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Liver Digest

A weekly update of PLRC happenings

August 22, 2019

PITTSBURGH LIVER RESEARCH CENTER

A partnership of University of Pittsburgh & UPMC

www.livercenter.pitt.edu

Featured Faculty - Dr. Michael Nalesnik

In this issue

Genomics & Systems Biology Core - New Services

The GSBC has introduced the Oxford Nanopore sequencer and a single cell sequencing machine.

Oxford Nanopore long-read sequencing

- Whole genome sequencing for de novo assembly, structural variation, single nucleotide variation and resequencing analyses
- Transcriptome sequencing for RNA isofrom and fusion transcript analyses using direct RNA sequencing and ligation sequencing
- Targeted sequencing include amplicons, sequence capture and exome
- Epigenetic sequencing including methylation, histone modification and non-coding RNA activity
- Microbiome sequencing

10X Genomics single cell sequencing

- Our center is equipped with Controller™ from 10x Genomics, Inc. We provide collaboration on the analysis of 3’ barcoded and 5’ barcoded single-cell transcriptome sequencing to classify cell type on individual cell level. We also provide exome, copy number and ATAC sequencing for the chromosome structure analysis, all on the single-cell level. Single-cell classification provides high-resolution analysis on cell population. It is a new tool that enables us to understand organ development, cancer evolution and immune cell activation in individual cell level.

These are available for use by PLRC members. Please submit your request using the form available on the website: **https://www.livercenter.pitt.edu/sites/default/files/PLRC%20GSBC%20request%20form%2005.17.2019.**

pdf

Omics Data on Liver Diseases

Special thanks to **Dr. Silvia Liu**, manager of the Genomics & Systems Biology Core, for compiling and organizing Omics data on various liver diseases. The information is available on the PLRC website (**http://www.livercenter.pitt.edu/omics-data-liver-diseases**), with links to each study and its resulting data set.

RSVP for the PLRC Meet & Greet - September 12

Please **click here** to RSVP for the **PLRC's Meet and Greet!**
(*Please respond by August 28*)
September 12, 2019
4:30-7:30 p.m.
Ballroom A - University Club
123 University Place
Pittsburgh, PA 15260

Faculty Highlights

PLRC members collaborating on manuscripts are noted in red.

Press Release:

Dr. Soto-Gutierrez's article published in *Stem Cells and Development* in July 2019 has been featured in a press release by the Mary Ann Liebert, Inc. Publishers.

Simple Protocol for Assessing Maturation of Hepatic-Like Cells from Induced Pluripotent Stem Cells

New Rochelle, NY, August 13, 2019—Researchers have developed a guide to help labs standardize the production of mature hepatic-like cells (HPCs) from stem cells and easily compare gene expression of HPCs to actual human liver tissue. This moderately high throughput protocol can enable a relatively quick assessment of the efficacy of stem cell differentiation and help guide the optimization of

differentiation conditions in regenerative medicine applications. The protocol and its implications are published in ***Stem Cells and Development***, a peer-reviewed journal from Mary Ann Liebert, Inc., publishers. **Click here** to read the full-text article for free on the ***Stem Cells and Development*** website.

"**Guide to the Assessment of Mature Liver Gene Expression in Stem Cell-Derived Hepatocytes**" was coauthored by **Stephen Strom**, Karolinska Institutet (Stockholm, Sweden) and **Alejandro Soto-Gutierrez**, University of Pittsburgh (PA), and colleagues from Karolinska Institutet, Royan Institute for Stem Cell Biology (Tehran, Iran), and University of Pittsburgh. The researchers used real time-quantitative polymerase chain reaction (rt-qPCR) to determine the mRNA expression of more than 60 genes expressed in fetal and mature human liver samples, normalized to an internal control. They measured gene expression in iPCs produced in their own lab and in those purchased from commercial labs. Genes evaluated included those for liver-specific plasma proteins, cytochrome P450 enzymes, transporters, multi-drug resistant proteins, and the genes requires for pluripotency and the ability of iPCs to differentiate into different cell types.

"The ability to direct the differentiation of a stem cell population to a target mature cell type and demonstrate the persistence and validity of that achievement is still beyond the skill of most stem cell biologists, and, more worryingly, the field continues to tolerate the assumption that a given paper has achieved this without proof," says Editor-in-Chief **Graham C. Parker**, PhD, The Carman and Ann Adams Department of Pediatrics, Wayne State University School of Medicine, Detroit, MI. "In their landmark paper, Stephen Strom and colleagues provide a benchmark technique for other laboratories to compare their stem cell derived hepatocyte-like cells to actual human liver samples."

Research reported in this publication was supported by the National Institutes of Health under Award Numbers DK099257 and DK117881. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

About the Journal

Stem Cells and Development is an authoritative peer-reviewed journal published 24 times per year in print and online. The Journal is dedicated to communication and objective analysis of developments in the biology, characteristics, and therapeutic utility of stem cells, especially those of the hematopoietic system. A complete table of contents and free sample issue may be viewed on the ***Stem***

Cells and Development website.

About the Publisher

Mary Ann Liebert, Inc., publishers Mary Ann Liebert, Inc., publishers is a privately held, fully integrated media company known for establishing authoritative peer-reviewed journals in many promising areas of science and biomedical research, including *Cellular Reprogramming*, *Tissue Engineering*, and *Human Gene Therapy*. Its biotechnology trade magazine, GEN (*Genetic Engineering & Biotechnology News*), was the first in its field and is today the industry's most widely read publication worldwide. A complete list of the firm's 80 journals, books, and newsmagazines is available on the **Mary Ann Liebert, Inc., publishers** website.

Original Article:

Shaikh OS, Rogal S, Malik A, Sharma V, Cacciarelli T. Liver Transplant From Increased-Risk Donors in the Era of Direct-Acting Antivirals for Hepatitis C. Exp Clin Transplant. 2019 Jul 19. doi: 10.6002/ect.2019.0065. PubMed PMID: 31324136.

ABSTRACT

OBJECTIVES: The opioid epidemic and the associated deaths have increased the availability of increased-risk donor organs. Here, we assessed factors associated with increased-risk donor liver transplant and determined their impact on survival and response to direct-acting antivirals.

MATERIALS AND METHODS: We analyzed anti-hepatitis C virus-positive deceased-donor liver transplant recipients from August 2013 through December 2017. We compared recipient and donor clinical and virologic features, response to direct-acting antivirals, and graft and patient survival rates in increased-risk versus traditional or non-increased risk donor organ transplants.

RESULTS: Of 153 transplant recipients, 89 (58%) were anti-hepatitis C virus positive, with 42/89 receiving increased-risk donor livers (mean age 62 years, 1 female, 80% white, and 60% with hepatoma). On univariable analysis, receipt of increased-risk donor liver was associated with simultaneous liver-kidney transplant, lower Model for End-Stage Liver Disease score, hepatitis C virus RNA positivity, pretransplant direct-acting antiviral nonresponse, and younger donor age. On multivariable analysis, only donor age and Model for End-Stage Liver Disease score were associated with increased-risk donor transplant. Among

increased-risk donors, 12 (29%) were hepatitis C virus RNA positive, including one who was anti-hepatitis C virus antibody negative. Among recipients, 62 were hepatitis C virus RNA positive (35 with increased-risk livers), with 50 recipients (81%) having genotype 1. Posttransplant, recipient genotype changed in 6 and was mixed in 4 recipients. Of 55 recipients treated with direct-acting antivirals, 54 (98%) achieved viral clearance. Overall 1-year graft and patient survival was 93%.

CONCLUSIONS: Increased-risk donor organs provided high levels of utility in liver transplant recipients who were anti-HCV positive, showing optimal graft and patient survival. Increased-risk donors were younger and preferably transplanted in hepatitis C virus RNA-positive recipients with lower Model for End-Stage Liver Disease score. Posttransplant direct-acting antiviral therapy was highly efficacious irrespective of pretransplant recipient and donor virologic status.

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

Original Article:
Watson AR, Dai H, Zheng Y, Nakano R, Giannou AD, Menk AV, **Stolz DB,** Delgoffe GM, **Thomson AW**. mTORC2 Deficiency Alters the Metabolic Profile of Conventional Dendritic Cells. Front Immunol. 2019 Jul 2;10:1451. doi: 10.3389/fimmu.2019.01451. eCollection 2019. PubMed PMID: 31338091; PubMed Central PMCID: PMC6626913.

ABSTRACT
In myeloid dendritic cells (DC), deletion of the mechanistic target of rapamycin complex 2 (TORC2) results in an augmented pro-inflammatory phenotype and T cell stimulatory activity; however, the underlying mechanism has not been resolved. Here, we demonstrate that mouse bone marrow-derived TORC2-deficient myeloid DC (TORC2-/- DC) utilize an altered metabolic program, characterized by enhanced baseline glycolytic function compared to wild-type WT control (Ctrl) DC, increased dependence on glycolytic ATP production, elevated lipid content and higher viability following stimulation with LPS. In addition, TORC2-/- DC display an increased spare respiratory capacity (SRC) compared to WT Ctrl DC; this metabolic phenotype corresponds with increased mitochondrial mass and mean mitochondrial DNA copy number, and failure of TORC2-/- DC mitochondria to depolarize following LPS stimulation. Our data suggest that the enhanced metabolic activity of TORC2-/- DC may be due to compensatory TORC1 pathway

activity, namely increased expression of multiple genes upstream of Akt/TORC1 activity, including the integrin alpha IIb, protein tyrosine kinase 2/focal adhesion kinase, IL-7R and Janus kinase 1(JAK1), and the activation of downstream targets of TORC1, including p70S6K, eukaryotic translation initiation factor 4E binding protein 1 (4EBP1) and CD36 (fatty acid translocase). These enhanced TORC1 pathway activities may culminate in increased expression of the nuclear receptor peroxisome proliferator-activated receptor γ (Pparγ) that regulates fatty acid storage, and the transcription factor sterol regulatory element-binding transcription factor 1 (Srebf1). Taken together, our data suggest that TORC2 may function to restrain TORC1-driven metabolic activity and mitochondrial regulation in myeloid DC.

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

Original Article:

Ludwig DR, Fraum TJ, Cannella R, Tsai R, Naeem M, LeBlanc M, Salter A, Tsung A, Fleckenstein J, Shetty AS, **Borhani AA, Furlan A**, Fowler KJ. Expanding the Liver Imaging Reporting and Data System (LI-RADS) v2018 diagnostic population: performance and reliability of LI-RADS for distinguishing hepatocellular carcinoma (HCC) from non-HCC primary liver carcinoma in patients who do not meet strict LI-RADS high-risk criteria. HPB (Oxford). 2019 Jun 28. pii: S1365-182X(19)30525-8. doi: 10.1016/j.hpb.2019.04.007. PubMed PMID: 31262487.

ABSTRACT

BACKGROUND: Hepatocellular carcinoma (HCC) can be diagnosed using imaging criteria in patients at high-risk for HCC, according to Liver Imaging Reporting and Data System (LI-RADS) guidelines. The aim of this study was to determine the diagnostic performance and inter-rater reliability (IRR) of LI-RADS v2018 for differentiating HCC from non-HCC primary liver carcinoma (PLC), in patients who are at increased risk for HCC but not included in the LI-RADS 'high-risk' population.

METHODS: This retrospective HIPAA-compliant study included a 10-year experience of pathologically-proven PLC at two liver transplant centers, and included patients with non-cirrhotic hepatitis C infection, non-cirrhotic non-alcoholic fatty liver disease, and fibrosis. Two readers evaluated each lesion and assigned an overall LI-RADS diagnostic category, additionally scoring all major, LR-M, and ancillary features.

RESULTS: The final study cohort consisted of 27 HCCs and 104 non-HCC PLC in 131 patients. The specificity of a 'definite HCC' designation was 97% for reader 1 and 100% for reader 2. The IRR was fair for overall LI-RADS category and substantial for most major features.

CONCLUSION: In a population at increased risk for HCC but not currently included in the LI-RADS 'high-risk' population, LI-RADS v2018 demonstrated very high specificity for distinguishing pathologically-proven HCC from non-HCC PLC.

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

Funding Opportunities

Regenerative Medicine Innovation Project (RMIP) Investigator-Initiated Clinical Trials (UG3/UH3 Clinical Trial Required)

(RFA-HL-20-030)
National Heart, Lung, and Blood Institute
National Center for Advancing Translational Sciences
National Eye Institute
National Institute on Aging
National Institute of Allergy and Infectious Diseases
National Institute of Arthritis and Musculoskeletal and Skin Diseases
National Institute on Deafness and Other Communication Disorders
National Institute of Dental and Craniofacial Research
National Institute of Diabetes and Digestive and Kidney Diseases
National Institute of Neurological Disorders and Stroke

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