Liver Digest

A weekly update of PLRC happenings

October 3, 2019

PITTSBURGH LIVER RESEARCH CENTER

A partnership of University of Pittsburgh & UPMC

www.livercenter.pitt.edu

Featured Faculty - Dr. Wendy Mars

In this issue

- Funding Opportunities

RFA - PLRC P&F Awards

We are pleased to announce the 2020 Request for Applications for Pittsburgh Liver Research Center Pilot & Feasibility grants. The RFA is available on the PLRC website as well (**https://www.livercenter.pitt.edu/plrc-grants**)

Letters of intention to apply are due **October 15, 2019**.
Applications are **due December 3, 2019.**

Next Week's Seminar

STI Internal Grand Rounds
Fri., October 4, 2019
8:00 - 9:00 a.m.
LHAS Auditorium, 7th Floor Montefiore

Amit D. Tevar, MD
Associate Professor of Surgery
Liver, Kidney and Pancreas Transplant Surgery
Director of Kidney and Pancreas Transplantation
Thomas E. Starzl Transplantation Institute
University of Pittsburgh Medical Center

"HCV and PHS Increased Risk Donors: Quantifying Risk for Your Patient"

Sponsored by
Thomas E. Starzl Transplantation Institute
University of Pittsburgh School of Medicine
Department of Surgery, Division of Transplantation
Center for Continuing Education in the Health Sciences

For a list of upcoming seminars, please visit the PLRC

website: **https://www.livercenter.pitt.edu/seminars**

Faculty Highlights

PLRC members collaborating on manuscripts are noted in red.

R01 Grant Awarded:

Dr. Mo Ebrahimkhani has been awarded an R01 to study "Integration of systems and synthetic biology to advance development of human tissues ex vivo." This is a collaborative project with Co-Investigators Dr. Samira Kiani (University of Pittsburgh, effective January 1, 2020) and Dr. Patrick Cahan (Johns Hopkins University). Congratulations!

ABSTRACT
The rapidly evolving field of stem cell bioengineering and organoids technology face key challenges: a) stem cell derived tissues are stalled developmentally and show fetal-stage phenotypes b) they often lack key subsets of vascular endothelial and stromal cells derived from different germ layers c) There is a lack of toolset to quantitatively assess cell or tissue identity and d) to guide morphogenetic events towards their native adult phenotypes. In this proposal, we will address these issues by undertaking an integrative synthetic biology and systems biology approach. We recently generated novel human fetal liver organoids ex vivo using human iPSCs. Our approach entails genetic engineering of human iPSCs via overexpression of GATA6 transcription factor in pluripotent media with bFGF and TGF-β. Through this strategy, we showed development of human endodermal and mesodermal, intercellular communications, co-differentiation and self-organization of cultures into a multi- cell type fetal liver organoid. We will employ this tissue as a unique testbed to develop and address biotechnology challenges for in vitro maturation, assessment and engineering of organoids. Through aim 1 of this proposal we will develop and validate a set of genetic toolset to drive cell-fate reprogramming of the multicellular tissue. Through aim 2, we will establish a computational platform to quantitatively assess liver organoids and to identify transcriptional regulators of stage specific development
IMPACT: We tackle improvement and assessment of human organoids ex vivo, two key challenges in stem cell bioengineering. The successful completion of this study will result in a hypothesis-driven framework for rational engineering and advancement of stem cell-derived tissues ex vivo. Our study will also

generate synthetic liver tissues with close proximity to adult human liver. It reduces dependence on animal experiments and increases access to refined human tissues.

More information on Dr. Ebrahimkhani's work is available on his website: **https://www.ebrahimkhanilab.com/**

R01 Grant Awarded:

Dr. Kari Nejak-Bowen has been awarded an R01 to study "Beta-catenin-driven hepatobiliary reprogramming as a therapeutic modality for cholangiopathies." Congrautlations!

ABSTRACT

Cholangiopathies are chronic, progressive diseases of the biliary tree, and can be either acquired or genetic. Regardless of etiology, cholangiopathies share common pathologic mechanisms, including inflammation, aberrant ductular proliferation, fibrosis, ductopenia, and cholestasis, which can over time result in tumorigenesis, cirrhosis, or liver failure. Despite recent advances in our understanding and diagnosis of these diseases, there are no proven therapeutic treatments for the majority of cholangiopathies. Thus, mechanistic-based studies that emphasize therapeutic development are desperately needed. Previous work has identified the Wnt/β-catenin signaling pathway as a modulatable target in mouse models of biliary injury such as 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) diet. Overexpression of a mutated non-degradable form of β-catenin in transgenic (TG) mice subjected to long-term DDC results in a significant reduction in serum alkaline phosphatase, a common prognostic marker for biliary injury, and a concurrent increase in bile flow. Notably, this improvement was associated with widespread expression of biliary marker A6 in the hepatocytes (HC) of these TG mice. Further analysis revealed that TG had increased expression of biliary markers in HC as early as 1 month after DDC. During biliary injury, HC are known to alter their phenotype and acquire cholangiocyte (CC)-like features, a process known as cellular reprogramming. HC reprogramming may contribute to biliary repair by modifying bile to reduce toxicity, serving as a source of de novo CC to repair the biliary epithelium, or creating new channels to facilitate bile flow. Thus, the overarching hypothesis of the proposal is that activation of Wnt/β-catenin signaling in HC during cholestasis will induce reprogramming to a CC-like phenotype, and that this process will aid in restoring bile flow and

reducing the severity of cholestatic liver disease. In aim 1, we will unambiguously determine if β-catenin-overexpressing HC fully differentiate into functional CC or maintain an intermediate phenotype during cholestasis by isolating permanently labeled HC and their progeny and analyzing them through phenotypic characterization, functional tests, and transcriptomic analysis. In aim 2, we will characterize the mechanism by which Wnt/β-catenin activates a biliary phenotype in HC by using unbiased methods to identify the transcription factors downstream of β-catenin in cholestasis, as well as evaluating the contribution of Yap signaling as a potential downstream effector of β-catenin using an in vivo two-gene reporter system. In aim 3, we will determine whether activating β-catenin in HC will enhance transdifferentiation into fully- functional CC in the absence of a functional biliary system. First, we will determine the effect of inhibiting or overexpressing β-catenin on the formation of biliary structures in vitro (HC-derived organoid cultures). Next, we will exogenously activate β-catenin using a Wnt agonist and assess its efficacy in alleviating cholestatic injury in mice with biliary insufficiency. Finally, we will utilize an immune-deficient, bile duct deficient model of liver repopulation and determine if transplanted TG HC have an advantage over wild-type HC in rescuing the phenotype. Thus, the proposed studies will further our understanding of the role of β-catenin in HC reprogramming and biliary repair, and will provide highly significant information for therapeutic and translational use.

Original Article:

Rosselot C, Kumar A, Lakshmipathi J, Zhang P, Lu G, Katz LS, **Prochownik EV**, Stewart AF, Lambertini L, Scott DK, Garcia-Ocaña A. Myc Is Required for Adaptive ß-Cell Replication in Young Mice but Is not Sufficient in One-Year-Old Mice Fed with a High-Fat Diet. Diabetes. 2019 Jul 10. pii: db181368. doi: 10.2337/db18-1368. PubMed PMID: 31292135.

ABSTRACT

Failure to expand pancreatic β-cells in response to metabolic stress leads to excessive workload resulting in β-cell dysfunction, dedifferentiation, death, and development of type 2 diabetes. In this study, we demonstrate that induction of Myc is required for increased pancreatic β-cell replication and expansion during metabolic stress-induced insulin resistance with short-term high-fat diet (HFD) in young mice. β-Cell-specific Myc knockout mice fail to expand adaptively and show impaired glucose tolerance and β-cell dysfunction. Mechanistically, PKCζ, ERK1/2, mTOR, and PP2A are key regulators of the Myc

response in this setting. DNA methylation analysis shows hypomethylation of cell cycle genes that are Myc targets in islets from young mice fed with a short-term HFD. Importantly, DNA hypomethylation of Myc response elements does not occur in islets from 1-year-old mice fed with a short-term HFD, impairing both Myc recruitment to cell cycle regulatory genes and β-cell replication. We conclude that Myc is required for metabolic stress-mediated β-cell expansion in young mice, but with aging, Myc upregulation is not sufficient to induce β-cell replication by, at least partially, an epigenetically mediated resistance to Myc action.

For full text, please **click here**.

Original Article:

Liu C, **Chikina M**, Deshpande R, Menk AV, Wang T, Tabib T, Brunazzi EA, Vignali KM, Sun M, **Stolz DB**, Lafyatis RA, Chen W, Delgoffe GM, Workman CJ, Wendell SG, Vignali DAA. Treg Cells Promote the SREBP1-Dependent Metabolic Fitness of Tumor-Promoting Macrophages via Repression of CD8(+) T Cell-Derived Interferon-γ. Immunity. 2019 Jul 13. pii: S1074-7613(19)30287-0. doi: 10.1016/j.immuni.2019.06.017. PubMed PMID: 31350177.

ABSTRACT

Regulatory T (Treg) cells are crucial for immune homeostasis, but they also contribute to tumor immune evasion by promoting a suppressive tumor microenvironment (TME). Mice with Treg cell-restricted Neuropilin-1 deficiency show tumor resistance while maintaining peripheral immune homeostasis, thereby providing a controlled system to interrogate the impact of intratumoral Treg cells on the TME. Using this and other genetic models, we showed that Treg cells shaped the transcriptional landscape across multiple tumor-infiltrating immune cell types. Treg cells suppressed CD8+ T cell secretion of interferon-γ (IFNγ), which would otherwise block the activation of sterol regulatory element-binding protein 1 (SREBP1)-mediated fatty acid synthesis in immunosuppressive (M2-like) tumor-associated macrophages (TAMs). Thus, Treg cells indirectly but selectively sustained M2-like TAM metabolic fitness, mitochondrial integrity, and survival. SREBP1 inhibition augmented the efficacy of immune checkpoint blockade, suggesting that targeting Treg cells or their modulation of lipid metabolism in M2-like TAMs could improve cancer immunotherapy.

For full text, please **click here**.

Original Article:

Celik N, Kelly B, Soltys K, **Squires JE, Vockley J**, Shellmer DA, Strauss K, **McKiernan P**, Ganoza A, **Sindhi R**, Bond G, Mazariegos G, Khanna A. Technique and outcome of Domino Liver Transplantation from patients with Maple Syrup Urine Disease: Expanding the donor pool for Live Donor Liver Transplantation. Clin Transplant. 2019 Sep 25:e13721. doi: 10.1111/ctr.13721. PubMed PMID: 31556146.

ABSTRACT

AIM/BACKGROUND: Domino liver transplantation (DLT) using liver allografts from patients with metabolic disorders enhances organ utilization. Short and long-term course and outcome of these patients can impact the decision to offer this procedure to patients, especially those with diseases that can potentially be cured with liver transplant. We reviewed the outcomes of DLT from maple syrup urine disease (MSUD) patients in our large academic pediatric and adult transplant program.

METHODS: All patients receiving DLT were analyzed retrospectively with a minimum of one-year follow-up period for patient and donor characteristics, early and late postoperative complications and patient and graft survival with their MSUD donors in terms of age, weight, MELD/PELD scores, cold ischemia time, postoperative leucine levels and peak ALT (alanine aminotransferase) levels during the first 48 postoperative hours.

RESULTS: Between 2006 and May 2019, 21 patients underwent domino liver transplantation with live-donor allografts from MSUD patients. Four patients transplanted for different metabolic diseases are focus of a separate report . Seventeen patients with minimum one year follow up period are reported herein. The indications were primary sclerosing cholangitis (PSC, n=4), congenital hepatic fibrosis (CHF, n=2), alpha-1 antitrypsin deficiency (A-1 ATD, n=2), progressive familial intrahepatic cholestasis (PFIC, n=2), cystic fibrosis (n=1), primary biliary cirrhosis (PBC, n=1), neonatal hepatitis (n=1), embryonal sarcoma (n=1), Caroli disease (n=1), hepatocellular carcinoma (HCC, n=1), and chronic rejection after liver transplantations for PSC (n=1). All patients and grafts survived at median follow-up of 6.4 years (range 1.2-12.9 years). Median domino recipient age was 16.2 years (range 0.6-64.6 years) and median MSUD recipient age was 17.6 years (range 4.8-32.1 years). There were no vascular complications during the early postoperative period, one patient had

portal vein thrombosis 3 years after DLT and a meso-Rex bypass was successfully performed. Small for size syndrome (SFSS) occurred in reduced left lobe DLT recipient and was managed successfully with conservative management. Biliary stricture developed in 2 patients and was resolved by stenting. Comparison between DLT and MSUD recipients' peak postoperative ALT results and PELD/MELD scores showed lower levels in DLT group (p-value<0.05).

CONCLUSIONS: Patient and graft survival in DLT from MSUD donors was excellent at short and long-term follow up. Metabolic functions have been normal in all recipients on a normal unrestricted protein diet. Ischemia preservation injury based on peak ALT was significantly decreased in DLT recipients. Domino transplantation from pediatric and adult recipients with selected metabolic diseases should be increasingly considered as an excellent option and alternative to deceased donor transplantation, thereby expanding the living donor pool. This, to date, is the largest world experience in DLT utilizing livers from patients with MSUD.

For full text, please **click here**.

Funding Opportunities

Research and Career Development Awards
American Association for the Study of Liver Diseases (AASLD)

- Pinnacle Research Award in Liver Diseases
- Clinical, Translational and Outcomes Research Award
- Bridge Award
- Pilot Research Award
- Afdhal / McHutchison LIFER Award
- Autoimmune Liver Diseases Pilot Research Award
- Advanced/Transplant Hepatology Award
- NP/PA Clinical Hepatology Fellowship

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