View this email in your browser

Liver Digest

A weekly update of PLRC happenings

January 16, 2020

PITTSBURGH LIVER RESEARCH CENTER

A partnership of University of Pittsburgh & UPMC

www.livercenter.pitt.edu

Featured Faculty - Dr. Abhinav Humar

In this issue

- Upcoming Seminars
- PLRC Mini-Retreat - February 3
- Faculty Highlights

PLRC Seminar

PLRC Seminar Series

Tue, 1/28/2020

12:00 to 1:00 pm

S123 BST

Suthat Liangpunsakul, MD **(click on name to go to bio page)**

Professor of Medicine

Professor of Biochemistry and Microbiology

Indiana University School of Medicine

Alterations in circadian machinery and ω-oxidation of fatty acids in the pathogenesis of alcohol-induced liver injury

Pizza will be provided.

This activity has been approved for AMA PRA Category 1 credit #6242-Liver Center Seminars.

Senior Vice Chancellor's Research Seminar

Fri, 01/31/2020
12:00 to 1:00 pm
S100A BST

Kari Nejak-Bowen, PhD, MBA

Assistant Professor of Pathology
Enrichment Director, PLRC

Novel Insights into the Role of Beta-Catenin in Biliary Pathophysiology

---

The full schedule of Enrichment activities is posted on **https://www.livercenter.pitt.edu/events**.

PLRC Mini-Retreat

Mon, 02/03/2020 -
10:30 - 4:00
S100A BST

10:30-11:30—P&F presentations (5 minutes presenting and 5 minutes Q&A)

- 10:30-10:40—Christian Fernandez
- 10:40-10:50—Sungjin Ko
- 10:50-11:00—Anita McElroy
- 11:00-11:10—Zach Freyberg
- 11:10-11:20—Sadeesh Ramakrishnan
- 11:20-11:35—Reben Raeman & Jai Behari

11:35-12:00—Lunch

12:00-1:00—P&F presentations (5 minutes presenting and 5 minutes Q&A)

- 12:00-12:10—Marlies Meisel

12:10-12:20—Amir Borhani group

12:20-12:30—Andres Duarte-Rojo

12:30-12:40—Hossam Abdelsamed

12:40-12:50—Vikrant Rachakonda group

12:50-1:30—Break

1:30-3:00—Grant-writing presentation – Gavin Arteel/Nick Giannoukakis

3:00-3:30—Open discussion & Q&A

3:30-4:00—Concluding remarks & EAB meeting debriefing - Dr. Paul Monga

---

Faculty Highlights

PLRC members collaborating on manuscripts are noted in red.

Conference Proceedings:Akkina R, Barber DL, **Bility MT**, Bissig KD, Burwitz BJ, Eichelberg K, Endsley JJ, Garcia JV, Hafner R, Karakousis PC, Korba BE, Koshy R, Lambros C, Menne S, Nuermberger EL, Ploss A, Podell BK, Poluektova LY, Sanders-Beer BE, Subbian S, Wahl A. Small Animal Models for Human Immunodeficiency Virus (HIV), Hepatitis B, and Tuberculosis: Proceedings of an NIAID Workshop. Curr HIV Res. 2019 Dec 22. doi: 10.2174/1570162X18666191223114019. PubMed PMID: 31870268.

ABSTRACT

The main advantage of animal models of infectious diseases over in vitro studies is the gain in understanding of the complex dynamics between the immune system and the pathogen. While small animal models have practical advantages over large animal models, it is crucial to be aware of their limitations. Although the small animal model at least needs to be susceptible to the pathogen under study

to obtain meaningful data, key elements of pathogenesis should also be reflected when compared to humans. Well-designed small animal models for HIV, hepatitis viruses, and tuberculosis require additionally a thorough understanding of similarities and differences in the immune responses between humans and small animals and should incorporate that knowledge into the goals of the study. To discuss these considerations, the NIAID hosted a workshop on 'Small Animal Models for HIV, Hepatitis B, and Tuberculosis' on May 30, 2019. Hightlights of the workshop are outlined below.

To access full text, please **click here**.

Original Article:
Wang X, Wang R, Bai S, Xiong S, Li Y, Liu M, Zhao Z, Wang Y, Zhao Y, Chen W, **Billiar TR**, Cheng B. Musashi2 contributes to the maintenance of CD44v6+ liver cancer stem cells via notch1 signaling pathway. J Exp Clin Cancer Res. 2019 Dec 30;38(1):505. doi: 10.1186/s13046-019-1508-1. PubMed PMID: 31888685.

ABSTRACT
BACKGROUND: Liver cancer stem cells (LCSCs) contribute to hepatocellular carcinoma (HCC) development, metastasis, and drug resistance. MSI2 and Notch1 signaling are involved in the maintenance of CSCs. However, it is unknown whether MSI2 and Notch1 are involved in the maintenance of CD44v6+ LCSCs. Therefore, we investigated the clinical significance and function of MSI2 and its relationship with Notch1 signaling in the maintenance of stemness properties in CD44v6+ LCSCs.

METHODS: The expression of MSI2 and CD44v6 were detected by fresh specimens and a HCC tissue microarray. The tissue microarray containing 82 HCC samples was used to analyze the correlation

between CD44v6 and MSI2. CD44v6+/- cells were isolated using microbeads sorting. We explored the roles of MSI2 and Notch1 signaling in CD44v6+ LCSCs by sphere formation assay, transwell assay, clone formation assay in vitro, and xenograft tumor models in vivo. A Notch RT2 PCR Array, Co-immunoprecipitation, and RNA-immunoprecipitation were used to further investigate the molecular mechanism of MSI2 in activating Notch1 signaling.

RESULTS: Here, we found MSI2 expression was positively correlated with high CD44v6 expression in HCC tissues, and further correlated with tumor differentiation. CD44v6+ cells isolated from HCC cell lines exhibited increased self-renewal, proliferation, migration and invasion, resistance to Sorafenib and tumorigenic capacity. Both MSI2 and Notch1 signaling were elevated in sorted CD44v6+ cells than CD44v6- cells and played essential roles in the maintenance of stemness of CD44v6+ LCSCs. Mechanically, MSI2 directly bound to Lunatic fringe (LFNG) mRNA and protein, resulting in Notch1 activation.

CONCLUSIONS: Our results demonstrated that MSI2 maintained the stemness of CD44v6+ LCSCs by activating Notch1 signaling through the interaction with LFNG, which could be a potential molecular target for stem cell-targeted therapy for liver cancer.

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

Original Article:
Zhang R, Nakao T, **Luo J**, Xue Y, Cornuet P, **Oertel M**, Kosar K, Singh S, **Nejak-Bowen K**. Activation of WNT/Beta-Catenin Signaling and Regulation of the Farnesoid X Receptor/Beta-Catenin Complex After Murine Bile Duct Ligation. Hepatol Commun. 2019 Oct 14;3(12):1642-1655. doi: 10.1002/hep4.1430. eCollection 2019 Dec. PubMed PMID:

31832572; PubMed Central PMCID: PMC6887668.

ABSTRACT

We have recently shown that loss of β-catenin prevents the development of cholestatic liver injury and fibrosis after bile duct ligation (BDL) due to loss of the inhibitory farnesoid X receptor (FXR)/β-catenin complex, which results in decreased hepatic bile acids (BAs) through activation of FXR. To further understand the role of Wnt/β-catenin signaling in regulating BA metabolism and cholestasis, we performed BDL on mice in which hepatocyte Wnt signaling is deficient but β-catenin is intact (low-density lipoprotein receptor-related protein [LRP]5/6 knockout [DKO]) as well as mice that have enhanced hepatocyte β-catenin expression (serine 45 mutated to aspartic acid [S45D] transgenic [TG] mice). Despite decreased biliary injury after BDL, hepatic injury, fibrosis, and inflammation were comparable in DKO and wild-type (WT) mice. Notably, the FXR/β-catenin complex was maintained in DKO livers after BDL, coincident with significantly elevated hepatic BA levels. Similarly, TG mice did not display accelerated injury or increased mortality despite overexpression of β-catenin. There was no augmentation of FXR/β-catenin association in TG livers; this resulted in equivalent hepatic BAs in WT and TG mice after BDL. Finally, we analyzed the effect of BDL on β-catenin activity and identified an increase in periportal cytoplasmic stabilization and association with T-cell factor 4 that correlated with increased expression of distinct downstream target genes. Conclusion: Localization of β-catenin and expression of Wnt-regulated genes were altered in liver after BDL; however, neither elimination of Wnt/β-catenin signaling nor overexpression of β-catenin in hepatocytes significantly impacted the phenotype or progression of BA-driven cholestatic injury.

Original Article:

Welch SR, Ritter JM, **McElroy AK**, Harmon JR, Coleman-McCray JD, Scholte FEM, Kobinger GP, Bergeron É, Zaki SR, Nichol ST, Spengler JR, Spiropoulou CF. Fluorescent Crimean-Congo hemorrhagic fever virus illuminates tissue tropism patterns and identifies early mononuclear phagocytic cell targets in IFNAR-/-mice. PLoS Pathog. 2019 Dec 2;15(12):e1008183. doi: 10.1371/journal.ppat.1008183. PubMed PMID: 31790513.

ABSTRACT

Crimean-Congo hemorrhagic fever virus (CCHFV, order Bunyavirales, family Nairoviridae, genus Orthonairovirus) is the tick-borne etiological agent of Crimean-Congo hemorrhagic fever (CCHF) in humans. Animals are generally susceptible to CCHFV infection but refractory to disease. Small animal models are limited to interferon-deficient mice, that develop acute fatal disease following infection. Here, using a ZsGreen1- (ZsG) expressing reporter virus (CCHFV/ZsG), we examine tissue tropism and dissemination of virus in interferon-α/β receptor knock-out (Ifnar-/-) mice. We demonstrate that CCHFV/ZsG retains in vivo pathogenicity comparable to wild-type virus. Interestingly, despite high levels of viral RNA in all organs assessed, 2 distribution patterns of infection were observed by both fluorescence and immunohistochemistry (IHC), corresponding to the permissiveness of organ tissues. To further investigate viral dissemination and to temporally define cellular targets of CCHFV in vivo, mice were serially euthanized at different stages of disease. Flow cytometry was used to characterize CCHFV-associated alterations in hematopoietic cell populations and to classify infected cells in the blood, lymph node, spleen, and liver. ZsG signal indicated that mononuclear phagocytic cells in the lymphatic tissues were early

targets of infection; in late-stage infection, overall, the highest levels of signal were detected in the liver, and ZsG was found in both antigen-presenting and lymphocyte cell populations.

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

Original Article:

Pan Y, Ballance H, Meng H, Gonzalez N, Kim SM, Abdurehman L, York B, Chen X, Schnytzer Y, Levy O, Dacso CC, McClung CA, O'Malley BW, **Liu S, Zhu B**. 12-h clock regulation of genetic information flow by XBP1s. PLoS Biol. 2020 Jan 14;18(1):e3000580. doi: 10.1371/journal.pbio.3000580. eCollection 2020 Jan. PubMed PMID: 31935211.

ABSTRACT

Our group recently characterized a cell-autonomous mammalian 12-h clock independent from the circadian clock, but its function and mechanism of regulation remain poorly understood. Here, we show that in mouse liver, transcriptional regulation significantly contributes to the establishment of 12-h rhythms of mRNA expression in a manner dependent on Spliced Form of X-box Binding Protein 1 (XBP1s). Mechanistically, the motif stringency of XBP1s promoter binding sites dictates XBP1s's ability to drive 12-h rhythms of nascent mRNA transcription at dawn and dusk, which are enriched for basal transcription regulation, mRNA processing and export, ribosome biogenesis, translation initiation, and protein processing/sorting in the Endoplasmic Reticulum (ER)-Golgi in a temporal order consistent with the progressive molecular processing sequence described by the central dogma information flow (CEDIF). We further identified GA-binding proteins (GABPs) as putative novel transcriptional regulators driving 12-h rhythms of gene expression with more diverse phases. These 12-h rhythms of gene expression are

cell autonomous and evolutionarily conserved in marine animals possessing a circatidal clock. Our results demonstrate an evolutionarily conserved, intricate network of transcriptional control of the mammalian 12-h clock that mediates diverse biological pathways. We speculate that the 12-h clock is coopted to accommodate elevated gene expression and processing in mammals at the two rush hours, with the particular genes processed at each rush hour regulated by the circadian and/or tissue-specific pathways.

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

Our mailing address is:
Pittsburgh Liver Research Center
200 Lothrop St. | Pittsburgh, PA 15261