestation of metabolic syndrome, by virtue of its accessory receptor CD36 which mediates cellular long chain fatty acid uptake. We sought to test the hypothesis that *Tlr2* contributes to the development hepatosteatosis and metabolic syndrome using a model of diet induced obesity.

Methods: Five week old C57BL/6 males and homozygous *Tlr2* deleted (*Tlr2*^{-/-}) mice on the C57BL/6 background were randomized to five weeks of *ad libitum* conventional rodent chow (CRC; kcal %: fat 16%, CHO 62%, protein 22%) or a custom diet designed to mirror an American-style diet (AD; Dyets #101588, kcal %: fat 32%, CHO 51%, protein 17%). Adiposity was assessed by DEXA and energy intake and expenditure through indirect calorimetry. Fasting serum was obtained at sacrifice for insulin, glucose, lipids and ALT. Portions of the liver and adipose tissue were allocated for histology and gene expression analysis by QPCR. Statistical analyses were conducted with two-factor ANOVA and Fisher’s LSD post hoc test.

Results: *Tlr2*^{-/-} mice were completely protected from AD induced adiposity and hepatic steatosis (Fig 1) and had lower serum cholesterol, LDL and triglycerides compared to AD fed controls. Consistent with the phenotype, *Tlr2*^{-/-} mice were largely spared AD induced adipocyte hypertrophy (Fig 2), macrophage infiltration and inflammatory gene expression. Indirect calorimetry and food consumption monitoring revealed that all groups had isocaloric intake and that energy expenditure did not explain the observed differences in adiposity. Furthermore, hepatic gene expression showed that transcriptional regulation of *Srebp1c*, *Fas* and *Ppara* were unlikely to be responsible for the disparate degree of steatosis documented (Fig 3), however surprisingly; serum free fatty acids were increased in *Tlr2*^{-/-} mice irrespective of diet. Taken together, these data raise the possibility that altered lipid absorption or uptake accounts for the lack of adiposity and steatosis in the *Tlr2*^{-/-} group.

Conclusion: *Tlr2* deletion confers dramatic protection against hepatosteatosis and other key clinical and molecular features of metabolic syndrome in a physiological model of obesity. Differences in lipid absorption and tissue uptake warrant investigation, particularly the cooperation between TLR2 and CD36.

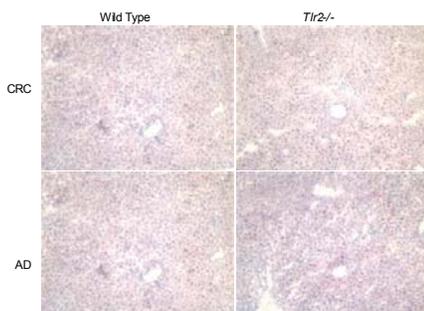


Fig 1. Liver, oil-red-o stain, original magnification 10X

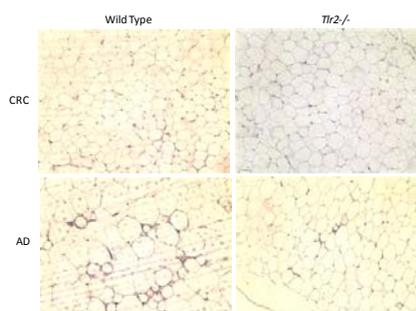


Fig 2. Adipose Tissue, H&E, original magnification 10X

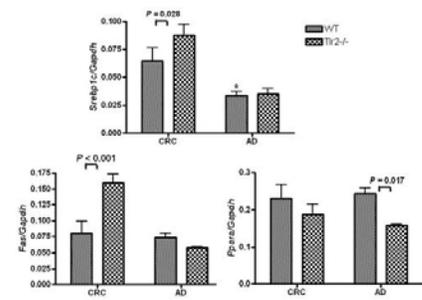


Fig 3. Hepatic Gene Expression. Means ± SEM, n = 4-5/group, *P = 0.003 vs. WT CRC

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**Massive lower gastrointestinal bleeding after abdominal paracentesis
treated with endoscopic therapy**

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A 62-year-old man with a history of hypertension and diabetes mellitus was admitted to the hospital for new onset dyspnea, lower extremity edema and abdominal ascites. He had no known heart, kidney, or liver disease. He denied use of non-steroidal anti-inflammatory drugs or history of gastrointestinal bleed. His physical exam was notable for elevated jugular venous distention, abdominal distention with shifting dullness and no hepatosplenomegaly. Laboratory workup was notable for thrombocytopenia (85 K/uL), BNP 2676 pg/ml, positive Hepatitis C antibody, hemoglobin 11.6 g/dL INR 1.4, normal urinalysis and basic metabolic profile. A bedside abdominal paracentesis was attempted in the right lower quadrant but only revealed 2 ml of bloody aspirate.

Four hours after paracentesis, the patient developed hematochezia with hypotension, tachycardia, and right lower quadrant tenderness. A nasogastric lavage returned only clear aspirate. Repeat labs revealed a drop in hemoglobin to 6 g/dl. He was transferred to the intensive care unit and received aggressive volume resuscitation and 4 units of packed red blood cells. He continued to pass bright red blood per rectum and urgent colonoscopy was performed. Colonoscopy revealed blood throughout the colon and a visible vessel with active bleeding from a puncture site in the ascending colon. Three endoclips were deployed with immediate hemostasis and no further bleeding was observed during his hospitalization. A CT scan did not reveal any free air or blood in the peritoneum.

We present the first reported case of massive intraluminal lower gastrointestinal bleeding caused by diagnostic paracentesis treated with urgent colonoscopy and endoclip placement. Bedside paracentesis is a commonly performed bedside procedure with rare serious complications. Significant bleeding, defined by a drop of hemoglobin of at least 1.5g/dL has been reported to occur in 0-2.7% of procedures and bowel perforation with abscess or peritonitis occur in 0.83% of procedures. We present a case of massive lower GI bleeding after paracentesis. Although this is an uncommon complication, prompt recognition and urgent colonoscopy may allow for effective endoscopic treatment.

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**The incidence and prevalence of inflammatory bowel disease
among veterans: A national cohort study**

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Background/Aims: The incidence and prevalence of Inflammatory Bowel Disease (IBD) has been reported in primarily Caucasian populations. Users of the Veterans Affairs (VA) health care system are diverse in both age and ethnic distribution. There is increasing recognition of IBD among non-Caucasian patients and the elderly. The incidence and prevalence of IBD among (VA) users are unknown. The aim of this study was to identify the incidence and prevalence of IBD among VA users using the national VA datasets.

Methods: We identified veterans with IBD during fiscal years 1998 to 2009 in the national VA administrative datasets, specifically the Patient Treatment Files (PTF) and the Outpatient Care (OPC) files. VA users with ulcerative colitis (UC) and Crohn’s disease (CD) were identified by International Classification of Diseases, 9th Revision (ICD-9) diagnosis codes. Prevalent cases of IBD for each fiscal year were identified by presence of any VA encounter during that year in a patient with an ICD-9 code for UC or CD prior to or during that year. Incident cases of IBD for each fiscal year were identified by first appearance of a diagnosis code for UC or CD in subjects with no prior diagnosis code for either UC or CD. Prevalence was calculated based on the total number of VA users in each fiscal year. Incidence was calculated based on the total number of VA users in each fiscal year minus prior prevalent IBD cases. Prevalence and incidence were adjusted for age and gender by direct standardization of the distribution in 1998.

Results: The prevalence of both UC and CD are steadily rising among VA users. Age and gender adjusted prevalence of UC increased from 163 per 100,000 VA users in 1998 to 397 per 100,000 in 2009. The adjusted prevalence of CD increased from 112 per 100,000 VA users in 1998 to 285 per 100,000 in 2009. There was no temporal trend in the incidence of UC among VA users between 1998 and 2009, with a range from 27 to 74 per 100,000 per year. Likewise, there was no temporal trend in the incidence of CD among VA users in the same time period, with a range of 22 to 56 per 100,000 per year.

Conclusions: Prevalence of both UC and CD are increasing among VA users. As seen in other studies in non-VA populations, there were no consistent temporal trends of incidence of UC or CD among VA users in the past 10 years. This study shows that IBD is prevalent among VA users and the temporal trend of incidence reflects that of the non-VA population.

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MicroRNAs dysregulation in Apc(min/+) mouse adenomas.

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BACKGROUND: The most frequent genetic mutation in hereditary and sporadic cases of colorectal cancer (CRC) is found in the adenomatous polyposis coli (Apc) tumor suppressor gene. The Apc(min/+) mouse, which develops small intestinal adenomas, is a model of human polyposis coli, a risk factor for developing CRC. MicroRNAs (miRNAs) are small regulatory molecules that usually repress gene expression by blocking translation and/or causing mRNA degradation, and they are dysregulated in cancers, including CRC. We hypothesized that the loss of functional APC in Apc(min/+) adenomas would identify miRNAs that could be dysregulated in early stages of CRC and might contribute to disease progression.

METHODS: High quality RNA was extracted from adenomas and the normal appearing intestinal epithelial tissue of C57Bl/6 Apc(min/+) mice (n=3), and from the epithelium of wild type (wt) littermate controls (n=2). Small RNAs (<200nt) from these samples were used for dual color competitive microarray hybridization (LC Science). Spot intensities were normalized, and student's t-test was used to compare expression between adenomas and normal tissues. Additionally, an Apc(min/+) adenoma and a wt control small RNA samples were profiled through next generation sequencing using the Illumina GAI instrument. Quantitative RT-PCR validated the microarray and sequencing results of 8 miRNAs.

RESULTS: By miRNAs microarray we discovered 42 miRNAs that were significantly upregulated and 39 downregulated ≥ 1.5 -fold ($p < 0.05$) in the adenomas compared to the Apc(min/+) normal and wt tissues. The spot intensities of miRNAs found in normal appearing intestinal epithelial tissue from the Apc(min/+) mice clustered with wt epithelium rather than the adenomas from the same mice. From our sequencing data, of the miRNAs that were at least 0.1% of the total usable reads (ur) per sample, we found 7 miRNAs with ≥ 1.5 -fold increased expression in Apc(min/+) adenomas (1,517,313 ur), and 22 miRNAs with ≥ 1.5 -fold increased expression in the wt epithelium (20,084,379 ur). As predicted, the tumor suppressors miR-16 and -15a were downregulated, and the oncomir miR-21 was upregulated in the adenomas by both microarray and sequencing. Q-RT-PCR confirmed Apc(min/+) adenoma-enriched expression of miR-21, -143, and -152, and Apc(min/+) normal epithelia and wt epithelia-enriched expression of miR-16, -22, -31, -142-5p, -194.

CONCLUSION: Adenomas from Apc(min/+) mice show aberrant miRNA expression when compared to normal intestinal tissue found in the same Apc(min/+) mice and wt littermate controls. This suggests that one functional allele of the Apc gene might be sufficient to maintain a normal miRNA expression profile in the mouse intestine, and loss of APC function could result in dysregulation of miRNAs that contribute to CRC progression.

2008 DDC Pilot/Feasibility Awardee

**Texas Medical Center Digestive Diseases Center
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Epigenetics in GI Health and Disease**

**Identification of rotavirus nsp4 ion channel inhibitors
using a high-throughput bacterial bioassay**

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In rotavirus-infected cells nonstructural protein 4 (NSP4) releases endoplasmic reticulum (ER) Ca²⁺ to elevate cytoplasmic Ca²⁺ levels. Release of ER Ca²⁺ is due to NSP4 viroporin activity and since increased Ca²⁺ is necessary for rotavirus replication, we hypothesize NSP4 ion channel inhibitors will block replication and could be effective antiviral drugs to treat rotavirus infection. Thus, we characterized the NSP4 ion channel in the ER membrane using patch clamp electrophysiology, and developed a bacterial bioassay to identify potential small molecule NSP4 inhibitors.

We expressed NSP4-EGFP in Sf9 insect cells using recombinant baculovirus and showed it localized to the outer nuclear envelope. In preliminary patch clamp experiments performed on isolated nuclei, no channel activity was observed on patches from uninfected cells. However, a channel exhibiting conductivity of 29.6 pico-Siemens in 225 mM K⁺ and open probability of ~96% was identified in NSP4-EGFP-containing nuclear envelope patches.

The bacterial bioassay utilizes the inhibition of *E. coli* growth upon expression of viroporins; however, addition of viroporin inhibitors partially restores growth. NSP4 was placed under the arabinose-inducible promoter to tightly control expression and expression induced with 3% arabinose to inhibit growth. LOPAC Ca²⁺ channel modulator compounds were applied directly onto plates inoculated with a lawn of *E. coli* bearing the NSP4 vector. Compounds were ‘hits’ if growth halos were visible around the site where compounds were applied. Four compounds were identified, with hexamethylene amiloride (HMA) giving the largest halo.

Thus, we have identified an “always open” ER ion channel in NSP4-expressing cells, which is consistent with the increased ER Ca²⁺ permeability previously reported by our lab. HMA-inhibition of NSP4 is being validated by demonstrating specific inhibition of NSP4 ion channel activity using patch clamp. Finally, HMA inhibits HIV, HCV, SARS, and Dengue virus viroporins, suggesting it may be possible to develop a broad-spectrum anti-viroporin drug.

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Plasma soluble receptor for advanced glycation end-products and risk of colon polyps in men

Li Jiao, Zhigang Duan, Hashem B. El-Serag

Background: Receptor for advanced glycation end products (RAGE) plays an important role in promoting chronic inflammation. Soluble form of RAGE (sRAGE) represents a naturally occurring competitive inhibitor of RAGE-mediated events. We previously found that sRAGE was inversely associated with risk of colorectal cancer in a prospective study among male smokers. However, the relationship between sRAGE and colorectal polyps has not been examined.

Methods: In this hospital-based case-control study, we examined the association of plasma levels of sRAGE, along with four soluble receptors of inflammatory cytokines including tumor necrosis factor- α receptor I and II (TNF- α RI and TNF- α RII) and interleukin-6 receptor (IL-6R), with risk of colorectal polyps in general and colon adenoma specifically. During 2008- 2010, we identified men with pathologically confirmed colorectal polyp as cases and those with no polyps as controls among prospectively enrolled men who underwent screening colonoscopy. We excluded men with clinical confirmed type 2 diabetes and serum creatinine > 1.2 mg/dl. A total of 73 cases (50 with adenoma) and 62 controls were included in the present analysis. Cases and controls were frequency-matched according to age and race. Plasma samples were obtained at time of colonoscopy and exposure information was collected using interview-administered questionnaire. We used multiplex-ELISA to determine plasma level of serological biomarkers. Multivariate logistic regression model adjusting for age, race, smoking status and body mass index was used to estimate odds ratio (OR) and its 95% confidence interval (CI).

Results: The median age was 61.0 years for both groups. sRAGE had no correlation with TNF-RI, TNF-RII, and sIL-6R levels among controls. Cases had significantly lower levels of sRAGE than controls (median 29.3 versus 34.5, P value for rank sum test = 0.01). When highest compared with lowest tertile of sRAGE, the OR (95% CI) was 0.28 (0.11-0.73) (P trend = 0.004) for colon polyps and 0.22 (0.06-0.85) (P trend = 0.001) for colon adenoma. sIL-6R had statistically non-significant positive association with risk of colon polyps (OR = 1.65, 95% CI = 0.63-4.32, P trend = 0.30) and adenoma (OR = 2.49, 95% CI = 0.81 -7.60, P trend = 0.09). Plasma levels of TNF-RI and TNF-RII had no association with risk of colon polyps.

Conclusion: This study suggests a protective role of sRAGE, an anti-inflammatory factor, for precursor lesions of colorectal cancer in men. Our finding is in line with the previous study that showed an inverse association between sRAGE and colorectal cancer. As a modifiable target, the role of sRAGE in colorectal carcinogenesis deserves further investigation.

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**Stigmasterol augments LPS-induced suppression of BSEP expression in liver:
An explanation for the combined effects of phytosterols and inflammation
in parenteral nutrition associated liver disease (PNALD)**

Heidi E. Karpen, M.D., Aurelie Sauter, B.S., Astrid Kusters, Ph. D., Saul Karpen, M.D., Ph. D.

BACKGROUND: PN administration is associated with the development of steatosis and cholestasis. Etiologies include linked contributions from immaturity, inflammatory signaling and phytosterols, components of lipid emulsions. Due to a paucity of appropriate models, these contributions have not been adequately explored. To address this, we utilized a new model of phytosterol accretion in liver: ABCG8^{-/-} mice fed phytosterol-laden chow.

HYPOTHESIS: The phytosterol stigmasterol (Stigma), a recently described potent antagonist of FXR, the main nuclear receptor (NR) for bile acids, augments inflammation-mediated suppression of FXR-activated genes in liver.

DESIGN/METHODS: C57BL/6 G8^{+/+} and G8^{-/-} male/female mice: 4 week oral feeding from weaning with 2.5% Stigma-enriched or control chow (olive oil), given low-dose LPS or saline i.p. and harvested at 1, 4 and 16 hours (n=6-8/arm). Studies included serum chemistry, histology and gene expression by qRTPCR. ANOVA and 2-tailed t-test, all p values < 0.05

RESULTS: Stigma feeding had no effect on growth or liver cytokine expression in any subgroup, but increased ALT (600± 500 vs. 92± 32) and conjugated bilirubin levels (0.3± 0.2 vs. 0.03± 0.02) only in female G8^{-/-} mice. Stigma feeding led to hepatic steatosis, and necrosis after LPS, only in G8^{-/-} mice, which was more marked in females. LPS equally suppressed NTCP, MRP2, and BSEP RNA levels regardless of G8 genotype or sex. Stigma feeding of G8^{-/-} mice, however, led to additive suppression, most markedly for BSEP expression, and a pronounced gender differential.

BSEP RNA Expression (LPS/Control)

BSEP RNA	G8 ^{+/+} Male	G8 ^{+/+} Female	G8 ^{-/-} Male	G8 ^{-/-} Female
Vehicle Chow	0.31 ± 0.2	0.16± 0.11	0.16± 0.07	0.29± 0.11
Stigmasterol	0.37 ± 0.12	0.25± 0.12	0.04± 0.01	0.02± 0.01

CONCLUSIONS: Livers from G8^{-/-}, but not G8^{+/+} mice, fed a 2% Stigma diet had enhanced suppressive effects of LPS on FXR-mediated gene expression, especially BSEP. Female G8^{-/-} mice displayed enhanced susceptibility to liver injury when fed a Stigma diet. These data suggest that combinatorial inhibition of liver gene expression may underlie the effects of inflammation and phytosterols in PNALD. Moreover, this model provides a new means to explore the effects of phytosterols on a variety of cellular functions in liver.

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β 2SP interacts with Smad3 and regulates TGF- β signaling in liver tumorigenesis

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M.D. MD Anderson Cancer Center

Objective: The TGF- β signaling pathway is involved in the control of multiple biological processes, including cell proliferation, differentiation, migration and apoptosis. It is one of the most commonly altered cellular signaling pathways in human cancers. TGF- β signaling is initiated by the binding of TGF- β ligand to the type II TGF- β receptors (TBRII). This binding allows the subsequent incorporation of the TGF- β type I receptor (TBRI). With the help of adaptor proteins, activated TBRI then recruits and phosphorylates two downstream transcription factors, Smad2 and Smad3, allowing them to bind to Smad4. Our previous study demonstrates that β 2SP (Spectrin beta2) regulates TGF- β signaling by mediating the association between TGF- β type I receptor and Smad3. However, the detailed molecular mechanism is still elusive. In the present study, we aim to investigate the molecular mechanism of the interaction between β 2SP and the elements of TGF β signaling pathway, as well as the biological significance of their interaction in liver tumorigenesis.

Materials & Methods: SNU398, SNU449, SNU475, HepG2, MEF and 293T cells were used. lentivirus infection was employed to generate stable cell lines. Co-immunoprecipitation and colocalization assays (Confocal and fluorescence microscopy) were performed to investigate the protein-protein interactions. RT-PCR, superarray assay, luciferase assay, ChiP assay, EMSA assay were carried out to evaluate the effects on transcription. MTT assay, Boyden chamber assay, soft agar assay were used to determine the biological significance of loss of β 2SP.

Results: Our data showed that β 2SP interacts with both TGF- β receptor I and Smad3, Association of β 2SP with TGF- β receptor I and Smad3 is mediated by its N-termini and spectrin-domains, respectively. β 2SP regulates the phosphorylation and nuclear translocation of Smad3. β 2SP also modulates Smad3-mediated transcription of TGF β signaling pathway target genes. Loss of β 2SP attenuates the tumor suppression effects of TGF β signaling and predisposes cells to tumorigenesis.

Conclusion: β 2SP interacts with both TBR1 and Smad3, and facilitates the recruitment and phosphorylation of Smad3 by TBR1. Loss of β 2SP impairs the Smad-dependant TGF β signaling, and increases the tumor promotion effects of TGF- β pathway.

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Epigenetics in GI Health and Disease**

***Lactobacillus reuteri* DSM 17938 reduces inflammation in necrotizing enterocolitis and increases regulatory T cells in intestines and mesenteric lymph nodes in neonatal rats**

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Background: Abnormal gut microbiota and aberrant immune responses can lead to necrotizing enterocolitis (NEC) in the premature infant. *Lactobacillus reuteri* DSM 17938 has been demonstrated to produce anti-inflammatory signals in the intestines of newborn rats. We hypothesized that DSM 17938 will reduce the inflammation and modulate immune responses to prevent NEC. Regulatory T cells (Tregs) have shown to be pivotal players in the maintenance of immune tolerance and anti-inflammatory responses. They are characterized by the expression of the transcription factor forkhead box P3 (Foxp3). The ability of DSM17938 to modulate Tregs has not been investigated.

Aims: To examine the potential of DSM 17938 to prevent NEC and modulate Tregs in a neonatal rat model.

Methods: Experimental NEC was induced in newborn rats by formula feeding and exposure to hypoxia for 3 days. Newborn rat pups were divided into 5 groups: dam-fed, formula-fed, formula + DSM 17938 (10^6 cfu/g. b.w. /day), formula + hypoxia (NEC), and NEC + DSM 17938. Intestinal histology was evaluated for NEC scores. Cytokine levels in the ilea were assessed. To analyze Tregs, rat pups were dam or dam + DSM 17938. Lymphocytes from thymus, spleens, mesenteric lymph nodes (MLN) and small intestines (INT) of rat pups at day of life (DOL) 1, 2, 3, and 4 were labeled for T cell surface markers CD3, CD4, CD8, and intracellular Foxp3 and analyzed by flow cytometry.

Results: Feeding DSM 17938 (n = 38) to neonatal rats with NEC significantly increased survival rate compared to NEC without probiotic (n = 46) ($p < 0.0001$). The incidence and severity of NEC were decreased by the administration of DSM 17938. Increased cytokine levels of TNF- α , IL-1 β , and IL-13 in the intestines of rat pups with NEC were all significantly decreased in the pups fed with DSM 17938. Ilea of rats that were formula-fed without hypoxia (n = 29) also demonstrated mild inflammation compared to dam-fed controls (n = 22). Formula-associated inflammation was suppressed by DSM 17938 (n = 19). Tregs (CD3⁺CD4⁺CD8⁻ Foxp3⁺ cells) in spleen and MLN of newborn rats with dam-fed increased in a day-of-life-dependent manner. Administration of DSM 17938 to dam-fed rats significantly increased Tregs in MLN on DOL 2, 3, and DOL4 ($p < 0.001$) and in INT on DOL 1 ($p < 0.001$) and DOL2 ($p < 0.01$) compared to dam-fed without probiotic. The probiotic DSM 17938 did not affect Tregs in either spleen or thymus early in life, indicating initial induction of immune response locally.

Conclusions: *L. reuteri* DSM 17938 reduced the incidence and severity of NEC. The anti-inflammatory effect of DSM 17938 was associated with the interaction with intestinal mucosa as well as induction and/or migration of Tregs to MLNs and INTs. These results support the contention that *L. reuteri* DSM 17938 may represent a useful treatment to prevent NEC.

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Regulation of cellular and metabolic homeostasis by endocrine FGFs*

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The canonical FGF-heparan sulfate (HS)-FGFR tyrosine kinase plays important roles in embryonic development and adult tissue homeostasis through promotion of cell proliferation, growth and differentiation by autocrine and paracrine mechanisms. Its deregulations underlie a variety of tissue-specific diseases including cancer. In metabolic organs, on the other hand, the transmembrane co-factors Klotho (KL) and betaKlotho (KLB) re-direct the FGFR signaling to regulation of metabolic homeostasis without promoting cell proliferation, through direct interaction with both FGFRs and the endocrine FGFs including FGF19, 21 and 23 but not canonical FGFs. Both FGF19 and FGF21 can bind and activate the FGFR1-KLB complex, but only FGF19 can bind and activate FGFR4-KLB. The postprandial FGF19 (mouse FGF15) from ileum regulated by the bile acids-activated FXR targets hepatic FGFR4-KLB for a negative feedback control of bile acid/cholesterol synthesis and lipid metabolism. It may also target the adipose FGFR1-KLB for regulation of fatty acids metabolism. FGF21 is a PPAR α -regulated hepatokine in response to starvation and other liver perturbations including liver damage and fatty liver, however, it serves to target the FGFR1-KLB in adipose tissue but not FGFR4-KLB in liver for regulation of lipid metabolism and suppression of obesity. Ablation of FGFR1 **specifically in adipose tissue has no overt effects on adipocyte morphology and growth**. Ablation of FGFR4 in hepatocytes causes not only defects in cholesterol/bile acid, lipid and glucose metabolism, but also accelerated progression of carcinogen-induced hepatomas. Pharmacological administration of either FGF19 or FGF21 results in drastic weight loss in wildtype but not the adipocyte-specific FGFR1 knockout mice with diet-induced obesity. The inducible expression of FGFR4 in cells expressing KLB inhibits cell population growth and anchorage-independent colony formation through dose-dependent induction of apoptotic cell death by depression of AKT and mTOR activity. In hepatocytes where expression of both KLB and FGFR4 are coordinately expressed at high levels, KLB expression is significantly down-regulated during hepatocarcinogenesis. Restoration of both KLB and FGFR4 to FGFR4 $^{-/-}$ hepatoma cells induces apoptosis in response to FGF19. This indicated that FGFR4-KLB might play a hepatoma suppressive role in addition to its metabolic function. Our results show that concurrent with metabolic regulation, KLB may serve to suppress tumor-promoting effects from either KLB-independent FGFR or other pathways. This provides new insights into the mechanism by which the KLB -FGFR partnership through the FGFR tyrosine kinase activity coordinates cellular and metabolic homeostasis.

* This work was supported in part by the PHS grant DK56338, which funds the Texas Medical Center Digestive Diseases Center. We are grateful to Dr. Wallace McKeehan for support and in whose laboratory the initial work was carried out.

2010 DDC Pilot/Feasibility Awardee

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Endothelial nitric oxide synthase is a key mediator of hepatocyte proliferation in response to partial hepatectomy in mice

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Background and Hypothesis: Endothelial nitric oxide synthase (eNOS) plays major roles in vascular physiology and pathophysiology. Recent studies confirm eNOS expression in hepatocytes in addition to endothelial cells. However, functional significance of eNOS in hepatocyte proliferation in response to partial hepatectomy (PH) has remained unexplored. Epidermal growth factor receptor (EGFR) is a key mediator of mitogenic signaling of multiple hepatic mitogens in regenerating livers. Therefore, the purpose of this study was to test the **hypothesis** that eNOS plays a critical role in EGF-mediated hepatocyte priming and proliferation in regenerating livers.

Methods: Wild type (WT) and eNOS knockout (KO) mice were subjected to 70% PH. The resected lobes (0 min) and remnant livers (15 min to 72h post-PH) were analyzed for eNOS activation (p-eNOS-Ser1177), hepatocyte priming (p-c-Jun-Ser63, c-Jun) and proliferation (cyclin A, PCNA western blotting; BrdU immunostaining). Primary hepatocytes isolated from WT and KO mice were treated with EGF (20 ng/mL; 1, 24h) in order to evaluate the role of eNOS in hepatocyte priming and proliferation *in vitro*. Hepatocytes were pre-treated with inhibitors targeting EGFR, PI3K/AKT signaling prior to EGF treatment (5-120 min) for the analysis of phosphorylation of EGFR, AKT, eNOS and c-Jun.

Results: PH induced eNOS phosphorylation and activation in remnant livers (peaked at 30 min). Hepatocyte priming and proliferation was significantly attenuated in the KO livers, as evidenced by impaired p-c-Jun (0.6 fold; 30min), cyclin A (0.3 fold; 45h), PCNA (0.8 fold; 45h; 0.4 folds; 72h) expression and BrdU incorporation (0.3 fold; 45h), as compared to WT (1.0). In response to EGF stimulation, KO hepatocytes had attenuated induction of p-c-Jun (0.3) at 1h, cyclin D1 (0.6), PCNA (0.7) and BrdU incorporation (0.5) at 24h. Inhibition of EGFR and PI3K-AKT signaling effectively blocked EGF-induced p-eNOS expression (0.2; 1h) and hepatocyte proliferation (cyclin D1, 0.2; PCNA, 0.1; 24h).

Conclusions: Our results suggest that eNOS activation is essential for efficient hepatocyte priming and proliferation in response to PH. EGF-induced eNOS activation and proliferation is dependent on intact EGFR-PI3K-AKT signaling in hepatocytes.

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**SOX9 acts as a tumor suppressor by direct regulation of *Igfbp4*,
CEACAM1, and *Vegfa* in colorectal cancer cells**

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Background and Aims: The transcription factor SOX9 plays an important role in the cell lineage specification and cellular differentiation of various tissues. In the intestinal epithelium, SOX9 is expressed in crypt cells including stem cells, transit-amplifying cells, and Paneth cells, and in most human colorectal cancer (CRC) cells SOX9 is highly expressed. We previously reported that SOX9 is required for Paneth cell differentiation in the mouse small intestine. In the absence of SOX9, the major Paneth cell specific genes are not detected and there is more proliferation observed in the crypts. However, little is known about the molecular mechanisms of how SOX9 regulates Paneth cell differentiation and proliferation in the normal intestine and in CRC. Using the mice in which *Sox9* is inactivated specifically in the intestinal epithelium (*Sox9*^{intestine}), we performed microarray experiments to identify genes that are regulated by SOX9 in the intestinal crypt cells, which included some known tumor suppressor genes as well as tumor promoters, namely *Igfbp4*, *CEACUM1*, and *Vegfa*, in addition to Paneth cell specific genes.

Results: Overexpression of SOX9 transactivated *Igfbp4* promoter in Caco2 cells, a human intestinal cell line, and the direct regulation of the *Igfbp4* promoter by SOX9 was also supported by reporter assays. In fact, *Igfbp4* was downregulated in *Sox9*^{intestine} intestinal crypts by *in situ* hybridization. In addition, transactivation of *Igfbp4* promoter was enhanced by Znf219, which was recently reported as a SOX9 co-activator by others. We speculated that SOX9 might act as a tumor suppressor by positively regulating some tumor suppressor genes and negatively regulating tumor promoters. In order to test this hypothesis, we crossed the *Sox9*^{intestine} mouse line with *Apc* mutated mouse line (*Apc*^{min/+}) and obtained statistical data that shows that in the absence of SOX9, there are more and larger adenomas. Intestinal adenoma samples from double mutant mice for *Sox9* and *Apc* showed down regulated levels of *Igfbp4* and *CEACUM1*, and upregulated *Vegfa* compared to littermate control (*Apc*^{min/+}) by quantitative RT-PCR.

Conclusions: We demonstrate, for the first time, that SOX9 directly regulates tumor suppressor, *Igfbp4* and suppresses tumor growth in *Apc*^{min/+} adenomas.

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Dietary cellulose supplementation during childhood induces transient trophic, anticolitic and microbiomic effects in the large intestine of mice

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Background: Nutritional exposures may influence the development of several common disorders. Dietary fibers have trophic and anticolitic effects on the large intestine in rodents and can exert beneficial effects in humans. Indirect epidemiologic data suggests that dietary fibers may protect against inflammatory bowel diseases (IBD) where intestinal mucosal microbes are recognized to play an etiologic role. The critical developmental period for nutritional exposures to induce their phenotype modifying effects may vary from disease to disease. The peak onset of IBD is in young adulthood. Therefore, the pediatric time period may be important in respect to the nutritional developmental origins of this disease group. Here, we studied the effects of transient dietary cellulose supplementation during childhood on young adult colitis susceptibility and on the colonic mucosal microbiome in mice.

Methods: C57BL/6J male mice were studied. The animals received a synthetic high fiber (12.5%), or control (2.5% fiber) diet from postnatal days (P) 30 to P80. Thereafter, the high fiber group was reversed to control diet for 10 (R10) or 40 (R40) days. Colitis was induced by dextran sulfate sodium (DSS). Outcome measures of colitis included weight loss and colonic length at sacrifice. The mucosal microbiome was studied by massively parallel pyrosequencing of *16S rRNA*.

Results: Cellulose supplementation induced transient trophic effects on the colon (increased length) that persisted 10 days following reversal, but diminished by 40 days. Colitis susceptibility inversely correlated with colonic length measures. Mice reversed for 10 days from the high fiber diet were protected compared to controls, while colitis susceptibility was similar between the 40 day reversal and control groups. High fiber diet stimulated a significant separation of the colonic mucosal microbiome from controls. This separation decreased, but persisted to some extent into 10 days following reversal.

Conclusions: Dietary cellulose supplementation during childhood induces transient trophic, anticolitic and microbiomic effects in the large intestine of mice. These findings may have potential implications for dietary fiber supplementation in humans.

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Genetic modifiers of liver disease associated with alpha1-antitrypsin deficiency

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Background: Inefficient degradation of aggregation-prone alpha1-antitrypsin (AAT) mutant Z has been implicated in the pathogenesis of liver disease associated with AAT deficiency. However, the underlying molecular mechanism remains largely unknown. Opportunistic removal of alpha1, 2-linked mannose units by ERManI, a putative endoplasmic reticulum (ER) resident mannosidase, plays a rate-limiting role in extracting misfolded N-linked glycoproteins, such as mutant AAT, from the ER for degradation by cytosolic 26S proteasomes. ERManI functions in a stoichiometric manner, therefore its concentration must be carefully regulated to ensure selective degradation of misfolded glycoproteins. Studies in our laboratory have revealed several post-transcriptional mechanisms involved in regulating the intracellular concentration of ERManI, including proteolytic down-regulation, hindered proteolysis, and the repositioning of molecules between intracellular compartments. Recently, we identified a single nucleotide polymorphism (SNP), rs4567, as a modifier of age-at-onset of the end-stage liver disease through translational suppression of ERManI. The allelic change of rs4567, which is localized to the 3'-untranslated region of ERManI mRNA, is predicted to alter the binding efficiencies of adjacent sequence to several microRNAs. This finding raised the possibility that microRNAs might be involved in the pathogenesis of liver disease associated with AAT deficiency. The current study is focused on the role of microRNAs in regulating rs4567-mediated translational suppression of ERManI, as well as the overall cellular response to the intracellular accumulation of AAT-Z.

Methods: ERManI cDNA, as well as luciferase reporter constructs bearing 3'UTR of ERManI with different alleles at rs4567, were generated. Each construct was co-transfected with candidate miRNA into mammalian cells and the effects of the miRNAs on translational efficiency of ERManI or the reporter were monitored using either pulse-chase experiment or luciferase assay, respectively. In addition, the role of AAT-Z on the expression of the candidate miRNAs was evaluated using real-time PCR.

To examine the miRNA expression profile in the disease models, total RNAs were extracted from adult (10-week old) male wild type (C57BL6/wt) and Z transgenic (C57BL6/Z) mice (n=5), or from HEK293 cells stably expressing wild type (M) or mutant AAT (Z) (n=3). The miRNA expression in each sample was examined using Agilent miRNA microarray platforms followed by statistical analysis.

Results: As one of the candidate miRNAs targeting rs4567, miR-362 showed a ~30% translational suppression of ERManI or luciferase reporter. In addition, Z overexpression induced a significant increase in the miR362 level.

Conclusion: Our results indicate that miR-362 is involved in rs4567-mediated translational suppression of ERManI. However, the low level of suppression by miR362 cannot fully account for the suppressive effect of rs4567 on ERManI translation. Additional miRNAs, or alternative mechanisms may exist and are currently under investigation.

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***PRAJA1*, a ring finger protein as a function E3 ligase mediated modulation
of TGF- β signaling by degradation of SMAD3 and β 2SP (ELF)**

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Purpose/Background: Cytokines of the transforming growth factor- β (TGF- β superfamily are multifunctional proteins that regulate growth, differentiation, apoptosis, and morphogenesis of various types of cells. Also, TGF- β signaling pathway plays an essential role in regulating tumorigenesis. Prior to initiation and in early stage during tumor progression TGF- β acts upon the epithelium as a tumor suppressor, however at later stages it is often a tumor promoter. Smads are signal mediators for the members of the TGF- β pathway. Upon phosphorylation by the TGF- β receptors, Smad3 translocates into the nucleus, recruits transcriptional coactivators and corepressors, and regulates transcription of target genes. In previous study Smad3 interacts with a Ring finger protein, through its C-terminal MH2 domain in a ligand dependent manner.

Methods: Here we performed expression of PRAJA1 and b2SP by western blot in HCC cells, and for understand the interaction of PRAJA1 and Smad3 used several methods such as pull-down, IP, luciferase assay, and QRT-PCR.

Results: PRAJA is expressed in abundance in tumors from hepatocellular carcinoma (HCC) when compared to the normal. In here we found PRAJA1 interact with not only MH2 domain but also show weak interaction MH1-link region in HCC cells. In previous report, we demonstrated under the influence of TGF- β , both b2SP and Smad3 are ubiquitinated and degraded by PRAJA1. Also, we have investigated the structure-function relationship between b2SP, Smad3 and PRAJA1 during HCC progression in presence or absence of TGF- β signaling. Co-localization via confocal microscopy and immunoprecipitation reactions demonstrate association of PRAJA with both Smad3 and b2SP in a TGF- β -dependent manner. Both b2SP and Smad3 interact with the N-terminal fragment of PRAJA1 (1-150 AA) whereas PRAJA1 couldn't interact with MH1 domain of Smad3. QRT-PCR and Northern blot analysis both confirm the induction of TGF- β induced gene *c-fos* during the above interactions.

Conclusion: Here, we study that PRAJA1 a Ring finger E3 ubiquitin ligases protein could interact with Smad3 and b2SP, which are Smad3 and Smad4 adaptor protein and a tumor suppressor. PRAJA1 hold Ring finger domain in carboxyl terminal. PRAJA1 associates with both Smad3 and b2SP at independent sites different from its RING finger domain, which is responsible for its ubiquitination function. These studies reveal a mechanism for tumorigenesis whereas defects in interacting proteins for Smads, such as b2SP as well as PRAJA, serve as an important future therapeutic target in HCC.

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**Intestinal cell specific contributions of nitric oxide
to the pathogenesis of necrotizing enterocolitis**

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Background: Necrotizing enterocolitis (NEC) is an intestinal emergency mainly of premature infants and the pathogenesis is unknown. Nitric oxide (NO) is one of the important mediators of NEC. however, its role is unclear. Argininosuccinate lyase (ASL) is the only enzyme in the body capable of generating arginine, the substrate for NO.

Objective: To study the contribution of specific intestinal cells to the local NO pool and its importance in the pathogenesis of NEC using a novel conditional knockout mouse model in which endogenous NO production is impaired at a *cellular level* in different intestinal compartments.

Design/Methods: Via homologous recombination, a conditional null allele of *Asl* was generated. Cell specific knockout (KO) of *Asl* was generated in enterocytes and macrophages by breeding with transgenic mice expressing Cre recombinase in the respective cells. A model of NEC was established in these mutant and wild type mice by subjecting premature mouse pups to exclusive formula feed, hypoxia and hypothermia. NEC was graded based on histopathology. Inflammatory mediators, protein and mRNA expression of *Asl*, nitrites and nitrosylation were measured.

Results: *Asl* levels on Western blot and RT-PCR analysis were lower in the macrophage specific *Asl* KOs. In macrophage specific KOs, the *in vitro* cytokine response to lipopolysaccharide (LPS) was significantly blunted [IFN γ ($p < 0.008$) and TNF α ($p < 0.0001$)]. 12/28 (43%) of controls and 8/29 (28%) of macrophage specific KOs developed NEC ($p = 0.2$). 6/11 (55%) of controls and 8/10 (80%) of enterocyte specific KOs developed NEC ($p = 0.3$).

Conclusions: The macrophage specific KO of *Asl* demonstrated significantly reduced response to cytokines. Within these limited sample sizes, the macrophage specific KO of *Asl* showed a trend towards decreased incidence of NEC and the enterocyte specific KO of *Asl* exhibited a trend towards increased incidence of NEC. The current trends if confirmed with increasing sample sizes suggest that macrophage derived NO may increase susceptibility to NEC and enterocyte derived NO is protective against NEC. These results highlight the importance the cell specific contributions to the local NO milieu in the pathogenesis of NEC. This offers new prospects in the pharmacological and cell specific manipulations of ASL/NO metabolism in the prevention and treatment of NEC.

2011 DDC Pilot/Feasibility Awardee

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MicroRNAs in villus and crypt epithelium of the mouse small intestine

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BACKGROUND: microRNAs (miRNAs) are a relatively new class of small regulatory RNAs (~22nt) essential for cell proliferation and differentiation in all animal cell types and tissues studied. miRNAs generally repress gene expression by binding the 3'-untranslated region of target mRNAs, blocking translation and/or causing mRNA degradation. In the small intestine, epithelial cells migrate from the crypts, the site of cell proliferation, up the villi, where terminally differentiated cells function. We hypothesize that miRNAs confer a novel layer of regulation in small intestinal epithelium and speculate that there are gradients of miRNA expression along the crypt-villus axis.

METHODS: Small RNAs were extracted from C57Bl/6 mouse villus- and crypt-enriched epithelial fractions (n=3). These RNA samples were used for dual color competitive microarray hybridization with known miRNA and microconserved elements (MCEs), which may represent novel miRNAs found in progenitor cells. Spot intensities were normalized, and student's t-test was used to compare expression between villus and crypt enriched epithelium. One villus- and one crypt fraction were also profiled through next generation sequencing using the Illumina GAII instrument. Small intestinal cell expression of select miRNAs was determined by miRNA RT followed by quantitative PCR (RT-qPCR) specific for the miRNAs. MicroRNA mimics were transfected into the Caco-2 intestinal cell line to show any reduction in mRNA and protein expression by RT-qPCR and Western blot, respectively.

RESULTS: A total of 111 miRNAs were detected in RNA from intact mouse jejunum, 92 expressed in the villus epithelia, and 89 in the crypt. A small number of these miRNAs displayed 2-fold or greater differential expression in the villus or crypt fractions, 9 with greater expression in the villi, and 3 in the crypts (p<0.05). From our sequencing data, of the miRNAs that were at least 0.01% of the total usable reads (ur) per sample, we identified 3 miRNAs with ≥2-fold increased expression in the villus-enriched epithelium (1,798,790 ur), and 60 miRNAs with ≥2-fold increased expression in the crypt-enriched epithelium (2,307,614 ur). Q-RT-PCR confirmed the villus-enriched expression of miR-22, -142-5p, and -150, and the crypt-enriched expression of miR-152. Mimics for miR-152 transfected into Caco-2 cells demonstrated that Krüppel-like factor-4 (Klf4), a predicted target of the crypt-enriched miR-152, had reduced protein but not mRNA expression.

CONCLUSIONS: A small number of miRNAs show differential expression in epithelial cells along the crypt-villus axis. These miRNAs may play a role in regulating proliferation and differentiation of intestinal epithelial cells. For instance, our data suggests that the crypt-enriched miR-152 may repress Klf4, expressed in mature goblet cells and enterocytes. Knowing small molecule RNAs important for intestinal epithelial cell proliferation and differentiation and identifying their targets could help us find new therapeutic agents for intestinal diseases such as intestinal bowel disease and cancer, and for promoting intestinal regrowth and repair.

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**Maternal methyl-donor supplementation induces prolonged
murine offspring colitis susceptibility**

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Background: Developmental epigenetic changes, such as DNA methylation, have been recognized as potential pathogenic factors in inflammatory bowel diseases (IBD) the hallmark of which is an exaggerated immune response against luminal microbes. A methyl-donor diet (MD) can modify DNA methylation at select murine genomic loci during early development. The components of the MD are routinely incorporated into prenatal human supplements. Therefore, we studied the effects of maternal MD supplementation on offspring colitis susceptibility and colonic mucosal DNA methylation and gene expression changes in mice as a model. Additionally, we investigated the offspring mucosal microbiomic response to the maternal dietary supplementation.

Materials/Methods: Colitis was induced by dextran sulfate sodium (DSS). Colonic mucosa from offspring of MD supplemented mothers following reversal to control diet at weaning was interrogated by methylation specific microarrays and pyrosequencing at postnatal days 30 (P30) and P90. Transcriptomic changes were analyzed by microarray profiling and real time RT-PCR. The mucosal microbiome was studied by high throughput pyrosequencing of *16S rRNA*.

Results: Maternal MD supplementation induced a striking susceptibility to colitis in offspring. This phenotype was associated with colonic mucosal DNA methylation and expression changes. Metagenomic analyses did not reveal consistent bacteriomic differences between P30 and P90, but showed a prolonged effect of the diet on the offspring mucosal microbiome.

Conclusions: Maternal MD supplementation increases offspring colitis susceptibility that associates with persistent epigenetic and prolonged microbiomic changes. These findings underscore that epigenomic reprogramming relevant to mammalian colitis can occur during early development in response to maternal dietary modifications.

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**Role of TGF- β Smad3/4 adaptor protein β 2SP in TLR4
(Toll-like Receptor 4)-dependent Liver Oncogenesis**

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Objective: HCC (Hepatocellular Cancer) is the third leading cause of cancer deaths. Heavy alcohol intake is an established risk factor for HCC in patients with hepatitis B virus (HBV) and hepatitis C virus (HCV) infection, increasing the risk by 4~6 fold as compared to those without alcoholism. This synergism also exists for diabetic HCV/HBV patients suggesting a common mechanism induced by alcohol and diabetes. Inactivation of TGF- β Smad3/4 adaptor protein β 2SP is a well known risk factor for HCC in man and a causal oncogenic mechanism in animal models.

Materila & Methods: In this study, we tried to merge these two independent concepts into one and tests a novel hypothesis that enhanced TLR4 signaling and reciprocally suppressed TGF-beta signaling are in fact causally linked to render synergistic oncogenic signaling in the genesis of CSCs and liver tumors due to alcohol and HCV.

Results: Our studies reveal that TLR4 (Toll-like receptor 4) induced in hepatocytes by the HCV NS5A protein in Ns5a transgenic mice results in heightened activation of TLR4 by endotoxemia associated with alcoholism and diabetes, leading to TLR4-dependent induction of the stem cell factor Nanog, generation of Nanog+ CSCs (cancer stem cells), and liver oncogenesis. In essence, TLR4 signaling is now identified as a central mediator in synergistic liver tumor formation by HCV and alcohol.

Conclusion: Given the morbidity/mortality associated with HCC among HCV/HCB patients, the outcome of our study will not only cast mechanistic insights but also contribute to the development of new therapeutic and preventive modalities. We further believe that this novel concept of TLR4-TGFbeta interactions has far-reaching implications in oncogenesis of epithelial organs in general which commonly follow chronic inflammation.

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**Reata, E3 ligase inhibitor, potently inhibits hepatocellular carcinoma cell growth through
PRAJA/Smad3/ β -Spectrin and Keap1/Nrf2 signaling**

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Background: HCC is lethal and difficult to treat due to late diagnosis and few effective targeted therapeutics and unclear molecular profiling for this deadly disease. Novel targets for early diagnosis and effective therapy are therefore urgently needed. Our recent studies support a key role for TGF- β signaling Smad3/4 adaptor protein β 2SP in suppressing these tumors. We found that deletion of β 2SP results in a dramatic and spontaneous formation of liver (HCC) and gastrointestinal cancers. High levels of E3 ligases PRAJA and Keap1 occur in tumors in these animals. We have demonstrated that PRAJA, as the E3 ubiquitin ligase of β 2SP and Smad3, plays an important role in regulating the levels of β 2SP, thereby modulating TGF- β signaling. Meanwhile, Keap1-Nrf2 signaling is another important pathway in HCC tumorigenesis, since Nrf2 activation leads to antioxidant response and Keap1 represses Nrf2 activation by ubiquitination and degradation.

Aims: The aim of this study is to elucidate E3 ligases, PRAJA and Keap1 as therapeutic targets on HCC and to determine the effects of the specific E3 ligase inhibitors Reata on HCC cell growth and the mechanism action involved.

Methods: Immunohistochemistry, Q-PCR and Western blot were used to determine the expression level of PRAJA and Keap1 in hepatocellular cancer tissues and cell lines in human and in β 2SP^{-/-} mouse model. Genetic down-regulation of PRAJA and Keap1 by lentivirus shRNA in HCC cell lines were established and used for its functional study in vitro. MTS assay was used for cell proliferation assay. Down-stream genes promoter activations were examined by transient transfections. Co-immunoprecipitation and confocal immunofluorescence were used for protein-protein interactions.

Results: In this study, we demonstrated both PRAJA and Keap1 is dramatically upregulated in human hepatocellular cancer tissues and cell lines as well as in β 2SP^{+/-} mice tissues and β 2SP^{-/-} MEFs cells compared with β 2SP wild type tissues and cells, while level of β 2SP was decreased in human hepatocellular cancer tissues and cell lines. Down regulation of PRAJA and Keap1 by shRNA lentivirus in HCC cell lines decreased cell growth. A specific E3 ligase inhibitor, Reata1/2 (also named RTA402/405), originally identified an inhibitor for Keap1, blocking Keap1-dependent Nrf2 ubiquitination, can potently inhibits hepatocellular carcinoma cell growth in HepG2, Chang Liver, Hep3B and Huh7 HCC cell lines. To further elucidate the mechanisms by which Reata inhibits HCC cell growth, we found that Reata inhibits cell growth by blocking degradation of Nrf2 and increases Nrf2 expression in Hep3B cells. Interestingly, Molecular docking shows that Reata also binds PRAJA ring finger domain and prevents degradation of β 2SP and Smad3. Treatment of Reata at 0.5 μ M in Hep3B, SNU449 and SNU475 lead to increase β 2SP protein level.

Conclusion: These results suggest that Reata, E3 ligase inhibitor, potently inhibits Hepatocellular carcinoma cell growth through PRAJA/Smad3/ β -Spectrin and Keap1/Nrf signaling. Thus, PRAJA and Keap1 may be potential novel targets in the setting of dysfunctional of TGF- β signaling in hepatocellular carcinoma.

Texas Medical Center Digestive Diseases Center
3rd Annual “Frontiers in Digestive Diseases” Symposium:
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**Regulatory control of reuterin production varies between
probiotic *Lactobacillus reuteri* strains and is dependent upon PocR**

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Probiotic *Lactobacillus reuteri* is an indigenous resident to the human digestive tract and possibly a key member of the microbiota. Probiotic characteristics of *L. reuteri* suggest a role in shaping the microbial landscape and influencing host response to perturbations of its gastrointestinal niche. In general, human isolates of *L. reuteri* produce reuterin, a broad-spectrum antimicrobial agent capable of aiding in sculpting the neighboring microbial community. In *L. reuteri*, glycerol dehydratase (*gdh*) converts glycerol to reuterin, and some reuterin is converted to 1,3-propanediol by 1,3-propanediol oxidoreductase (1,3-*pdo*). While the synthesis and antimicrobial effects of reuterin are well established, the regulatory mechanisms of reuterin production and resistance are unknown. We have shown previously that different *L. reuteri* strains (DSM 17938 and ATCC PTA 6475) vary in their ability to produce reuterin. Further investigation revealed that *L. reuteri* 6475 is a constitutive producer, while *L. reuteri* 17938 induces reuterin production over time. Two-color microarray analyses showed increased expression in both strains of a putative AraC-like transcriptional regulator (*pocR*), indicating this gene might participate in reuterin production. Targeted insertional mutagenesis was used to generate *L. reuteri* mutants inactivated in *pocR*, and phenotypic analysis has deemed this gene essential for reuterin production. Comparisons between the transcriptomes of *pocR* mutants and wild-type strains under anaerobic conditions (with fold changes >1.5 and adjusted p-values <0.05) indicate that *pocR* is involved in the expression of operons required for reuterin production and vitamin B12 synthesis. The inactivation of *pocR* in 17938 resulted in the down-regulation of several transcripts related to reuterin production (18 genes) and vitamin B12 synthesis (26 genes), respectively. Similarly, the down-regulation of 19 genes related to reuterin production was observed in 6475 *pocR*, however only 10 vitamin B12 genes were down-regulated in this strain. Additionally, 1,3-*pdo* was down-regulated 3-fold in the 6475 *pocR* insertion mutant, but remained unaffected in the 17938 *pocR* mutant. These results suggest that *pocR* either directly or indirectly effects both reuterin production and degradation in 6475 but only reuterin production in 17938. These differences may contribute to the variation in reuterin production measured in these two strains. Further microarray studies have uncovered potential resistance mechanisms employed by *L. reuteri* for surviving against its own antimicrobial. These studies are ongoing and ultimately may enhance our understanding of microbial community-remodeling functions of probiotics.

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P2X7 purinergic receptor-mediated early activation of JNK signaling is essential for endotoxin-induced acute liver injury in mice

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Introduction. Endotoxin-induced acute liver injury is initiated via activation of inflammatory cascades, with eventual induction of hepatocellular apoptosis and necrosis. P2X7 is a ligand-gated ion channel activated by extracellular ATP, widely recognized as a ‘danger signal’ during tissue damage. Therefore, the purpose of this study was to test the **hypothesis** that P2X7 purinergic receptor activation is essential for the induction of endotoxin-induced acute liver injury.

Methods. Wild type (WT) and P2X7^{-/-} (KO) mice were injected (i.p.) with galactosamine (GalN, 700 mg/kg) and lipopolysaccharide (LPS, 100 µg/kg); controls received saline. Liver tissue sections (1, 5 hrs) were analyzed for apoptosis by TUNEL assay and serum analyzed for alanine transaminase (ALT) activity. Total liver homogenates were analyzed by western blotting for activation of signaling pathways. Nuclear protein extracts were analyzed by EMSA for the activation of transcription factors. Cytokine and chemokine expression were evaluated by qRT-PCR.

Results. At 5 hrs after GalN/LPS treatment serum ALT level was elevated 17-fold in the WT, as compared to saline controls, with significant attenuation in the KO mice (5-fold). A robust induction of JNK signaling was detected at 1 and 5 hrs of treatment in the WT livers. However, early activation (1 hr) of JNK and AP-1 DNA binding activity were significantly attenuated in the KO livers as compared to WT, with comparable induction at 5 hrs. Moreover, KO livers harvested at 5 hrs had elevated induction of ERK, and significantly less TUNEL-positive hepatocytes, as compared to WT livers. Pro-inflammatory cytokine and chemokine mRNA expression and NF-κB activation were comparable between the WT and KO.

Conclusions. Our findings suggest that P2X7 receptor activation plays an essential role in endotoxin-induced acute liver injury in mice. Extracellular ATP-mediated activation of P2X7 receptors may influence progression of hepatocellular injury via its effects on the net temporal activation of pro-apoptotic and pro-survival cell signaling pathways. These results highlight a previously unrecognized role of P2X7 receptors in the pathogenesis of acute liver failure, with implications for the development of targeted therapies.

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Higher total serum testosterone is associated with advanced hepatic fibrosis and inflammatory activity risk in chronically HCV-infected male veterans

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Background: Hepatocellular carcinoma (HCC) risk is greatly increased in males across disease etiologies. Several experimental and epidemiologic research studies found increasing testosterone levels were associated with increased HCC risk in males with chronic hepatitis B infection. However, much less is known about whether testosterone similarly increases risk of advanced hepatic fibrosis or inflammatory activity within a background of chronic hepatitis C virus (HCV) infection.

Methods: We prospectively recruited consecutive HCV+ veterans seen in the dedicated hepatitis C clinic at a single large urban VA medical center. Recruitment was limited to African-American and Caucasian males ages 20-70, chronically mono-infected with HCV and not currently receiving treatment. Venipuncture was performed to: 1) confirm viral status, 2) complete the validated Fibrosure test as a proxy measure for hepatic biopsy assessed pathology, and 3) to measure total serum testosterone level. We performed two sets of logistic regression analyses to evaluate the association between total testosterone and advanced fibrosis (F3/F4 and F4) and advanced inflammatory activity (A2/A3 and A3) respectively. All multivariate analyses included adjustment for age, ethnicity, current alcohol use and viral load.

Results: We recruited N=218 HCV+ male veterans between May 2009 and October 2010. Mean age was 56.5 years, 54% were African-American, and mean total serum testosterone was 5.6 ng/ml (SD 2.30). In univariate analysis, HCV+ veterans with advanced fibrosis (n=70) had higher mean serum testosterone (p=0.06) and were younger (57.6 vs. 55.9 yrs; p=0.02) than those with mild fibrosis (n=148). In contrast, HCV+ veterans with advanced inflammatory activity (n=55) were less likely to be African-American than those with mild inflammatory activity (p=0.08). Multivariate analysis demonstrated a 1 ng/ml increase in total serum testosterone was associated with a significant 21% increase in advanced fibrosis risk after adjusting for age, ethnicity, and viral load (OR=1.21, 95% CI 1.05-1.38, p=0.006). A 1 ng/ml increase in total serum testosterone was also associated with a 14% increased advanced inflammation risk that closely approached significance (OR=1.14, 95% CI 0.999-1.38, p=0.052).

Conclusion: Increased serum testosterone is associated with significantly increased risk of advanced hepatic fibrosis and inflammation in male veterans with chronic HCV. Larger prospective studies are needed to confirm our findings in male veterans and to assess if a similar association exists in HCV+ females.

2010 DDC Pilot/Feasibility Awardee

**Texas Medical Center Digestive Diseases Center
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**TGF- β pathway regulates transcription of telomerase (TERT)
in hepatocellular cancer formation.**

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Objective: Telomerase is a specialized reverse transcriptase that synthesizes repeat DNA sequences at telomeres- the specialized ends of chromosomes regulated by multiple proteins that include TGF- β , c-Myc and Smad3. Absence of telomerase activity in most normal human cells results in the progressive shortening of telomeres with each cell division, ultimately leading to chromosomal instability and cellular replicative senescence or growth arrest. Emerging evidence indicates that β 2SP, a Smad3/Smad4 adaptor protein required for TGF- β signaling, is a powerful tumor suppressor. β 2SP^{+/-}, β 2SP^{+/-}/Smad3^{+/-} and β 2SP^{+/-}/Smad4^{+/-} mice dramatically develop foregut cancers. Because TERT is markedly activated in β 2SP^{+/-} hepatocellular cancer tissues compared to Smad3^{-/-} tissues, we hypothesized that β 2SP interaction with Smad3 could be an important factor in regulating TERT expression.

Material & Methods: SNU398, SNU449, SNU475, HepG2, MEF and 293T cells were used and transfected with luciferase plasmid using renilla as control. CHIP was measured to detect the CTCF, Smad3 and β 2SP binding site of hTERT promoter. Immunohistochemistry, RTPCR and Westerblot were used to detect β 2SP, Smad3 and CTCF expression.

Results: We tested human TERT (hTERT) expression levels in several HCC cell lines that have different levels of β 2SP. hTERT expression levels negatively correlate with β 2SP by Western Blot analysis. Loss of β 2SP in murine from β 2SP^{+/-} and β 2SP^{-/-} embryonic fibroblasts (MEFs) significantly activated mTERT expression. Over-expression of β 2SP and/or Smad3 decreases the RNA level of telomerase (hTERT) in PLC/PRF/5 and SNU-398 (hepatocarcinoma) cell lines. Co-transfection of a β 2SP expression vector and a 0.9kb hTERT promoter-luciferase construct significantly inhibited the hTERT promoter in SNU-398 cells. Smad3 siRNA blocked the β 2SP repression of TERT transcription. Deletion studies of β 2SP and Smad3 binding elements in the human TERT promoter obtained consistent results which show increased promoter activities by luciferase assay. Furthermore, chromatin immunoprecipitation (CHIP) assay and mass spectrum analysis suggests the binding of Smad3/ β 2SP protein to hTERT promoter.

Conclusion: Our data suggest that divergent pathways converge on β 2SP and Smad3 that then regulate TERT transcription. Inactivation of the TGF- β signaling pathway with TERT activation provides a strong strategy for generating targeted therapeutics at TERT to these lethal human cancers.

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Special Thanks To

Chris Barone
Sue Crawford
Dede Fox

This event is supported by in part by a grant from the National Institute Diabetes and Digestive and Kidney Diseases (NIDDK) through its *Silvio O. Conte Digestive Diseases Research Core Center* program, grant number P30 DK56338.
