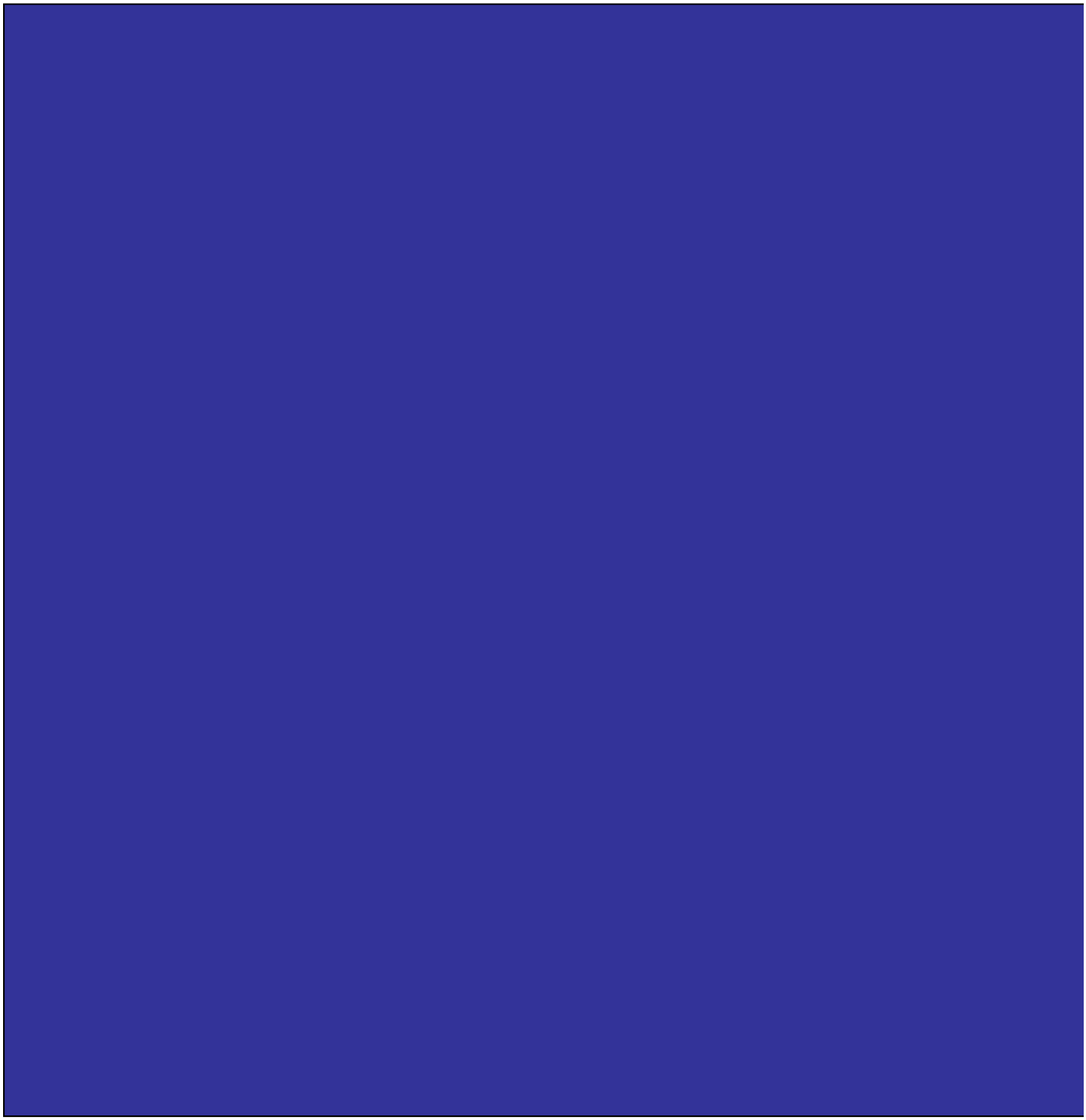

Texas Medical Center Digestive Diseases Center
3rd Annual Frontiers in Digestive Diseases:
Epigenetics in GI Health and Disease

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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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 traoTD-.36 -1.145 T...3(t)l(on (.3(t)l...3(o-10.5(5.7(d1

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L I S T O F A B S T R A C T S

Fasting Insulin and Gamma Glutamyl Transferase (GGT) Predict NonalcoholicSteatohepatitis (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 exists 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.

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

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[1]Translational Biology Molecular Medicine Program, Baylor College of Medicine [2]Pediatrics
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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 ^{14}C-MTA. Mice were euthanized at 5, 10, 30 and 90 min and the radioactive ^{14}C-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 (^{14}C) 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.

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

Caroline Bauchart-Thevret, PhD[1], Barbara Stoll, PhD[1], Nancy Benight[1], David Lazar[2], Oluyinka Olutoye, MD/PhD[2] and Douglas Burrin, PhD[1].

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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 ^{13}C-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 VO_2 uptake, VCO_2 production, respiratory exchange ratio and heat production. At d 9 (Table), we found lower ($P<0.05$) breath $^{13}CO_2$ enrichments and $^{13}CO_2$ production, % of $^{13}CO_2$ recovery/h and cumulative % recovery of ^{13}C-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 $T_{1/213}$

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

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]

[1]Department of Surgery, UTHSC-Houston; [2]UTMB, 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-β1 (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 protectvie 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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Dual functions of p21-activated kinase in regulating myosin light chain

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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 eTJ/TT8 pcd.0009 Tc.0327 TwD.00 in e(hISMC(activi4(ur lacued d5.5(ia-igate loped. HISMCs were)5.6jelity wa

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 enveloped. The membrane envelop 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+}

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

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Gastroenterology, Baylor College of Medicine

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. Esophagogastroduodenscopy 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 becauseof the potential risk of bleeding from gastric variceal hemorrhage.

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

Hemarajata, Peera; Saulnier, Delphine; Zhang, Fan; Thomas, Carissa; Versalovic, James

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

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Massive lower gastrointestinal bleeding after abdominal paracentesis treated with endoscopic therapy

Charles Hotte, MD, Jason Hou, MD

Michael E. DeBakey Veterans Affairs Medical Center, Houston, TX

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

*Jason K. Hou, MD[1,3,4], Jennifer R. Kramer, PhD, MPH[1,2], Peter Richardson, PhD[1,2], Minghua Mei, PhD[1,2], Hashem El-Serag, MD, MPH[1,2,3,4]

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

Hutson, Anne M[1], Sam, Christina A[1], Mori-Akiyama, Yuko[2], and Gunaratne, Preethi H[3, 4]

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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 dysregylated 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 GAII 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-RTPCR 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

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) Ca^{2+} to elevate cytoplasmic Ca^{2+} levels. Release of ER Ca^{2+} is due to NSP4 viroporin activity and since increased Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} 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.

2011 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

Yu Mei, Sundararajah Thevananther.

Pediatrics/Gastroenterology, Hepatology & Nutrition, Texas Children's Liver Center, Baylor College of Medicine

Background and Hypothesis:

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

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Department of Pathology, Texas Children's Hospital

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, *Igbp4* 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:

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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-RTPCR 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 Krüpple-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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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-kB 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.

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.

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