Liver Injury, Repair and Cancer		
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2	**14:41**	**A Role for Hepatic Lymphoid Structures in the Outcome of HBV Infection** *Jillian Jespersen, Anuj Gaggar, Jean Publicover and Jody Baron*
3	**14:52**	**A Combined Proteomics/Genomics Approach Links Hepatitis C Virus Infection with the Nonsense-Mediated mRNA Decay Surveillance Pathway** *Gagandeep Renuka Kumar, Holly R. Ramage, Erik Verschueren, Jeffrey R. Johnson, Jon Von Dollen, Tasha Johnson, Priya Shah, Julie Horner, Nevan J. Krogan and Melanie M. Ott*
4	**15:03**	**Chemical screen identifies inhibitors of β-catenin-driven liver tumorigenesis** *Kimberly J. Evason, Macrina T. Francisco, Vladislava Juric, Maria del Pilar Lopez Pazmino, Gillian G. Hale, John D. Gordan, Sanjay Kakar, Jan Spitsbergen, Didier Y.R. Stainier and Andrei Goga*
5	**15:14**	**Phase II trial of temsirolimus (TEM) plus sorafenib (SOR) in hepatocellular carcinoma (HCC) (NCT01687673)** *Robin K. Kelley, Halla S. Nimeiri, Johd D. Gordan, Jimmy Hwang, Ryan M. McWhirter, Advaita Kanakamedala, Chloe E. Atreya, Laura Kulik, Mary F. Mulcahy, Al B. Benson III and Alan P. Venook*
6	**15:25**	**Nitrosative Regulation of Glycolysis** *Limin Lu and Wei Wei*
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8	**15:47**	**Direct Orthotopic Surgical Implantation of Primary Mouse Hepatocyte Organoids – An Early Experience** *Vivian X. Zhou, Macarena Lolas and Tammy T. Chang*

Hepatic Physiology and Metabolism		
9	**14:30**	**NASH Patient iPSC-Derived Hepatocytes are Primed for Lipogenesis** *Aras N. Mattis, Caroline C. Duwaerts, Jacquelyn J. Maher and Holger Willenbring*
10	**14:41**	**Prolonged feeding of high-calorie diets with unique carbohydrate/fat combinations reveals starch/oleate as a major inducer of hepatic steatosis and adipose tissue necrosis** *Caroline C. Duwaerts, Andrew A. Pierce, Chris Her, Scott M. Turner, Carine Beysen, Mark Fitch, Soo-Jin Cho, James P. Grenert and Jacquelyn J. Maher*
11	**14:52**	**Hepatocyte-specific deletion of the ER stress protein XBP1 results in hepatic steatosis and acute hepatocellular injury after a fructose challenge** *Caroline C. Duwaerts, Russell K. Soon, Chris Her and Jacquelyn J. Maher*
12	**15:03**	**Disruption of the human LRH1 SUMOylation cycle exacerbates liver fibrosis in a mouse model of NAFLD** *Diego A. Miranda, Miyuki Suzawa and Holly A. Ingraham*
13	**15:14**	**Role of SQSTM1/p62 in Cytochrome P450 Autophagy** *Yi Liu and M. Almira Correia*
14	**15:25**	**MR Assessment of Liver Fat and Bone Marrow Fat in HIV versus HIV/HCV Subjects** *Kyle Tillinghast, Natalie Korn, A Sharma, Susan Noworolski and Phyllis C. Tien*
15	**15:36**	**Racial Disparities in Hepatic and Visceral Fat in Men with HIV, Hepatitis C, and HIV/Hepatitis C Co-infection** *Natalie Korn, Linda Nix, Kyle Tillinghast, Susan Noworolski and Phyllis C. Tien*
16	**15:47**	**Muscle Function, Quantity, and Quality in Liver Transplant Candidates: from the Functional Assessment in Liver Transplantation (FrAILT) Study** *Connie W. Wang, Sandy Feng, Kenneth Covinsky, Hilary Hayssen, Benjamin M. Yeh and Jennifer C. Lai.*

ABSTRACT # 1
Theme: Liver Injury and Repair

Modulating Therapeutic Targets in a Murine Model of Hepatitis B

Johnson A, Publicover J, Goodsell A, and Baron J

Hepatitis B virus (HBV) is a noncytopathic hepadnavirus that causes acute and chronic hepatitis. HBV is a major human pathogen with ~400 million chronically infected people and ~1 million deaths annually due to HBV-related liver disease. The immune response to HBV controls viral clearance versus chronic infection. It is also the immune response to HBV that leads to the necroinflammatory process involved in chronic hepatitis, cirrhosis, and hepatocellular carcinoma. The major predisposing factor to developing chronic HBV infection is age of infection. Only 5-10% of immune-competent adults who are acutely infected with HBV develop chronic hepatitis, as compared to over 90% of vertically exposed neonates, and 30% of exposed children aged 1-5.

Development of experimental models to study HBV clearance and disease pathogenesis is crucial to provide the scientific foundation for focused hypotheses to be testing in human patients. The Baron lab has developed age-sensitive mouse models of primary HBV infection that mimic key aspects of HBV clearance and persistence in humans, to enable the dissection of immune mechanisms underlying viral clearance or viral persistence. Thus far, we have identified liver macrophages, CXCL13, and OX40 ligand as potential therapeutic targets in HBV pathogenesis.

To this end, we have developed an experimental treatment approach with immune modulators known to facilitate viral clearance in humans, as well as with immune modulators known to increase CXCL13, OX40 ligand and facilitate maturation of macrophages. Preliminary data has shown an increased diversity of T cell responses and HBV-antigen clearance in 50% of treated mice.

ABSTRACT #2
Theme: Liver Injury and Repair

A Role for Hepatic Lymphoid Structures in the Outcome of HBV Infection

Jillian Jespersen, Anuj Gaggar, Jean Publicover, Jody Baron

Hepatitis B virus (HBV) infection is an important public health threat, with over 350 million people infected worldwide. Infection with HBV results in one of two outcomes: 1) acute infection followed by viral clearance or 2) persistent infection, which is often associated with chronic hepatitis. HBV outcome is largely dependent upon the age at which a person is exposed to the virus – approximately 95% of adults exposed to HBV experience acute, self-resolving disease, while 90% of infants < 1 year and 30% of children age 1-5 become chronically infected with HBV. Because HBV itself is non-cytopathic, differences in the immune response of the infected persons rather than activities of the virus are responsible for disease outcome. In order to study the immune response to HBV, our lab has generated a transgenic mouse model that closely mimics HBV clearance and persistence in humans. Using this model we have uncovered several key immunologic characteristics that differ between adult and young mice during the immune response against HBV, including the ability of adult but not young mice to form transient lymphoid clusters within the liver that aid in immune priming. We have identified several candidate genes that we believe to be important in the formation of these clusters and activation of immune cells that ultimately lead to successful viral clearance, namely CCR6 and its ligand CCL20 as well as TNFSF14 (LIGHT) and its receptors TNFSFR14 (HVEM) and LTβR.

ABSTRACT #3
Theme: Liver Injury and Repair

A Combined Proteomics/Genomics Approach Links Hepatitis C Virus Infection with the Nonsense-Mediated mRNA Decay Surveillance Pathway

Holly R. Ramage, Gagandeep Renuka Kumar*, Erik Verschueren, Jeffrey R. Johnson, John Von Dollen, Tasha Johnson, Billy Newton, Priya Shah, Julie Horner, Nevan J. Krogan, and Melanie M. Ott*

** Equal Contribution*

Although recent advances in direct-acting antivirals promise a sharp decline in disease burden by hepatitis C virus (HCV) infection in the developed world, the virus will remain relevant as a major pathogen in less developed countries and as an important model system for *Flaviviridae* biology in antiviral research. We recently completed a comprehensive virus-host interaction screen, which identified 139 host factors that bind to the 10 HCV-encoded proteins in hepatoma. We also confirmed, in an associated RNAi screen, that ~70% of newly identified interacting host factors are functionally relevant in modulating HCV infection. Pathway analysis identified RNA processing as a top pathway targeted by HCV infection in cells. We focused our efforts on the nonsense-mediated decay (NMD) pathway as a host RNA surveillance mechanism that we find specifically interfaces with HCV proteins and is inactivated during HCV infection. Moreover, we find that HCV has evolved to convert an NMD-associated host factor, WIBG, into a necessary viral cofactor that supports HCV infection. NMD is a quality-control mRNA surveillance process that degrades transcripts containing premature termination codons (PTC) that might otherwise produce toxic, C-terminally truncated proteins. NMD also regulates ~3–10% of the host transcriptome. We hypothesize that disrupting the NMD pathway in HCV-infected cells disables a host restriction mechanism and induces selective transcriptional reprogramming of the infected cell in support of persistent HCV infection. Because WIBG interacts with the viral core protein, we further hypothesize that this host factor has a previously unknown role in viral assembly and possibly viral translation.

ABSTRACT #4
Theme: Liver Injury and Repair

Chemical screen identifies inhibitors of β-catenin-driven liver tumorigenesis

Kimberley J. Evason, Macrina T. Francisco, Vladislava Juric, Maria del Pilar Lopez Pazmino, Gillian G. Hale, John D. Gordan, Sanjay Kakar, Jan Spitsbergen, Didier Y. R. Stainier, and Andrei Goga

ABSTRACT/SUMMARY:
Hepatocellular carcinoma (HCC) is one of the most lethal human cancers. The search for targeted treatments has been hampered by the lack of relevant animal models for the genetically diverse subsets of HCC, including the 20-40% of HCCs that are defined by activating mutations in the gene encoding β-catenin. To address this chemotherapeutic challenge, we created and characterized transgenic zebrafish expressing hepatocyte-specific activated β-catenin. Using this novel transgenic model, we screened for druggable pathways that mediate β-catenin-induced liver growth and identified two c-Jun N-terminal kinase (JNK) inhibitors and two antidepressants that suppressed this phenotype. We found that activated β-catenin was associated with JNK pathway hyperactivation in zebrafish and in human HCC. In zebrafish larvae, JNK inhibition decreased liver size and increased survival specifically in the presence of activated β-catenin. The β-catenin-specific growth-inhibitory effect of targeting JNK was conserved in human liver cancer cells. Our other class of hits, antidepressants, has been used in patient treatment for decades, raising the exciting possibility that these drugs could potentially be repurposed for cancer treatment. We found that amitriptyline decreased tumor burden in a mouse HCC model. Our studies implicate JNK inhibitors and antidepressants as potential therapeutics for β-catenin-induced liver tumor formation.

ABSTRACT #5
Theme: Liver Injury and Repair

Phase II trial of temsirolimus (TEM) plus sorafenib (SOR) in hepatocellular carcinoma (HCC) (NCT01687673)

Robin K Kelley, Halla S Nimeiri, John D Gordan, Jimmy Hwang, Ryan M McWhirter, Advaita Kanakamedala, Chloe E Atreya, Laura Kulik, Mary F Mulcahy, Al B Benson III, Alan P Venook

Background: The multikinase inhibitor SOR prolongs survival in patients with unresectable HCC. In HCC preclinical models, the addition of an inhibitor of the mammalian target of rapamycin (mTOR) pathway to SOR is synergistic. We previously completed a phase I study of the mTOR inhibitor TEM combined with SOR in 25 HCC patients demonstrating safety and early efficacy. This two-center, phase II study was developed to examine the efficacy of the combination and to explore candidate biomarkers. The study was funded by The National Comprehensive Cancer Network and activated October 2012.

Methods: Design: Single-arm, one stage phase II trial. Primary endpoint: Time to progression (TTP) by RECIST 1.1. Other endpoints: Response rate, overall survival, rate of alpha fetoprotein decline ≥ 50%, toxicity, and exploratory biomarkers (mTOR pathway protein expression in tumor cells, circulating tumor cells, and blood and tumor micro-RNA profiles). Sample size: 25 evaluable patients to test null hypothesis of median TTP < 3 months vs. alternate hypothesis of ≥ 6 months, with 1-sided alpha 10%, power 88%. Key eligibility: HCC not amenable to curative therapies, histologically-confirmed, measurable disease. No prior systemic therapy. ECOG ≤ 1. Child-Pugh score ≤ 7, bilirubin ≤ 2 mg/dL. Treatment and procedures: TEM 10 mg IV weekly plus SOR 200 mg PO BID Q28d, with collection of archival tumor samples and blood samples at baseline, on treatment, and at progression.

Accrual status: Twenty of 25 planned evaluable patients have enrolled. An interim analysis for safety after 30% enrollment met pre-specified target to continue.

ABSTRACT #6
Theme: Liver Injury and Repair

Nitrosative Regulation of Glycolysis

Limin Lu and Wei Wei

Summary
The development of human hepatocellular carcinoma (HCC) is often associated with both activation of inducible nitric oxide synthase (iNOS) and genetic deletion of S-nitroso-glutathione reductase (GSNOR), the enzyme deactivating nitric oxide bioactivity. We reported previously that GSNOR deficiency in mice causes nitrosative inactivation of a major DNA repair enzyme and promotes both carcinogen-induced and spontaneous HCC. We have found most recently that under nitrosative stress, GSNOR critically protects glycolytic, but not mitochondrial, ATP production. Both enzymatic assay and metabolic analysis has identified a key glycolytic enzyme that is highly susceptible to inactivation by GSNO. Interestingly, the enzyme isoform expressed in HCC is at least ten-times more resistant than the normal liver isoform, suggesting that the isoform change in HCC cells increases their resistance to nitrosative stress and promotes carcinogenesis. Responsible for GSNO hypersensitivity is a unique Cys conserved in the normal isoform. Thus, GSNOR deficiency concurrent with iNOS activation, through excessive S-nitrosylation, has a profound effect on both DNA repair and cell glycolysis in inflammation and HCC development.

ABSTRACT #7
Theme: Liver Injury and Repair

Hepatocyte plasticity underlies de novo bile duct formation

Johanna R. Schaub, Kari A. Huppert, Charles White, Ashley E. Cast, Stacey S. Huppert, Holger Willenbring

The liver is unique among mammalian organs with its capacity for regeneration. It is well established that hepatocytes can self-duplicate to replace lost and injured hepatocytes, but emerging evidence suggests hepatocytes can also give rise to biliary cells. This change in cell fate can be induced by activation of biliary lineage-determining pathways such as by overexpression of the Notch intracellular domain (NICD), but also occurs spontaneously in liver injury models. This hepatocyte plasticity may be a mechanism to protect hepatocytes from injury through decoy metaplasia, but whether hepatocyte-to-biliary cell conversion is also relevant for regeneration of the biliary system is unclear. Here we use a mouse model of human Alagille syndrome—lacking peripheral intrahepatic bile ducts at birth—to show that hepatocytes can convert into normal cholangiocytes and undergo tubulogenesis. The resulting hepatocyte-derived biliary system is fully functional as demonstrated by reversal of cholestasis and liver injury in these mice. We provide evidence for the role of hypoxia signaling in initiation of hepatocyte-to-cholangiocyte conversion. These data show the importance of hepatocyte plasticity beyond a role in hepatocyte regeneration. Our results have therapeutic implications as understanding the mechanisms underlying hepatocyte plasticity will inform approaches to induce bile duct regeneration in the yet incurable bile duct paucity diseases.

ABSTRACT #8
Theme: Liver Injury and Repair

Direct Orthotopic Surgical Implantation of Primary Mouse Hepatocyte Organoids – An Early Experience

Vivian X. Zhou, Macarena Lolas, and Tammy T. Chang

Generation of liver organoids has raised the possibility that tissue formed *ex vivo* may be developed for implantation therapy for liver insufficiency. Although ectopic implantation of liver organoids demonstrated engraftment and some function, orthotopic implantation into the liver parenchyma has not been established. To address this, we are developing a surgical technique to implant hepatocyte organoids directly into the liver parenchyma. In our studies, hepatocyte organoids are generated by three-dimensional culture in rotating wall vessel bioreactors using primary mouse hepatocytes isolated from ROSA26 C57BL/6 mice, in which β-galactosidase is expressed under a ubiquitous promoter. Confocal microscopy analysis demonstrates that hepatocytes within these organoids show cortical actin organization and produce extracellular matrix. We implanted these organoids into the livers of wild-type C57BL/6 mice by direct parenchymal injection and, 3 days later, analyzed the recipient livers by X-gal, hematoxylin, and eosin staining to identify implanted organoids. For comparison, we transplanted freshly isolated primary hepatocytes as single-cell suspensions either by splenic injection or direct liver parenchyma injection. We found that engraftment efficiency of organoids was superior to that of single cells. Organoid engraftment and survival were improved if inflammation was minimized during surgical implantation and if space was created in the parenchyma for the organoids to reside. Two-thirds partial hepatectomy at the time of implantation did not appear to improve engraftment of the organoids. Based on these results, we will continue to refine our surgical technique with the ultimate goal of developing regenerative liver organoid implantation therapy to treat end-stage liver disease.

ABSTRACT #9
Theme: Hepatic Physiology and Metabolism

NASH Patient iPSC-Derived Hepatocytes are Primed for Lipogenesis

Aras N. Mattis, Caroline Duwaerts, Jacquelyn J. Maher, and Holger Willenbring

With the rising obesity epidemic, non-alcoholic fatty liver disease (NAFLD) is increasing in prevalence affecting nearly 20% of the US population. Ten percent of NAFLD patients go on to develop hepatic inflammation leading to fibrosis, cirrhosis and an increased risk for hepatocellular carcinoma. We found patients believed to have an increased genetic predisposition for the disease, obtained skin punch biopsies and established fibroblast cell lines. As a first step, we tested and confirmed that our three NASH fibroblast patient lines were negative for the PNPLA3 I148M mutation that is known to be a susceptibility marker for NAFLD. We next generated three induced pluripotent cell subclones from each patient fibroblast line using non-integrating episomal vectors expressing the Yamanaka reprograming factors. We validated pluripotency in these new cell lines and then using a well-established 22-day protocol, differentiated these into iPS-derived hepatocytes (iHeps) expressing many features of human hepatocytes including albumin, enzymes of lipid metabolism, and immature cytochrome P450s. We challenged these iHeps using different concentrations of oleate and palmitate, and found that compared to controls, NASH iHeps showed increased steatosis when challenged with palmitate. Quantitative PCR and RNA sequencing suggested the cells are primed for de novo lipogenesis. We also evaluated activated proteins of cellular stress and apoptosis and found that our iHeps showed at least a two-old increase in phosphorylated JNK and eIF2α. These iHeps represent an improved in vitro model of NASH that provides a patient-derived approach with features similar to those found in human NASH biopsies.

ABSTRACT # 10
Theme: Hepatic Physiology and Metabolism

Prolonged feeding of high-calorie diets with unique carbohydrate/fat combinations reveals starch/oleate as a major inducer of hepatic steatosis and adipose tissue necrosis

Caroline C. Duwaerts, Andrew A. Pierce, Chris Her, Scott M. Turner, Carine Beysen, Mark Fitch, Soo-Jin Cho, James P. Grenert and Jacquelyn J. Maher

BACKGROUND: Chronic consumption of excess calories induces obesity and hepatic steatosis. Still, it is unclear whether specific macronutrients or macronutrient combinations pose a unique risk for fatty liver. The objective of this study was to test the effects of different isocaloric combinations of carbohydrates (CHO) and fats on hepatic outcome in mice. **METHODS:** Mice were fed one of four experimental diets containing 40% CHO:40% fat for 6 mos. CHO was provided either as sucrose or starch, while fat was provided either as palmitate or oleate. The 4 groups were designated starch/palmitate, sucrose/palmitate, starch/oleate and sucrose/oleate. One day prior to euthanasia mice received either D_2O or D_{31}-palmitate to monitor the synthesis and trafficking of hepatic lipids. **RESULTS:** Of all 4 experimental diets, starch/oleate induced the greatest metabolic dysfunction as evidenced by the highest serum insulin levels, worst glucose tolerance, and highest serum cholesterol. Mice fed starch/oleate also had the worst hepatic steatosis (211±40mg/g vs. 7-128 mg/g triglyceride) and the highest ALT (77±13UI/L vs. 47-58 UI/L). *De novo* lipogenesis was disproportionately high in the starch/oleate group, as was the fraction of hepatic lipid coming from adipose tissue. In line with the latter finding, mice fed starch/oleate demonstrated significant adipose tissue involution associated with histologic necrosis (scored 2.0±0.6 vs. 0.75-1.22, scale 0-3) and exaggerated suppression of adipokines. **SUMMARY**: Starch and oleate, the main macronutrients in the Mediterranean diet, are actually more harmful to the liver than other dietary CHO/fat combinations, through their ability to stimulate hepatic de novo lipogenesis and adipose tissue involution.

ABSTRACT #11
Theme: Hepatic Physiology and Metabolism

Hepatocyte-specific deletion of the ER stress protein XBP1 results in hepatic steatosis and acute hepatocellular injury after a fructose challenge.

Caroline C. Duwaerts, Russell K. Soon*, Chris Her and Jacquelyn J. Maher*

BACKGROUND: The ER stress transducer IRE1 and its target XBP1 are important regulators of hepatic lipid metabolism, but the precise contribution of IRE1 and XBP1 to hepatic lipid homeostasis is unclear. The objectives of this study were to determine the impact of hepatocyte-specific XBP1 deletion in adult mice and investigate whether XBP1 deficiency promotes liver injury in response to a lipogenic challenge. **METHODS**: $XBP1^{\Delta hep}$ mice were generated by infecting adult $XBP1^{fl/fl}$ mice with AAV8-Ttr-Cre. $XBP1^{fl/fl}$ and $XBP1^{\Delta hep}$ mice were then fed chow or high-fructose for 1-4 weeks. **RESULTS:** $XBP1^{\Delta hep}$ mice exhibited exaggerated IRE1 activation and low serum lipids, as reported previously. ALT and liver histology were normal. Fructose feeding for 1 week induced lipogenic gene expression in $XBP1^{fl/fl}$ mice but caused a much milder induction in $XBP1^{\Delta hep}$ mice. ALT and hepatic lipid remained normal in both groups. Four weeks of fructose feeding induced hepatic steatosis in $XBP1^{fl/fl}$ mice. In $XBP1^{\Delta hep}$ mice, steatosis was exaggerated and ALT was elevated (185 vs. 52). $XBP1^{\Delta hep}$ livers exhibited lobular disarray, apoptosis and regeneration. ER stress was detected in all $XBP\text{-}1^{\Delta hep}$ livers in the form of eIF2α phosphorylation. In fructose-fed $XBP1^{\Delta hep}$ mice, CHOP was also induced. Transcriptome analysis revealed that the gene most highly induced in fructose-fed $XBP1^{\Delta hep}$ livers was another eIF2α target, ATF3. **CONCLUSION:** Hepatocyte-specific XBP1 deletion suppresses lipogenic gene expression, but not fructose-induced hepatic steatosis. In fact, XBP1 deletion enhances fructose-induced steatosis and promotes hepatocellular injury. Liver injury in $XBP1^{\Delta hep}$ mice coincides with activation of eIF2α and downstream targets ATF3 and CHOP.

ABSTRACT #12
Theme: Liver Injury and Repair

Disruption of the human LRH1 SUMOylation cycle exacerbates liver fibrosis in a mouse model of NAFLD.

Diego A. Miranda, Miyuki Suzawa and Holly A. Ingraham.

Studies from our lab demonstrate that human LRH1 sumoylation is attenuated in mouse livers after acute exposure to high fat diet; however, the in vivo importance of sumoylation of hLRH1 in the progression of NAFLD has yet to be tested. To do this, I have expressed the double (K192R/K270R (2KR)) SUMO-deficient mutant in liver specific LRH1 knockout (LKO) mice followed by 4 weeks of high fat diet feeding. Epitope tagged human LRH-1 was expressed via adeno-associated virus (AAV) 8 driven by the thyroxine binding globulin promoter. Western blot analysis of LKO livers infected with AAV-hLRH1 or 2KR confirm that hLRH-1 and 2KR are specifically expressed in liver. Importantly, all major sumoylated species are observed with wild type, but not with 2KR demonstrating for the first time that hLRH-1 is fully sumoylated in vivo. Interestingly, hepatic expression hLRH1 but not 2KR in LKO mice significantly improved hepatic triglyceride levels as compared to LKO mice. 2KR mice exhibited a significant increase in serum bile acids and a slight but not significant increase in serum ALT indicating early hepatic injury. Liver remodeling by activation of hepatic stellate cells (HSCs) has been reported in various models of liver injury. Indeed, 2KR livers exhibited increased liver remodeling as indicated by increased Sirius Red staining and increased expression of *Col1a1* and α*-SMA*, a marker of HSC activation. Collectively, our results indicate that sumoylation of hLRH1 is involved in the progression liver remodeling in NAFLD, this may serve as a new model for the study of liver fibrosis.

ABSTRACT #13
Theme: Liver Injury and Repair

Role of SQSTM1/p62 in Cytochrome P450 Autophagy

Yi Liu and M. Almira Correia

We have previously reported that primary hepatocytes treated with *N*-methyl-protoporphyrin (NMPP) exhibit a rapid degradation of P450s 3A and 2B. We now document that protoporphyrin IX (PPIX)- as well as Zinc-protoporphyrin IX (ZnPP)-treated hepatocytes, which are models of oxidative stress and acute hepatic protoporphyria, showed an even greater degradation of CYP2B. This degradation is associated with a significant aggregation of SQSTM1/p62, a cellular protein scaffold that selects cargo substrates for autophagy. This CYP2B degradation can also be partially reversed by the autophagic/lysosomal inhibitors 3-methyladenine (3MA) and NH_4Cl. These observations suggest that p62 mediates the autophagic-lysosomal degradation of CYP2B. To test this possibility, we co-expressed p62 together with CYP2B6 and CYP3A4 in HEK297T cells. To our surprise, under normal conditions, p62 co-expression significantly increased CYP2B6 as well as CYP3A4 protein levels. Co-immunoprecipitation assays demonstrated that CYP2B6 and p62 physically interacted with each other. A p62 truncation mutant without the ubiquitin-binding domain (UBA) also interacted with CYP2B, suggesting that their interaction does not depend on CYP2B ubiquitination. We then employed p62 $^{-/-}$ mouse primary hepatocytes to examine CYP2B6 levels after phenobarbital induction and found a significant decrease of CYP2B6 in p62$^{-/-}$ hepatocytes. These findings suggest a new p62 working model: p62 binds substrates (CYP2B6) under normal/basal conditions and stabilizes them, whereas under stress conditions, it shuttles them to their autophagic-lysosomal degradation. Supported by NIH grants, DK26506, GM44037 and DK26743 (Liver Center)

ABSTRACT #14
Theme: Hepatic Physiology and Metabolism

MR Assessment of Liver Fat and Bone Marrow Fat in HIV versus HIV/HCV Subjects

K Tillinghast, N Korn, A Sharma, SM Noworolski, PC Tien

Increased levels of liver fat (LF), and bone marrow fat (BMF) lead to higher rates of cirrhosis and fracture, respectively (Farrell and Larter 2006; Schellinger et al. 2004). HIV has previously been shown to increase LF (Hadigan et al. 2007) and decrease BMF (Mulkern et al. 2004), but the effects of HCV co-infection have yet to be investigated. In this study, we compared liver fat fraction (FF) and bone marrow FF measured by magnetic resonance spectroscopy (MRS) in 22 subjects with either HIV infection or HIV/HCV co-infection. The FF, where FF=Fat/(Fat+Water), was measured with 3T MRS [single-voxel PRESS; liver: TR/TE=2500/30ms, 20x20x20mm, water suppressed (NEX=64) and unsuppressed (NEX=8); L3 marrow: TR/TE=3000/30ms, 20x20x20mm, water suppressed (NEX=32) and unsuppressed (NEX=8)]. The breakdown of subjects was HIV (n=10, male=4, mean age=56.9) and HIV/HCV (n=12, male=1, mean age=56.5). Compared to HIV/HCV, HIV subjects had slightly less BMF (HIV median=0.820, range=0.719-0.915; HIV/HCV median=0.864, range=0.760-0.922; $p<0.138$, Wilcoxon Signed Rank Test), and slightly more LF (HIV median=0.0215, range=0.0070-0.1240; HIV/HCV median=0.013, range=0.002-0.195; $p<0.156$, Wilcoxon Signed Rank Test). In addition, two subjects in each category (HIV, HIV/HCV) had steatosis (FF>0.05). Removing those subjects we found that LF remained higher in HIV subjects (HIV median=0.018, HIV/HCV median=0.011, $P<0.060$) and we found BMF became more alike between groups (HIV=0.820, HIV/HCV=0.859, $P<0.214$). These results warrant further investigation in a larger population to understand the effects of HIV and HCV co-infection on both LF and BMF and their role in patient health.

ABSTRACT #15
Theme: Hepatic Physiology and Metabolism

Racial Disparities in Hepatic and Visceral Fat in Men with HIV, Hepatitis C, and HIV/Hepatitis C Co-infection

N Korn, L Nix, K Tillinghast, SM Noworolski, PC Tien

Racial differences in accumulation of hepatic and visceral fat are known, however a need exists to investigate these differences in common diseases. We compared hepatic fat fraction (FF) measured by magnetic resonance spectroscopy (MRS) where 'FF=Fat/(Fat+Water)' [single-voxel PRESS, TR/TE=2500/30ms, 20x20x20mm, fat suppressed (NEX=64) and unsuppressed (NEX=8)], and visceral fat by magnetic resonance imaging (MRI) [3-slice average fat volume [cc] from axial IDEAL FSE-XL, FOV=50x50cm, 10mm slices, NEX=0.5] in Caucasian (CA) and African American (AA) men with HIV, hepatitis C (HCV), and co-infection (HIV/HCV) against an age-matched control population. Of the 182 men recruited, the population breakdown was Control-CA (n=29, mean age=51.7); Control-AA (n=37, mean age=53.1); HIV-CA (n=44, mean age=53.3); HIV-AA (n=11, mean age=54.8); HCV-CA (n=26, mean age=57.2); HCV-AA (n=16, mean age=58.7); HIV/HCV-CA (n=11, mean age=54.4); HIV/HCV-AA (n=8, mean age=56.5). In controls, AA men showed a non-significant trend of less visceral fat (average AA=159cc, CA=197cc, $p<0.103$), and less hepatic FF (AA= 0.050, CA=0.059, $p<0.073$.) In HIV, AA men showed significantly less visceral fat (average AA=161cc, CA=221cc, $p<0.0.010$), and significantly less hepatic FF (AA= 0.028, CA=0.090, $p<0.002$.) In HCV, we observed little difference in visceral fat (average AA=197cc, CA=211cc, $p<0.766$), or hepatic FF (AA= 0.037, CA=0.029, $p<0.676$.) In HIV/HCV, we observed little difference in visceral fat (average AA=128cc, CA=116cc, $p<0.828$), or hepatic FF (AA= 0.019, CA=0.015, $p<0.152$.) In conclusion, AA men store less hepatic and visceral fat than CA men, a difference that was enhanced in the HIV population, while there was little difference in HCV and HIV/HCV populations.

ABSTRACT #16
Theme: Hepatic Physiology and Metabolism

Muscle Function, Quantity, and Quality in Liver Transplant Candidates: from the Functional Assessment in Liver Transplantation (FrAILT) Study

Connie W. Wang, Sandy Feng, Kenneth Covinsky, Hilary Hayssen, Benjamin M. Yeh, Jennifer C. Lai

Background: Sarcopenia and muscle quality, as determined by computed tomography (CT) skeletal muscle index (SkMI) and mean attenuation (SkMA), respectively, are associated with adverse outcomes in liver transplant (LT) candidates, but incur the cost and inconvenience of repeat CT scans. Performance-based tests of physical function are also associated with adverse outcomes. The relationship between these tests and CT-based muscle indices remains poorly defined.
Methods: Adults listed for LT, enrolled in the Functional Assessment in Liver Transplantation (FrAILT) Study, and with an abdominal CT *within 3 months* of grip strength testing were included. SkMI=total cross-sectional area of psoas, paraspinal, and abdominal wall muscles at L3 / height2 (cm2/m2). SkMA=mean Hounsfield units (HU) of lean tissue within total skeletal muscle at L3. Linear regression assessed the relationships between grip strength, SkMI, and SkMA, Cox regression the relationship between these tests and waitlist mortality.
Results: 292 patients were included. Median grip strength was 30 kg, median SkMI was 49 cm2/m2, and median SkMA was 35 HU. Grip strength was significantly associated with SkMI (coef=0.3; $p<0.01$) and SkMA (coef=0.2; $p<0.01$). After MELD adjustment, grip strength was predictive of waitlist mortality (HR 0.95, $p<0.01$), but SkMI (HR 0.97, $p=0.08$) and SkMA (HR 0.97, $p=0.08$) were not.
Conclusions: Grip strength is associated with muscle quantity and quality. It predicts waitlist mortality, whereas CT-based muscle indices do not. Given that it can be conducted quickly and economically, grip strength may be an important tool to use in clinical and research settings as a surrogate marker for sarcopenia and muscle quality.