View this email in your browser

Liver Digest

A weekly update of PLRC happenings

July 18, 2019

PITTSBURGH LIVER RESEARCH CENTER

A partnership of University of Pittsburgh & UPMC

www.livercenter.pitt.edu

Featured Faculty - Dr. Vikrant Rachakonda

In this issue

PLRC P&F Recipients

Meet PLRC's most recent Pilot and Feasibility Recipients and know their funded research at **http://www.livercenter.pitt.edu**

Faculty Highlights

Original Article:

Gerlach JC, Thompson RL, Gridelli B, Schmelzer E. Effects of Delta-Like Noncanonical Notch Ligand 1 Expression of Human Fetal Liver Hepatoblasts on Hematopoietic Progenitors. Stem Cells Int. 2019 Mar 18;2019:7916275. doi: 10.1155/2019/7916275. eCollection 2019. PubMed PMID: 31011334; PubMed Central PMCID: PMC6442310.

ABSTRACT

Although the hepatic and hematopoietic progenitors of the liver are well characterized, the interactions between these two lineages remain mostly elusive. Hepatoblasts express delta-like noncanonical Notch ligand 1 (Dlk1), whose cleaved extracellular domain can become a soluble protein. We assessed the effects of DLK1 gene expression knockdown in cultures of total fetal liver cells. Furthermore, we separated Dlk1+ hepatoblasts from the total liver cell fraction and investigated effects of direct cell contact. Dlk1- cells were cultured either without Dlk1+ hepatoblasts, in direct contact with hepatoblasts, or separated from hepatoblasts by a porous membrane in inserts to inhibit cell contact but allow free exchange of molecules. Expression of the hepatic and hematopoietic genes, colony forming unit potential of various hematopoietic progenitors, and cell numbers and types were investigated. We found that DLK1 knockdown in total fetal liver cell cultures decreased total cell numbers. The expression of hepatic progenitor genes and mature hematopoietic genes was affected. Hematopoietic BFU-E and CFU-GM colony

numbers were reduced significantly. The depletion of Dlk1+ hepatoblasts in culture decreased the potential of all hematopoietic progenitors to form colonies of all types and reduced the percentage of mature hematopoietic cells. The addition of hepatoblasts in inserts to Dlk1- cells further decreased the potential to form the CFU-GM and CFU-GEMM colonies and the percentage of mature hematopoietic cells but increased total cell numbers. Conclusively, direct contact of Dlk1 supports hematopoietic progenitor expansion and functionality that cannot be reconstituted in coculture without direct cell contact.

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

Original Article:

Wan Z, Sun J, Xu J, Moharil P, Chen J, Xu J, Zhu J, Li J, Huang Y, Xu P, **Ma X**, **Xie W**, Lu B, **Li S**. Dual functional immunostimulatory polymeric prodrug carrier with pendent indoximod for enhanced cancer immunochemotherapy. Acta Biomater. 2019 May;90:300-313. doi: 10.1016/j.actbio.2019.03.048. Epub 2019 Mar 28. PubMed PMID: 30930305; PubMed Central PMCID: PMC6513707.

ABSTRACT

Immunotherapy based on checkpoint blockade has been regarded as one of the most promising approaches towards many types of cancers. However, low response rate hinders its application due to insufficient tumor immunogenicity and immunosuppressive tumor microenvironment. To achieve an overall enhanced therapeutic outcome, we developed a dual-functional immuno-stimulatory polymeric prodrug carrier modified with pendent indoximod, an indoleamine 2,3-dioxygenase (IDO) inhibitor that can be used to reverse immune suppression, for co-delivery of Doxorubicin (Dox), a hydrophobic anticancer agent that can promote immunogenic cell death (ICD) and elicit antitumor immunity. The resulted carrier denoted as POEG-b-PVBIND, consisting of poly (oligo (ethylene glycol) methacrylate) (POEG) hydrophilic blocks and indoximod conjugated hydrophobic blocks, is rationally designed to improve immunotherapy by synergistically modulating the tumor microenvironment (TME). Our data showed that Dox-triggered ICD promoted intra-tumoral infiltration of CD8+ T cells and IFN-γ-production by CD8+ T cells. Meanwhile, cleaved indoximod significantly increased CD8+ T cell infiltration while reducing the immunosuppressive T regulatory cells (Tregs). More importantly, Dox/POEG-b-PVBIND micelles led to significantly improved tumor regression in an orthotopic murine breast cancer model compared to both Dox-loaded POEG-b-PVB micelles (a control inert

carrier) and POEG-b-PVBIND micelles alone, confirming combination effect of indoximod and Dox in improving the overall antitumor activity. STATEMENT OF SIGNIFICANCE: Indoleamine 2,3-dioxygenase (IDO) is an enzyme that can induce immune suppressive microenvironment in tumors. As a well-studied IDO inhibitor, indoximod (IND) represents a promising agent for cancer immunotherapy and could be particularly useful in combination with other chemotherapeutic agents. However, three major problems hinder its application: (1) IND is barely soluble in water; (2) IND delivery efficiency is limited (3) simultaneous delivery of two agents into tumor site is still challenging. Currently, most reports largely focus on improving the pharmacokinetic profile of IND alone via different formulations such as IND prodrug and IND nanocrystal. However, there is limited information about IND based co-delivery systems, especially for delivering hydrophobic chemotherapeutic agents. Here, we developed a new dual-functional polymeric prodrug carrier modified with a number of pendent IND units (denoted as POEG-b-PVBIND). POEG-b-PVBIND shows immunostimulatory and antitumor activities by itself. More importantly, POEG-b-PVBIND polymer is able to self-assemble into nano-sized micelles that are highly effective in formulating and codelivering other hydrophobic agents including doxorubicin (Dox), sunitinib (Sun), and daunorubicin (Dau), which can elicit antitumor immunity via promoting immunogenic cell death (ICD). We have shown that our new combination therapy led to a significantly improved antitumor activity in an aggressive murine breast cancer model (4T1.2).

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

Original Article:

McKiernan PJ, Ganoza A, **Squires JE**, **Squires RH**, **Vockley J**, **Mazariegos G**, Soltys K, Sun Q, **Sindhi R**. Evolving Trends in Liver Transplant for Metabolic Liver Disease in the United States. Liver Transpl. 2019 Jun;25(6):911-921. doi: 10.1002/lt.25433. Epub 2019 Apr 15. PubMed PMID: 30753750.

ABSTRACT

Indications for liver transplantation (LT) in metabolic disease are evolving. We reviewed the US experience with primary LT for metabolic disease in the Scientific Registry for Transplant Recipients (October 1987 to June 2017) to determine the following: temporal changes in indications, longterm outcomes, and factors predicting survival. Patients were grouped by the presence of structural liver disease (SLD) and whether the defect was confined to the liver. There were 5996 patients who underwent LT for metabolic disease, 2354

(39.3%) being children. LT for metabolic disease increased in children but not in adults. Children experienced a 6-fold increase in LT for metabolic disease without SLD. Indications for LT remained stable in adults. Living donor liver transplantation increased between era 1 and era 3 from 5.6% to 7.6% in children and 0% to 4.5% in adults. Patient and graft survival improved with time. The latest 5-year patient survival rates were 94.5% and 81.5% in children and adults, respectively. Outcomes were worse in adults and in those with extrahepatic disease ($P < 0.01$), whereas SLD did not affect outcomes. Survival improved with younger age at LT until age <2 years. On multivariate analysis, diagnostic category, inpatient status, age at LT, and transplant era significantly predicted outcomes in all ages with male sex predicting survival in childhood only. Children without structural disease were less likely to die awaiting LT and had improved post-LT survival compared with children with chronic liver disease. In conclusion, LT for metabolic disease is increasingly used for phenotypic correction in children; extrahepatic manifestations significantly impact survival at all ages; where indicated, transplantation should not be unnecessarily delayed; and the development of new allocation models may be required.

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

Original Article:

Josepmaria Argemi, Maria U. Latasa, Stephen R. Atkinson, Ilya O. Blokhin, Veronica Massey, Joel P. Gue, Joaquin Cabezas, Juan J. Lozano, Derek Van Booven, **Aaron Bell**, Sheng Cao, **Lawrence A. Vernetti**, Juan P. Arab, Meritxell Ventura-Cots, Lia R. Edmunds, Constantino Fondevilla, Peter Stärkel, Laurent Dubuquoy, Alexandre Louvet, Gemma Odena, Juan L. Gomez, Tomas Aragon, Jose Altamirano, Juan Caballeria, **Michael J. Jurczak**, **D. Lansing Taylor**, Carmen Berasain, Claes Wahlestedt, **Satdarshan P. Monga**, Marsha Y. Morgan, Pau Sancho-Bru, Philippe Mathurin, Shinji Furuya, Carolin Lackner, Ivan Rusyn, Vijay H. Shah, Mark R. Thursz, Jelena Mann, Matias A. Avila, **Ramon Bataller**. Defective HNF4alpha-dependent gene expression as a driver of hepatocellular failure in alcoholic hepatitis. Nature Communications volume 10, Article number: 3126 (2019). Published 16 July 2019.

ABSTRACT

Alcoholic hepatitis (AH) is a life-threatening condition characterized by profound hepatocellular dysfunction for which targeted treatments are urgently needed. Identification of molecular drivers is hampered by the lack of suitable animal models. By performing RNA sequencing in livers from patients

with different phenotypes of alcohol-related liver disease (ALD), we show that development of AH is characterized by defective activity of liver-enriched transcription factors (LETFs). TGFβ1 is a key upstream transcriptome regulator in AH and induces the use of HNF4α P2 promoter in hepatocytes, which results in defective metabolic and synthetic functions. Gene polymorphisms in LETFs including HNF4α are not associated with the development of AH. In contrast, epigenetic studies show that AH livers have profound changes in DNA methylation state and chromatin remodeling, affecting HNF4α-dependent gene expression. We conclude that targeting TGFβ1 and epigenetic drivers that modulate HNF4α-dependent gene expression could be beneficial to improve hepatocellular function in patients with AH.

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

Methods Paper:

Zabulica M, Srinivasan RC, Vosough M, Hammarstedt C, Wu T, Gramignoli R, Ellis E, Kannisto K, Collin de l'Hortet A, Takeishi K, **Soto-Gutierrez A**, Strom SC. Guide to the Assessment of Mature Liver Gene Expression in Stem Cell-Derived Hepatocytes. Stem Cells Dev. 2019 May 24. doi: 10.1089/scd.2019.0064. [Epub ahead of print] PubMed PMID: 31122128.

ABSTRACT

Differentiation of stem cells to hepatocyte-like cells holds great promise for basic research, drug and toxicological investigations and clinical applications. There are currently no protocols for the production of hepatocyte-like cells from stem cells, such as embryonic stem cells or induced pluripotent stem cells, that produce fully mature hepatocytes with a wide range of mature hepatic functions. This report describes a standard method to assess the maturation of stem cell-derived hepatocyte-like cells with a moderately high throughput format, by analysing liver gene expression by quantitative RT-qPCR. This method also provides a robust data set of the expression of 62 genes expressed in normal liver, generated from 17 fetal and 25 mature human livers so that investigators can quickly and easily compare the expression of these genes in their stem cell-derived hepatocyte-like cells with the values obtained in authentic fetal and mature human liver. The simple methods described here will provide a quick and accurate assessment of the efficacy of a differentiation protocol and will help guide the optimization of differentiation conditions.

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

Funding Opportunities

Mechanisms of Tolerance (R21/R33 - Clinical Trial Required)

(PAR-19-311)

National Institute on Alcohol Abuse and Alcoholism

Our mailing address is:

Pittsburgh Liver Research Center

200 Lothrop St. | Pittsburgh, PA 15261