Bile acid-regulation of mast cells in models of Primary Sclerosing Cholangitis and cholangiocarcinoma

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Progress of research (11-2014 to 11-2015)

We are sincerely grateful for the award from the PSC Partners Seeking a Cure to support this work and believe we have made significant advances during the last year of funding. The overall goals of this proposal are to (i) demonstrate that mast cells can be regulated by bile acids (that are increased in patients with cholangiopathies) and (ii) that mast cells are critical regulators of both PSC and cholangiocarcinoma progression. In the past funding cycle we have progressed and our findings have been published, presented and included as preliminary data for both a submitted NIH RO1 proposal and VA Merit Award – both of which have been awarded, fall 2015. Below are our findings to date:

- We have found that hepatic mast cells infiltrate/migrate into the liver following liver damage including after bile duct ligated (BDL)-induced injury and in the genetic PSC model, MDR2$^{-/-}$ mice. Infiltration of mast cells increases biliary proliferation, fibrosis and liver damage. In addition, there is an increase in circulating histamine levels.
- In BDL and MDR2$^{-/-}$ rodent models we have demonstrated that blocking mast cell histamine by cromolyn sodium treatment decreases proliferating bile ducts, angiogenesis and the fibrotic reaction seen during PSC progression. This discovery pinpoints mast cell-derived histamine as a key contributor to PSC.
- Migration of hepatic mast cells is driven by the c-kit/stem cell factor (SCF) interaction. Cholangiocytes and cholangiocarcinoma secrete large amounts of SCF and mast cells are coated with c-kit receptors. By blocking SCF secretion in cholangiocytes we are able to block the migration of mast cells, *in vitro* and we are currently doing experiments to determine if this phenomenon occurs *in vivo*. **This paper has been published (see below).**
- During cholangiocarcinoma mast cells are also found to increase in number in the liver. Once in the tumor microenvironment (presumably via the c-kit/SCF interaction) there is an activation of angiogenesis, epithelial to mesenchymal transition (EMT) and the breakdown of the extracellular matrix (ECM). Further, we found that co-culture of human cholangiocarcinoma cells and mast cells increases cholangiocarcinoma growth, EMT switch and ECM breakdown, *in vitro*. When we pre-

treated mast cells with the inhibitor to histidine decarboxylase (reducing histamine secretion from mast cells) there was a significant decrease in angiogenesis, EMT and ECM degradation. These results pinpoint mast cell-derived histamine as a key contributor to cholangiocarcinoma progression. **This paper has been published (see below).**

- We have successfully developed a protocol to isolate hepatic mast cells from rodent livers. Our group is the first to accomplish this in mature rats (most mast cells are isolated from lung, peritoneal cavity, fetal liver or bone marrow). This tool is necessary for us to study the full paracrine effects of mast cells on different liver etiologies and can be extended later into various diseases.
- *In vivo* we have found that mast cells are increased in the liver in rats that have been treated with the bile acid, taurocholate; whereas treatment with ursodeoxycholate (to BDL rats) results in a decrease in mast cell infiltration. Mast cell marker expression is also decreased in livers from BDL + ursodeoxycholate feeding and histamine receptor expression (in mast cells and cholangiocytes) is also decreased. Currently we are treating mast cells *in vitro* with various bile acids and performing co-culture studies with cholangiocytes and cholangiocarcinoma cells to determine the effects on proliferation, fibrosis markers, EMT switch and ECM degradation. Our preliminary data suggests that pre-treatment with ursodeoxycholate in mast cells decreases cholangiocarcinoma proliferation and EMT switch.
- We have started injection wild-type (WT) mice with cultured mast cells. In livers and cholangiocytes isolated from these animals we have found that after mast cell injection there is an increase in biliary proliferation, fibrosis and liver damage. Current studies are also examining the effects of injecting mast cells into $HDC^{-/-}$ mice that have been subjected to BDL. These studies are underway and we have presented a poster regarding this work.
- In human liver biopsy samples from patients with late and advanced stage PSC, we have found that mast cells are present in large numbers and that histamine secretion is increased in serum from these patients. Further, in a cohort of patients treated with ursodeoxycholate, there is a decrease in the number of mast cells present in the liver and histamine secretion decreases suggesting that in human PSC, ursodeoxycholate may alter mast cell number and response.
- An outgrowth of these studies has been to examine the role that histamine receptor blockers have on PSC and cholangiocarcinoma progression. To that end, we have treated both wild-type and PSC mice with popular, over-the-counter H1 and H2 blockers. We found that using these blockers chronically, decreases the fibrotic reaction and biliary proliferation found in PSC mice, but has no negative effects on wild-type mice thus demonstrating that these drugs may be beneficial to patients with PSC.

Lay summary of progress:

Background: Primary Sclerosing Cholangitis (PSC) and the primary liver cancer, cholangiocarcinoma are devastating liver diseases that target cells within the liver called cholangiocytes. These diseases have limited treatment options. Patients that present with PSC have a higher incidence of developing cholangiocarcinoma. When cholangiocytes (that line bile ducts where bile flows out of the body) become

damaged and ducts become blocked, cholangiocytes will increase in number and this disrupts the normal state of the liver and bile flow. Bile acids are synthesized by the liver to aide in the digestion of foods and the secretion of bile. Patients with liver disease are frequently treated with bile acid therapy to improve liver function. Ursodeoxycholate is a secondary bile acid that is used in patient therapy for PSC and has been found to have beneficial effects in patients suffering from these diseases. Besides cholangiocytes, mast cells (inflammatory cells that release histamine in the body) are found in the liver and increase in number during liver disease. When mast cells are treated with bile acids there is a release or blockage of histamine release (depending on the type of bile acid).

Update on research: To date, we have found that mast cells infiltrate the liver during PSC and cholangiocarcinoma progression. Once these cells migrate to the liver they release numerous mediators that can negatively impact a patient's prognosis. One of the main factors released is histamine that can contribute to the progression of liver injury or tumor growth. Since we have found a large number of mast cells in the liver following injury, we recently developed a technique to isolate or extract these cells from rodent liver giving us a tool to better examine their effects on PSC and cholangiocarcinoma models. Further, following treatment with cromolyn sodium, a compound that blocks the release of histamine from mast cells, we have demonstrated that there is a decrease in liver fibrosis (which is a consequence of diseases like PSC) and tumor growth. We have found that treatment with ursodeoxycholate decreases the release of histamine from mast cells, which may also be an important finding in the treatment fibrosis or cholangiocarcinoma. Our results demonstrate that targeting mast cells may be a potential therapy for patients suffering from PSC and cholangiocarcinoma. Using human patient samples we have demonstrated that in PSC patients (advanced and late stages) there is an infiltration of mast cells compared to normal liver samples and histamine levels increase significantly in these patients.

Published results pertaining to the PSC Partners grant:

1. Inhibition of Mast Cell-Derived Histamine Decreases Human Cholangiocarcinoma Growth and Differentiation via c-Kit/Stem Cell Factor-Dependent Signaling. Johnson C, Huynh V, Hargrove L, Kennedy L, Graf-Eaton A, Owens J, Trzeciakowski JP, Hodges K, DeMorrow S, Han Y, Wong L, Alpini G, Francis H. *Am J Pathol.* 2015 Nov 18. pii: S0002-9440(15)00578-7. doi: 10.1016/j.ajpath.2015.09.016. [Epub ahead of print] **PMID: 26597881**

2. Bile acid signaling and biliary functions. *Acta Pharm Sin B.* Jones H, Alpini G, Francis H. 2015 Mar;5(2):123-8. doi: 10.1016/j.apsb.2015.01.009. Epub 2015 Feb 19. **PMID: 26579437**

3. Histamine restores biliary mass following carbon tetrachloride-induced damage in a cholestatic rat model. Johnson C, Hargrove L, Graf A, Kennedy L, Hodges K, Harris R, Francis T, Ueno Y, Francis H. *Dig Liver Dis.* 2015 Mar;47(3):211-7. doi: 10.1016/j.dld.2014.12.006. Epub 2014 Dec 17. **PMID: 25575430**

Papers in preparation pertaining to the PSC Partners grant:

1. Inhibition of mast cell-secreted histamine decreases biliary proliferation and hepatic fibrosis in the PSC mouse model, Mdr2$^{-/-}$. Hannah Jones, Laura Hargrove, Lindsey Kennedy, Fanyin Meng, Allyson Graf-Eaton, Jennifer Owens, Gianfranco Alpini, Christopher Johnson, Francesca Bernuzzi, Jennifer Demieville, Sharon DeMorrow, Pietro Invernizzi and Heather Francis *to be submitted to Hepatology, December 2015*

2. Isolation and characterization of hepatic mast cells from cholestatic rodent models. Laura Hargrove, Lindsey Kennedy, Fanyin Meng, Allyson Graf-Eaton, Jennifer Owens, Jennifer Demieville, Sharon DeMorrow and Heather Francis. *to be submitted to Gastroenterology, January 2016*

3. The introduction of mast cells increases biliary hyperplasia and hepatic fibrosis in normal and HDC$^{-/-}$ mice. Laura Hargrove, Lindsey Kennedy, Jennifer Owens, Jennifer Demieville and Heather Francis. *to be submitted to Laboratory Investigation, February 2016*

Presentations related to the PSC Partners grant:

1. ***L. Hargrove***, L Kennedy, J. Owens, **H. Francis**. Cholestatic-induced biliary proliferation and fibrosis are decreased in mice lacking mast cells: an innovative study using C-kit knockout mice. Hepatology 62:A812, 2015 **Presidential Poster of Distinction, AASLD annual meeting, San Francisco, CA

2. ***H. Jones***, L. Hargrove, L Kennedy, J. Owens, **H. Francis**. Prolonged usage of H1 or H2 histamine receptor antagonists decreases fibrosis and liver damage in Mdr2$^{-/-}$ mice: novel evidence of the therapeutic benefits of anti-histamines. Hepatology 62:A822, 2015 **Presidential Poster of Distinction, AASLD annual meeting, San Francisco, CA

3. ***L. Kennedy***, L. Hargrove, J. Owens, **H. Francis**. Mast cells interact with proliferating cholangiocytes to activate hepatic stellate cells and promote fibrosis via TGF-b1 signaling during cholestatic injury. Hepatology 62:A1363, 2015 **Presidential Poster of Distinction, AASLD annual meeting, San Francisco, CA

4. ***L. Hargrove***, L Kennedy, A. Graf, F. Meng, J. Owens, **H. Francis**. The introduction of mast cells by tail vein injection enhances biliary proliferation and fibrosis in cholestatic HDC$^{-/-}$ mice. Poster, Digestive Disease Week, Washington, DC

5. ***H. Jones***, L. Hargrove, L Kennedy, A. Graf, F. Meng, J. Owens, **H. Francis**. Mast cell-derived histamine induces the progression of fibrosis in the PSC model of Mdr2-/- mice. Poster, Digestive Disease Week, Washington, DC

6. ***L. Hargrove***, L Kennedy, S. DeMorrow, J. Owens, **H. Francis**. Knockout of histidine decarboxylase ($HDC^{-/-}$) gene reduces hepatic fibrosis in cholestatic bile duct ligated mice. Poster and oral presentation, Experimental Biology, Washington, D.C.

Abstracts submitted to DDW 2016

1. ***L. Hargrove***, L Kennedy, J. Demieville, S. DeMorrow, F. Meng, **H. Francis**. Loss of l-histidine decarboxylase (HDC) prevents the damage and fibrotic reaction of cholangiocytes (but not hepatocytes and hepatic stellate cells) during high fat diet feeding

2. ***L. Hargrove***, L Kennedy, J. Demieville, S. DeMorrow, F. Meng, **H. Francis**. UDCA treatment reverses biliary proliferation and hepatic fibrosis in Mdr2-/- mice and human PSC by decreasing mast cell infiltration and histamine release