

**Final progress report
Esperance Schaefer
NIDDK 1F32DK97855-1
Characterization of microRNA-lipid-HCV interactions
12/1/2012 – 11/30/2012**

PROGRESS TOWARDS STATED AIMS AND RESULTS

Aim 1: Determine the mechanisms by which apolipoprotein B100 supports the hepatitis C viral lifecycle

During the conduct of this grant, we have elucidated the primary mechanism by which apoB is required for HCV. Using the *APOB* $-/-$ Huh 7/CD81 high hepatoma cell line, we examined, stepwise, the HCV lifecycle to determine at which steps HCV required the presence of intracellular apoB100. In the conduct of these experiments, I clearly delineated the following:

- Intracellular levels of HCV are decreased as early as 2 hours following infection in the $-/-$ cells compared to $+/+$
- However, ApoB100 is not required for HCV entry. And, in fact, HCV entry is enhanced in the *APOB* knockout cells. This discordance between facilitated entry and diminished intracellular levels at an early time point is likely due to the enhanced expression of LDL-R in the $-/-$ cells (LDLR-mediated uptake has been described to progress along a non-productive pathway), which was demonstrated both by flow cytometry and immunofluorescence.
- Replication is not impaired in the $-/-$ cells. These data were also confirmed in a replicon model of HCV with siRNA mediated knockdown of apoB
- Production of HCV virion is diminished, as evidence by a significant decrease in the amount of HCV RNA released into the supernatant.
- Further, once the amount of HCV RNA was controlled for, the *APOB* $-/-$ produced virion had significantly decreased infectivity when compared to $+/+$ generated virion
- Mass-spectrometry demonstrated that the virion produced lack, entirely, cholesterol esters, and this defect may largely account for the impaired infectivity

Pending studies include the *in vitro* testing of a small molecule antagonist of apoB100 against HCV. These data have been presented at AASLD in 11/2012 (oral presentation, abstract #69) and 2013 (poster presentation, abstract #1487, awarded presidential poster of distinction), and are planned to be submitted for publication by the end of 2013.

AIM 2. Characterize the mechanisms by which microRNA-122 regulates expression of apolipoprotein B 100 and other intracellular lipids

We determined that mir-122 expression is required to prevent the proteasomal degradation of apoB100. When the proteasome is pharmacologically inhibited by MG132, levels of apoB100 are partially restored in the setting of miR122 blockade. We further investigated mRNA and protein levels of potential regulators of apoB100 post-translational stabilization and determined that:

- MiR-122 blockade significantly lowers MTTP mRNA expression
- There is concomitant decrease of MTTP expression at the protein level

- Expression levels of two other “chaperone” proteins of apoB100, GRP94 and HSP70 were *increased* in the setting of miR-122 antagonism

From the above data, we concluded that miR122 blockade decreases MTTP expression, which results in failed lipidation of apoB100 and its targeting for proteasomal degradation. Important pending studies include studies rescuing apoB100 expression by restoring MTTP protein expression after miR-122-related inhibition. The relationship between miR122 and MTTP remains likely indirect and this would require further characterization. These data are to be submitted for presentation at AASLD’s Henry M. and Lillian Stratton Basic Research Single Topic Conference: Non-coding RNAs in Liver Function and Dysfunction, being held in Miami, March 2014.