Yale SCHOOL OF MEDICINE

YALE LIVER CENTER

Vol. 5, No. 4 | Publication of The Yale Liver Center at Yale University School of Medicine | Winter/Spring 2017

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Inside this issue:

Workshop Highlights Impact of iPSCs on Research

At the 2016 *Liver Meeting®*, the Basic Research Workshop focused on how inducible pluripotent stem cells (iPSCs) can be used to produce liver cells and organoids.

The Liver Meeting® Today asked program chairs Mario Strazzabosco, MD, PhD, FAASLD and Jorge Bezerra, MD, FAASLD, to share some details about how these exciting new tools for experimental hepatology hold great promise for clinical hepatology.

LMT: *How was the focus selected for this year's workshop?*
The ability of iPSCs to generate liver cells has a major impact on hepatology research, and holds promise for breakthroughs in modeling of human disease, drug screening, and precision medicine. Recent advances include strategies to use iPSCs to generate cells with better functional profile of hepatocytes and generate liver and biliary organoids. The ability to generate stem cells from fully mature cells of adults was first demonstrated by Shinya Yamanaka, MD, PhD's group using human fibroblasts in 2006. In the approach, the investigators used genetic means to reprogram fibroblasts into cells with stem properties, thus coining the term "induced pluripotent stem cells." For this discovery, in 2012, Dr. Yamanaka was awarded the Nobel prize in Physiology and Medicine together with John B. Gurdon, DPhil. Application of this technology to the study of liver disease is more recent. The first successful attempt was reported by Rashid et al. JCI 2010, who generated iPSC cell lines from dermal fibroblast of patients suffering from 5 different metabolic diseases (alpha1-AT, Glicogen storage disease type 1a, familial hypercholesterolaemia, hereditary tyronaesimia and Crigler-Najjar syndrome) and differentiated them in hepatocyte-like cells. Other papers with improved technology and protocols for differentiation into cholangiocytes followed. Thus, AASLD decided that it was now timely and appropriate to have a discussion applying the tremendous opportunities that are emerging from this technology to the study of liver diseases.

LMT: *Why is the topic of how iPSCs can be used to produce liver cells and organoids particularly timely and relevant for basic science researchers?*
The technology provides experimental hepatologists access to much needed human cell models from patients with a number of genetic and acquired liver diseases. Availability of tissue models that are relevant to human diseases has always been an unmet need for researchers investigating the pathophysiology of liver diseases and their disease-relevant targets. The access to primary human liver material is insufficient and relies mostly on liver transplant tissues with a series of limitations in the isolation and culture of primary liver cells from these samples. While rodent models have been very helpful in discovery and pre-clinical research, and will continue to be useful, several cellular pathways and targets may be significantly different from humans.

The greatest advantage of iPSCs is that they can be easily derived, without invasive procedures, from a small blood sample or from a tissue biopsy (i.e. skin) of the patient. By retaining the same genetic and epigenetic identity as the donors, they maintain a very high replicative potential that provides an unlimited source of patient-specific cells able to differentiate into the liver cell of interest. The newest breakthrough is the use of iPSCs to generate 3D cellular structures known as liver or biliary organoids. Their 3D structure recapitulates the organization and biological properties of the primitive tissue of origin and compared with isolated primary cells in culture, they do not lose their properties when maintained and propagated in culture.

All together, this technology offers a new source of disease derived liver cells that can be used at the bench for studying the mechanisms of the disease or for clinical applications such as patient-specific drug screening or in the future for regenerative medicine.

LMT: *Is there any benefit for researchers with a translational or clinical focus to attend this basic science workshop?*
There is definitely a great benefit in learning about the possible range of applications, the benefits, and the pitfalls of this novel technology. At this point the technology is still novel, so it is a great field also for young scientists to enter and make an impact.

For example, the researcher with a clinical focus will learn about the advantage of the use of this technology with the possibility in the foreseeable future of repairing the disease causing mutation by gene editing and the following transplantation of corrected cells genetically matching with the patient.

On the other hand, the translational researcher, usually confined to study cell material from patients whose disease has already progressed to advanced stages at the time of diagnosis, will understand that iPSC derived cells that go through the same developmental stages as the patients' cells can recapitulate and capture the very early stages of the disease.

**Credit for this interview is given to AASLD/The Liver Meeting® Today*

Why is this Relevant for our Center Members?

Liver Center investigators (Romina Fiorotto from the Strazzabosco Lab, Carol Soroka from the Boyer lab, both recipients of Liver Center Pilot Project Awards) are using iPSC-derived biliary cells or organoids and **these technologies are available collaboratively to other Liver Center Members.** The project of banking monocytes from patients with liver diseases of interest is underway. This will allow liver center members to generate iPSCs and derive cholangiocytes for a number of acquired and congenital biliary diseases.

Director's Corner

The Yale Liver Center (YLC) is one of 18 Digestive Diseases Research Core Centers (DDRCC) supported by NIH/NIDDK. The YLC has been funded continuously for over 30 years and is one of only four that focus on the liver. Full story >

LIVER CENTER ANNOUNCEMENTS

Yale Liver Center Seminars

April 4, 2017
Holger Willenbring, MD, PhD
Professor of Surgery
Associate Director, Liver Center
University of California San Francisco
(Host: Dr. Mario Strazzabosco)
TAC S247, 5:00PM

May 2, 2016
Paul Kubes, PhD
Professor, Departments of Physiology & Pharmacology, Medicine and Microbiology, Immunology and Infectious Diseases
University of Calgary
(Host: Dr. Mario Strazzabosco)
TAC S247, 5:00PM

2016-2017 New Liver Center Members

Adam Arterbery, PhD (Pediatrics)
Christopher Ibarra, PhD (Transplant)
Dana Peters, PhD (Radiology & Biomedical Imaging)
Xuchen Zhang, MD, PhD (Pathology)

If you are interested in becoming a member of the Yale Liver Center, please contact Christine Abu-Hanna for an application.

Membership Criteria

MEMBERS' RECENT PUBLICATIONS

Type 2 inositol trisphosphate receptor gene expression in hepatocytes is regulated by cyclic AMP. Kruglov E, Ananthanarayanan M, Sousa P, Weerachayaphorn J, Guerra MT, Nathanson MH. Biochem Biophys Res Commun. 2017 PMID: 28327356

Alcohol and calcium make a potent cocktail. Iwakiri Y, Nathanson MH. J Physiol. 2017. PMID: 28295353

Effects of andrographolide on intrahepatic cholestasis induced by alpha-naphthylisothiocyanate in rats. Khamphaya T, Chansela P, Piyachaturawat P, Suksamrarn A, Nathanson MH, Weerachayaphorn J. Eur J Pharmacol. 2016. 789:254-64. PMID: 27475677

Activity of the C. elegans egg-laying behavior circuit is controlled by competing activation and feedback inhibition. Collins KM, Bode A, Fernandez RW, Tanis JE, Brewer JC, Creamer MS, Koelle MR. Elife. 2016.; pii: e21126.PMID: 27849154

Adenylyl cyclase 5 links changes in calcium homeostasis to cAMP-dependent cyst growth in polycystic liver disease. Spirli C, Mariotti V, Villani A, Fabris L, Fiorotto R, Strazzabosco M. J Hepatol. 2017; 66:571-580 PMID: 27826057

Insulin receptor Thr1160 phosphorylation mediates lipid-induced hepatic insulin resistance. Petersen MC, Madiraju AK, Gassaway BM, Marcel M, Nasiri AR, Butrico G, Marcucci MJ, Zhang D, Abulizi A, Zhang XM, Philbrick W, Hubbard SR, Jurczak MJ, Samuel VT, Rinehart J, Shulman GI. J Clin Invest. 2016. 126:4361-4371. PMID: 27760050

REV-ERBα Activates C/EBP Homologous Protein to Control Small Heterodimer Partner-Mediated Oscillation of Alcoholic Fatty Liver. Yang Z, Tsuchiya H, Zhang Y, Lee S, Liu C, Huang Y, Vargas GM, Wang L. Am J Pathol. 2016; 186:2909-2920. PMID: 27664470

Sirtuin 1 activation alleviates cholestatic liver injury in a cholic acid-fed mouse model of cholestasis. Kulkarni SR, Soroka CJ, Hagey LR, Boyer JL. Hepatology. 2016; 64:2151-2164. PMID: 27639250

Use of transient elastography in patients with HIV-HCV coinfection: A systematic review and meta-analysis. Njei B, McCarty TR, Luk J, Ewelukwa O, Ditah I, Lim JK. J Gastroenterol Hepatol. 2016; 31:1684-1693. PMID: 26952020

The cystic fibrosis transmembrane conductance regulator controls biliary epithelial inflammation and permeability by regulating Src tyrosine kinase activity. Fiorotto R, Villani A, Kourtidis A, Scirpo R, Amenduni M, Geibel PJ, Cadamuro M, Spirli C, Anastasiadis PZ, Strazzabosco M. Hepatology. 2016; 64:2118-2134. PMID: 27629435

Child-Turcotte-Pugh Class is Best at Stratifying Risk in Variceal Hemorrhage: Analysis of a US Multicenter Prospective Study. Fortune BE, Garcia-Tsao G, Ciarleglio M, Deng Y, Fallon MB, Sigal S, Chalasani NP, Lim JK, Reuben A, Vargas HE, Abrams G, Lewis MD, Hassanein T, Trotter JF, Sanyal AJ, Beavers KL, Ganger D, Thuluvath PJ, Grace ND, Groszmann RJ; Vapreotide Study Group. J Clin Gastroenterol. 2016 [Epub ahead of print] PMID: 27779613

Long noncoding RNA MEG3 induces cholestatic liver injury by interaction with PTBP1 to facilitate shp mRNA decay. Zhang L, Yang Z, Trottier J, Barbier O, Wang L. Hepatology. 2017; 65:604-615. PMID: 27770549

Empowering women: Perspective from a hepatologist. Garcia-Tsao G. Hepatology. 2016; 64:1831-1833. PMID: 27770471

The Child-Turcotte Classification: From Gestalt to Sophisticated Statistics and Back. Garcia-Tsao G. Dig Dis Sci. 2016; 61:3102-3104. PMID: 27696097

Exposure to inorganic arsenic can lead to gut microbe perturbations and hepatocellular carcinoma. Choiniere J, Wang L. Acta Pharm Sin B. 2016; 6:426-429. PMID: 27709011

A Macrophage Migration Inhibitory Factor Polymorphism Is Associated with Autoimmune Hepatitis Severity in US and Japanese Patients. Assis DN, Takahashi H, Leng L, Zeniya M, Boyer JL, Bucala R. Dig Dis Sci. 2016; 61:3506-3512. PMID: 27696094

JAK2 Disease-Risk Variants Are Gain of Function and JAK Signaling Threshold Determines Innate Receptor-Induced Proinflammatory Cytokine Secretion in Macrophages. Hedl M, Proctor DD, Abraham C. J Immunol. 2016; 197:3695-3704. PMID: 27664279

ACOX2 deficiency: A disorder of bile acid synthesis with transaminase elevation, liver fibrosis, ataxia, and cognitive impairment. Vilarinho S, Sari S, Mazzacuva F, Bilgüvar K, Esendagli-Yilmaz G, Jain D, Akyol G, Dalgiç B, Günel M, Clayton PT, Lifton RP. Proc Natl Acad Sci U S A. 2016; 113:11289-11293. PMID: 27647924

MARCH1 regulates insulin sensitivity by controlling cell surface insulin receptor levels. Nagarajan A, Petersen MC, Nasiri AR, Butrico G, Fung A, Ruan HB, Kursawe R, Caprio S, Thibodeau J, Bourgeois-Daigneault MC, Sun L, Gao G, Bhanot S, Jurczak MJ, Green MR, Shulman GI, Wajapeyee N. Nat Commun. 2016; 7:12639. PMID: 27577745

Neuronal Calcium Sensor 1 Has Two Variants with Distinct Calcium Binding Characteristics. Wang B, Boeckel GR, Huynh L, Nguyen L, Cao W, De La Cruz EM, Kaftan EJ, Ehrlich BE. PLoS One. 2016; 11:e0161414. PMID: 27575489

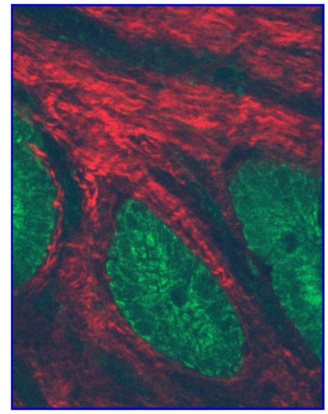
Pigment Epithelium-derived Factor (PEDF) Blocks Wnt3a Protein-induced Autophagy in Pancreatic Intraepithelial Neoplasms. Gong J, Belinsky G, Sagheer U, Zhang X, Grippo PJ, Chung C. J Biol Chem. 2016; 291:22074-22085. PMID: 27557659

Continued on Next Page

Featured Core: Clinical-Translational Core

Guadalupe Garcia-Tsao, MD
Director

Loren Laine, MD Associate Director	Dhanpat Jain, MD Associate Director	Randolph de la Rosa Rodriguez Research Coordinator



The overarching goal of the Clinical Core is to facilitate translational and patient-oriented research. The Clinical Translational Core aims to develop an infrastructure for patient-oriented research by streamlining regulatory and compliance processes, obtaining and storing high-quality samples linked to extensive clinical phenotypic information, offering expert consultation in the design and implementation of patient-oriented trials, and supplying statistical expertise for application of innovative analytic methods in translational and patient-oriented research.

Research Coordinator

- Expertise in effectively addressing NIH, IRB and HIPAA policies and reporting requirements concerning confidentiality, inclusion of women, children and ethnic/minority participation in clinical studies, data and safety monitoring requirements
- Ensure the protection of human research participants
- Ensuring that all regulatory requirements for Liver Center protocols are met
- Trains Liver Center staff and/or study coordinators on the requirements and best practices so that compliance can be maintained, ensuring that all Liver Center clinical studies follow local and federal guidelines

Clinical Registry

Patient Registry of the Clinical Core (PaRCC)

Patient Databases, that include a prospective database of patients attending the outpatient liver clinics at Yale and the CT-VA Healthcare System. Patients in the PaRCC have given specific consent to be contacted for possible participation in future clinical studies and most of them have also consented to provide samples for the serum and tissue bank (SaRCC). Other liver disease-specific databases available to Center members include those of patients with compensated and decompensated cirrhosis, acute-on-chronic liver failure, hepatitis C, hepatocellular carcinoma (HCC), autoimmune liver disease and inherited metabolic liver diseases (Gaucher and Wilson disease).

Sample Registry of the Clinical Core (SaRCC)

The Serum and Tissue Bank that is referred to as the SaRCC currently consists of over 10,000 frozen samples of serum and EDTA plasma (for DNA) obtained from patients included in the PaRCC. It also consists of liver biopsy slides and paraffin blocks stored at the Pathology Departments of both Yale and the VA. Both the PaRCC and the SaRCC are linked and maintained by the Clinical Core Research Coordinator with the objective of providing Center members with data regarding numbers of patients with specific diagnoses seen at the Center in a defined period of time to assess the feasibility of a specific study, contact patients for participation in a study and provide serum/tissue samples for use in research. The Clinical Core has also established collaboration with the Yale Center for Clinical Investigation (YCCI) through its Biorepository Core that is responsible for the processing, storing and tracking of blood and tissue samples through the Clinical Core, as well as maintaining the database for the samples in storage.

Investigators interested in obtaining more information or samples should contact Randolph de la Rosa Rodriguez.

Biostatistician

The Liver Center has two biostatisticians that provide assistance to basic researchers with clinical study design, particularly for small pilot projects that are the initial step in the translational process. For established clinical investigators using the clinical component, this resource provides consultation for clinical study/trial development particularly in establishing sample size calculations and advice regarding the most appropriate study design. Furthermore, biostatistical analysis of patients already in liver disease-specific databases continue to provide new insights into the natural history of these heterogeneous disorders.

For more information, please contact our biostatisticians Maria Ciarleglio or Yanhong Deng.

MEMBERS' RECENT PUBLICATIONS (continued)

Combination Therapy of All-Trans Retinoic Acid With Ursodeoxycholic Acid in Patients With Primary Sclerosing Cholangitis: A Human Pilot Study. Assis DN, Abdelghany O, Cai SY, Gossard AA, Eaton JE, Keach JC, Deng Y, Setchell KD, Ciarleglio M, Lindor KD, Boyer JL. J Clin Gastroenterol. 2017; 51:e11-e16. PMID: 27428727

Low-Dose Paclitaxel Reduces S100A4 Nuclear Import to Inhibit Invasion and Hematogenous Metastasis of Cholangiocarcinoma. Cadamuro M, Spagnuolo G, Sambado L, Indraccolo S, Nardo G, Rosato A, Brivio S, Caslini C, Stecca T, Massani M, Bassi N, Novelli E, Spirli C, Fabris L, Strazzabosco M. Cancer Res. 2016; 76:4775-84. PMID: 27328733

The liver throws the skeleton a bone (resorption factor). Chung C, Insogna KL. Hepatology. 2016; 64:977-9. PMID: 27312397

Pigment Epithelium-Derived Factor (PEDF) is a Determinant of Stem Cell Fate: Lessons from an Ultra-Rare Disease. Sagheer U, Gong J, Chung C. J Dev Biol. 2015; 3:112-128. PMID: 27239449

Veterans health administration hepatitis B testing and treatment with anti-CD20 antibody administration. Hunt CM, Beste LA, Lowy E, Suzuki A, Moylan CA, Tillmann HL, Ioannou GN, Lim JK, Kelley MJ, Provenzale D. World J Gastroenterol. 2016; 22:4732-40.

The Yale Liver Center is built on a tradition established by the late Gerald Klatskin, one of the country's founders of the discipline of Hepatology and a member of Yale's faculty for over 50 years.

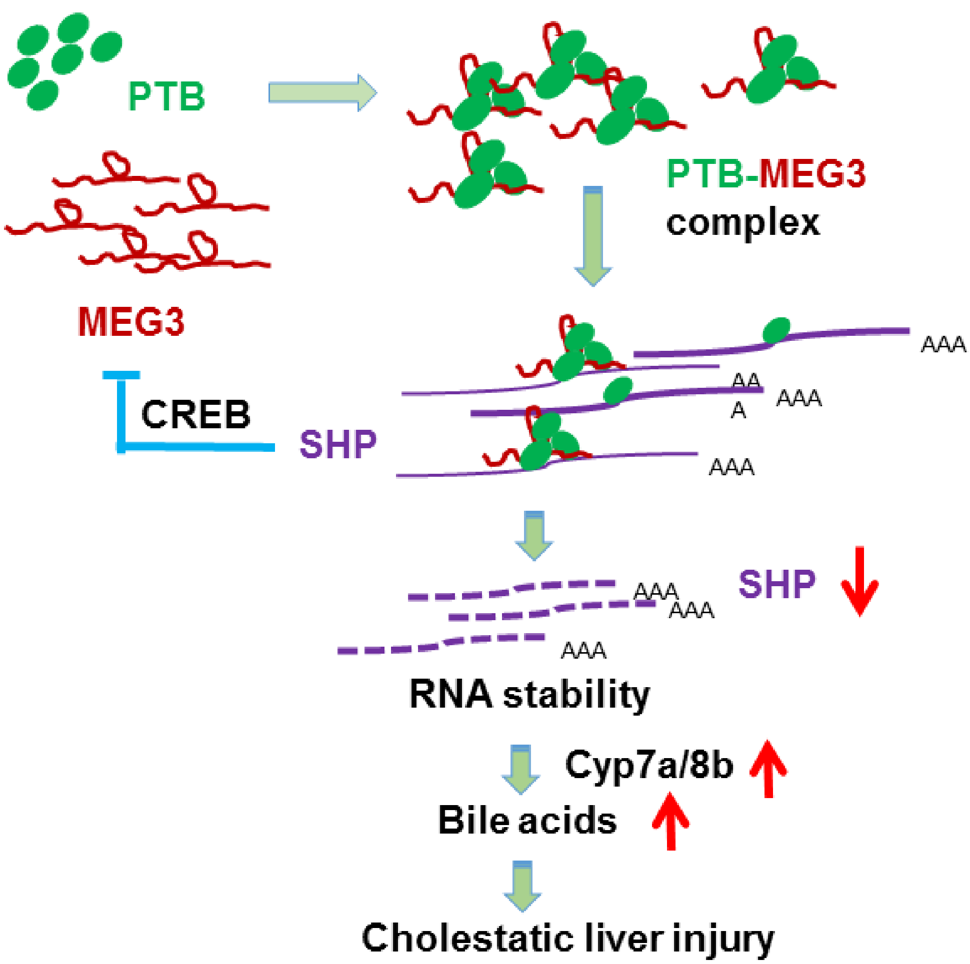
To site The Liver Center in your publications please use P30 DK034989

Featured Publications

Long Noncoding RNA MEG3 Induces Cholestatic Liver Injury by Interaction With PTBP1 to Facilitate Shp mRNA Decay

Zhang, L., Yang, Z., Trottier, J., Barbier, O., **Wang, L.**. *Hepatology;* 2017; 65: 604-615 PMID: 27770549

Long non-coding RNAs (lncRNAs) have emerged as important regulators of liver function and diseases. In this study, we identified a novel role for maternally expressed gene 3 (MEG3) in modulating bile acid homeostasis and cholestatic liver injury. We found that MEG3 serves as a guide RNA scaffold to recruit RNA binding protein PTBP1 to destabilize nuclear receptor SHP mRNA. This results in the loss of feedback inhibition of bile acid synthesis, leading to increased BA levels and cholestasis. On the other hand, SHP represses MEG3 expression via a cyclic adenosine monophosphate response element-binding protein (CREB)-mediated mechanism in a feedback regulatory fashion. Moreover, MEG3 and PTBP1 expression is upregulated in human fibrotic and cirrhotic livers, suggesting that their functional roles are intricately linked to an array of chronic liver diseases. Overall, this study highlights the importance of lncRNAs as new regulators of liver metabolic function.

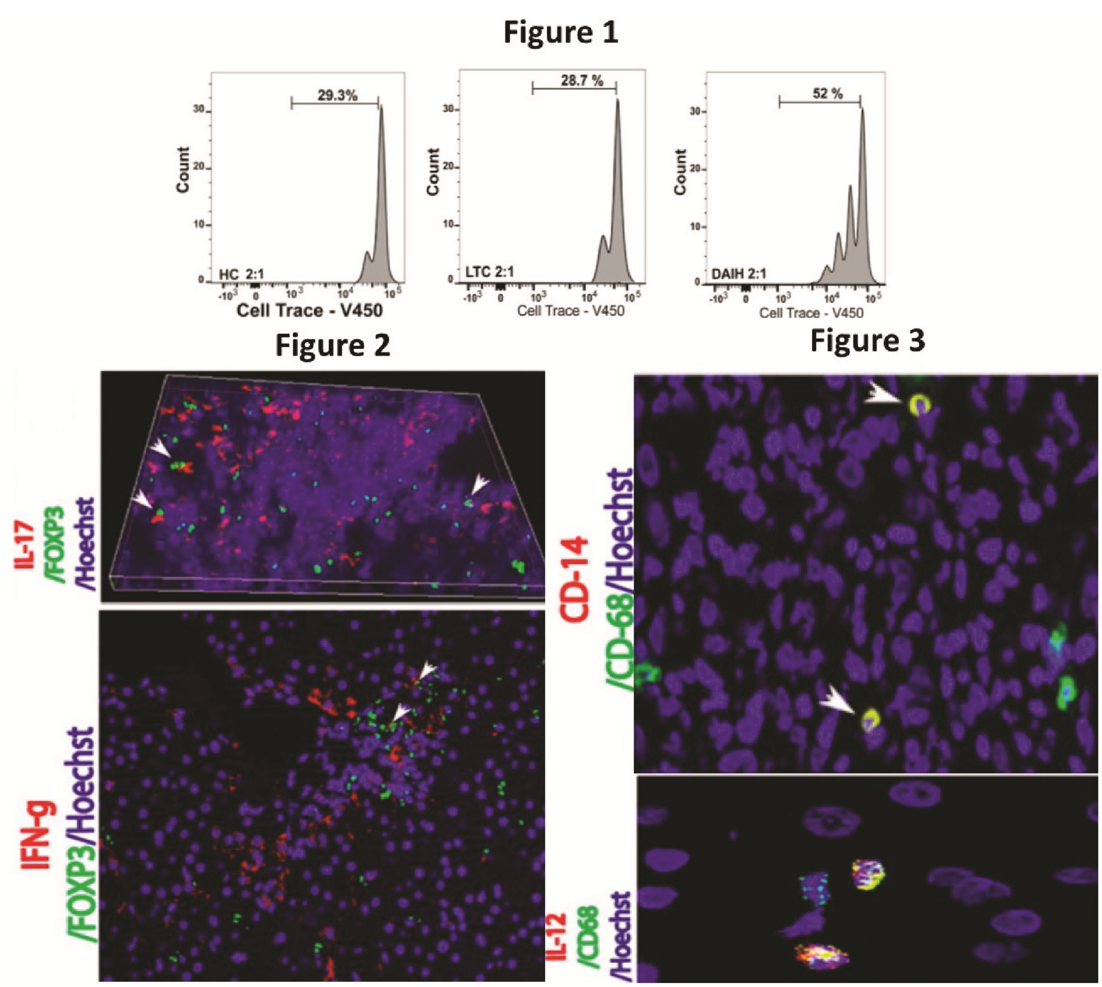


Featured Publications

Production of Proinflammatory Cytokines by Monocytes in Liver-Transplanted Recipients with De Novo Autoimmune Hepatitis Is Enhanced and Induces TH-1 like Regulatory T Cells

Arterbery, AS., Osafo-Adda, A., Avitzur Y., **Ciarleglio, M., Deng, Y.,** Lobritto SJ., Martinez M., Hafler DA., Kleinewietfeld M., **Ekong UD.** *J Immunol.* 2016; 196: 4040-51. PMID: 27183637

De novo autoimmune hepatitis (DAIH) is an important cause of late allograft dysfunction, unfortunately, the underlying pathogenesis remains unclear. We sought to identify cell types prevalent in DAIH and investigate how they might contribute towards disease pathogenesis. In this study, we show that FOXP3+ regulatory T cells (Tregs) from patients with DAIH display phenotypic characteristics of pro-inflammatory TH1 and TH17 cells and produce the inflammatory cytokines, IFN-g and IL-17; additionally, they are functionally impaired in in vitro suppression assays, failing to suppress T effector cell proliferation efficiently (figure 1). These pro-inflammatory cytokine secreting FOXP3+ regulatory T cells were similarly observed in livers of patients with DAIH (figure 2). We have also shown that IL-12 produced by CD14++ monocytes of these patients is responsible for driving the differentiation of regulatory T cells to IFN-g-producing regulatory T cells. Similar to the findings from blood, CD68+ monocyte/macrophages in livers of patients with DAIH also produce IL-12 (figure 3). Lastly, blockade of IL-12 or IFN-γ partially restores suppressive function of regulatory T cells suggesting that monocytes/macrophages contribute significantly to the inflammatory milieu in the liver that drives the induction of pro-inflammatory regulatory T cells in patients with DAIH.



TH1 and TH17 polarizing cytokines and their key signature cytokines, IFN-γ and IL-17A, make up the cytokine milieu within livers with dAIH. Figure 1) Tregs from patients with dAIH are functionally impaired, failing to suppress T effector cell proliferation efficiently *in vitro*; Figure 2) IFN-γ+/IL-17A+ cells present within the portal tract and co-expressing with FOXP3+ cells. Arrowheads point to co-expressing cells; Figure 3) CD14+ and CD68+ co-expressing cells (white arrows), and IL-12+ and CD68+ co-expressing cells.

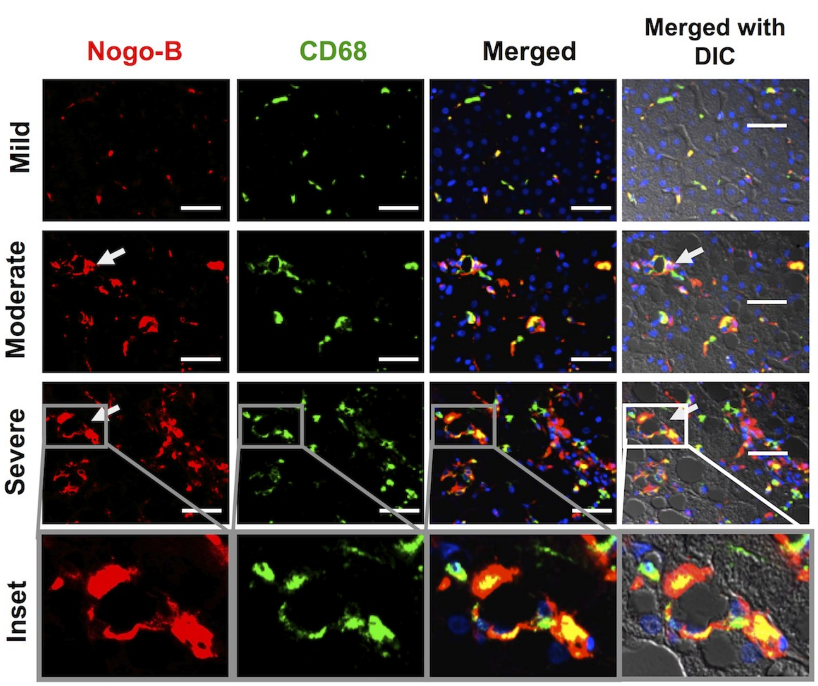
Featured Publications

An endoplasmic reticulum protein, Nogo-B, facilitates alcoholic liver disease through regulation of Kupffer cell polarization

Park, J.K.*, Shao, M.*, Kim, M.Y., Baik, S.K., Cho, M.Y., Utsumi, T., Satoh, A., Ouyang, X., Chung, C., **Iwakiri, Y.** Hepatology 2017. * Sharing first authorship

Chronic alcohol consumption leads to hepatic injury that can develop to liver cirrhosis and cancer. Kupffer cells (liver resident macrophages) mediate inflammatory responses in alcoholic liver disease (ALD) and contribute to the development of hepatic steatosis and injury in a paracrine manner. Macrophages have different functional states with pro-inflammatory M1 type and anti-inflammatory M2 type. The mechanisms that govern this M1/M2 polarization remain to be elucidated. Nogo-B, also known as Reticulon 4B, is an endoplasmic reticulum (ER) resident protein that regulates ER structure and function. Since ER stress is known to induce M2 macrophage polarization, we examined whether Nogo-B regulates M1/M2 polarization of Kupffer cells and alters the pathogenesis of ALD.

We showed a significant positive correlation between Nogo-B positive Kupffer cells and disease severity in ALD patients (Fig 1). Further, Nogo-B positive Kupffer cells correlated with M1 activation (iNOS) and negatively with M2 status (CD163) in these patients. WT mice exhibited significantly increased liver injury ($p<0.05$) and higher hepatic triglyceride levels ($p<0.01$), compared to Nogo-B KO mice in response to chronic ethanol feeding. Nogo-B in Kupffer cells promoted M1 polarization, whereas absence of Nogo-B increased ER stress and M2 polarization in Kupffer cells. We demonstrated that Nogo-B is permissive for M1 polarization of Kupffer cells, thereby accentuating liver injury in ALD in humans and mice. Our study suggests that Nogo-B in Kupffer cells may represent a new therapeutic target for ALD.



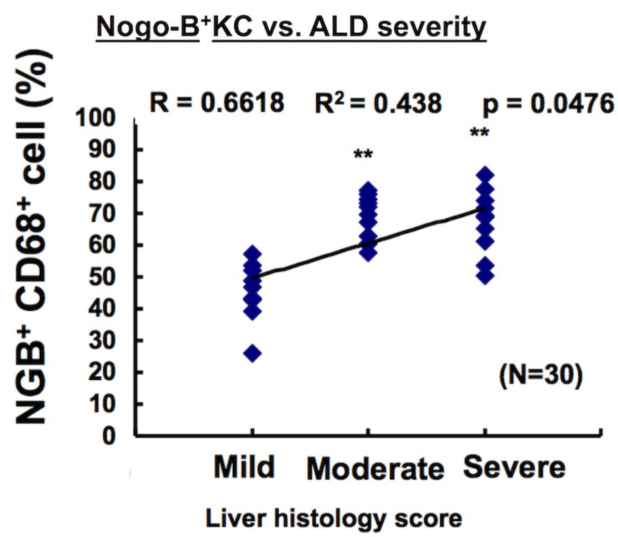


Figure 1. Nogo-B levels in Kupffer cells correlate with severity of alcoholic liver disease (ALD) in humans. Immunofluorescence of Nogo-B (red) and CD68-positive Kupffer cells (green) in mild, moderate and severe ALD. Arrows: Nogo-B-positive Kupffer cells. A positive correlation was observed between the ratio of Nogo-B^{high} Kupffer cells to total Kupffer cells and ALD severity (n=30, r=0.66, p=0.048). Scale bar = 50mm.