Regenerative Medicine Minnesota
Progress Report
Due: May 30, 2016
Grant Title: Post-Doctoral Scholar in Regenerative Medicine
Grant Number: MRM 2015 PDSCH 002
Requester: Nidhi Jalan-Sakrikar
Project Timeline: May 1, 2015 – April 30, 2016
Brief description: (please describe your course of study or research project)

My Research project goal was to test the hypothesis that "**injured cholangiocytes communicate with progenitor cells via exosomes, promoting cholangiocyte differentiation through mechanisms dependent upon Shh-mediated fibronectin release"**. We had designed three specific aims to accomplish our objectives:

Aim 1: Utilize exosome isolation and Shh pathway manipulation targeting the hedgehog receptors, in conjunction with various molecular techniques to test the subhypothesis that **injured cholangiocytes release Shh-containing exosomes which then signal to progenitor cells to promote fibronectin secretion and cholangiocyte differentiation**

We utilized exosome isolation from LPS-treated or untreated normal human cholangiocyte (NHC) cell line. Western blot analysis and immunogold electron microscopy revealed an increase in Shh ligand in exosomes isolated from LPS-treated cells. Consistent with this observation, exosomes from LPS-treated cells promoted the differentiation of iPSC to cholangiocytes along with fibronectin release compared to the control exosomes. Furthermore the effect of exosomes on the iPSC differentiation can be blocked by the pharmacological inhibitor (CPN) of Shh pathway. Employing shRNA against the Shh receptor, SMO, as well as CPN, we show that Shh pathway is critical in regulating both the differentiation of iPSC towards cholangiocytes and fibronectin release.

We further investigated transcription of the CK7, CK19 and FN genes. Both ChIP and luciferase assays showed that Gli-1 (the transcription factor downstream of Shh) binds to these promoters and activates their transcription.

Aim 2: Employ complementary molecular (integrin shRNA) and pharmacologic (FN peptide inhibitors) interventions to test the subhypothesis that **secreted fibronectin promotes autocrine cholangiocyte differentiation through αvβ6 integrin signaling in liver progenitor cells**

We utilized several clones of beta6 shRNA to knockdown the integrin subunit in the iPSC system, however none of the clones reduced the beta 6 levels in the iPSC to iDC differentiation. We are in the process of using siRNA technology to test the role of alpha v beta6 in autocrine signaling for cholangiocyte differentiation.

Alternatively, we isolated ductular reactive cells (DRCs) from mice and show that treatment of these cells with Shh is sufficient to differentiate them towards cholangiocyte fate as seen by increase in CK7, CK19 as well as FN release.

Since epigenetic events play a critical role in regulating gene transcription events we explored the role of EZH2, an H3K27me3 writer implicated in hepatoblast proliferation and differentiation in the embryonic liver. Based on RNA-seq analysis EZH2 is downregulated in iPSC to cholangiocyte differentiation. This loss in EZH2 is congruent with a prominent decrease in repressive H3K27me3 levels. Thus we overexpressed EZH2 using adenoviral system and found significant impairment in acquisition of the cholangiocyte markers as well as matrix

deposition. Furthermore in DRCs, EZH2 overexpression prevented the Shh-mediated activation of cholangiocyte differentiation and FN secretion. Similarly overexpression of EZH2 abolishes Shh-induced luciferase activity for both the FN and Ck19 promoters, whereas inhibition of EZH2 with a pharmacological compound enhanced Shh-induced luciferase activity.

Consistent with the above in vitro observation, we found that the EZH2 KO animals show ductular fibrosis at baseline level which was markedly exaggerated upon CDE-feeding which induces liver injury.

Overall our studies support a model whereby hedgehog signaling antagonizes EZH2 mediated transcriptional repression, thereby supporting cholangiocyte differentiation and depositional of provisional FN matrix.

Aim 3: Use fibroblasts from PSC patients to develop iDCs and test the subhypothesis that **the diseased iDCs upregulate the Shh pathway, enhance production of Shh-containing exosomes, and increase fibronectin secretion in progenitor cells.**

We successfully transduced fibroblasts from 2 different PSC patients to get iPSC clones. Utilizing the same protocol as for control iPSCs we are able to differentiate the patient iPSCs to iDCs. Characterization of the different phases of the differentiation reveal acquisition of the cholangiocyte markers CK7 and CK19 at the HS phase, however, the PSC patients exhibit enhanced levels of these markers at the final iDC phase. Furthermore, these PSC patient iDCs also show enhanced FN levels in the cells via immunoblotting. We are currently in the process of analyzing the conditioned media for secreted FN and isolating exosomes to check the Shh levels compared to the control iDC.

Outcome: (In 2-3 paragraphs, please explain how the RMM funds impacted your course of study, research project, and/or career path.)

Funds from Regenerative Medicine Minnesota were instrumental in moving this project forward. The innovative data generated on this fellowship has resulted presentations at national meetings and a first-author manuscript currently under revision.

Also I was able to successfully compete for the Satter Foundation fellowship for the upcoming year to further the project in understanding the molecular basis of PSC.

Please list any of the following that have resulted from your Regenerative Medicine Minnesota grant funding:

Publications:

1) Jie Lu, Yingqun Zhou, Tianyuan Hu, Hui Zhang, Miao Shen , Ping Cheng ,Weiqi Dai , Fan Wang ,Kan Chen ,Yan Zhang ,Chengfeng Wang ,Jingjing Li ,Yuanyuan Zheng ,Jing Yang ,Rong Zhu, Jianrong Wang , Wenxia Lu ,Huawei Zhang ,Junshan Wang , Yujing Xia , Thiago M. De Assuncao , **Nidhi Jalan-Sakrikar** , Robert C. Huebert, Bin Zhou , and Chuanyong Guo “Notch

Signaling Coordinates Progenitor Cell-Mediated Biliary Regeneration Following Partial Hepatectomy" *Scientific Reports, 2016 Mar 8, 6:22754.*

2) Merino-Azpitarte M, Lozano E, Perugorria MJ, Erice O,1 Santos-Laso A, Jiménez-Agüero R,1 Lacasta A, Briz O, **Jalan-Sakrikar N**, Huebert RC, Gradilone S, Aransay AM, Lavín JL, Fernández-Barrena MG, Marzioni M, Gores G, Marín JJ, Bujanda L, , Banales JM "SOX17 regulates cholangiocyte differentiation and acts as a tumor suppressor in cholangiocarcinoma" *Under Preparation*

- Publications and/or manuscripts submitted for publication
  1) **Nidhi Jalan-Sakrikar**, Thiago M. De Assuncao, Jie Lu, Luciana L. Almada, Gwen A. Lomberk, Martin E. Fernandez-Zapico, Raul A. Urrutia, Robert C. Huebert "Hedgehog-Dependant Antagonism of Enhancer of Zeste Homolog 2-Mediated Transcriptional Silencing is Required for Biliary Maturation" *Under Revision*

- Disclosures/patents
- Other grant applications and/or awards
  1) Satter Foundation Fellowship 2016-2017

Responsible Spending:
Please let us know how you spent the money. Any unspent funds must be returned.

The funds were used to support the salary of Dr. Jalan-Sakrikar and to purchase reagents for the project.