Project Title: A novel genetic reprogramming therapy for hepatocellular carcinoma using focused ultrasound-guided delivery of microRNA

Principal Investigator: Juergen K. Willmann, MD

6 month Progress Report: January 4th 2016

Project Background and Goals:
Hepatocellular carcinoma (HCC) continues to be one of the most dangerous forms of cancer characterized by late detection, poor prognosis and very high mortality rate. This is primarily because current treatment options for HCC patients are limited and their benefits are often overweighed by their associated side effects. Although chemotherapy can provide some initial respite in advanced stage HCC, eventually, drug resistant forms of the disease develop that can potentially be more invasive than the non-resistant forms. Hence, new strategies to treat advanced stage HCC that can overcome the shortcomings of currently available therapeutic options are critically needed. Towards that end, our preliminary studies have identified microRNA-122 and antimiR-21 as two highly potent oligonucleotides that, when co-delivered in HCC cells, can significantly decrease cell proliferation in non-resistant cells and re-sensitize drug resistant forms to drug treatment. The overarching goal of this project is to strategically optimize an ultrasound-guided delivery protocol that can be used to deliver significant amounts of biocompatible PLGA nanoparticles loaded with miRNA/antimiR's into tumor cells *in vivo* to induce strong therapeutic effects. Specifically, in the first six months of this project our goal was to develop an optimized ultrasound protocol that can then be used to deliver significant amounts of the miRNA-loaded PLGA nanoparticles homogenously throughout the tumor parenchyma in animal models of HCC.

Progress:
Since the liver is well accessible for ultrasound imaging in patients, ultrasound-guided delivery of miRNA-loaded PLGA nanoparticles is a promising new and clinically translatable therapeutic strategy for treating HCC in patients. Building on our previous optimization studies on ultrasound parameters for drug delivery in cancer, in the last 6 months we have undertaken further refinement of our ultrasound-guided delivery protocol to substantially enhance intra-tumoral delivery of miRNA-loaded PLGA-NP *in vivo* compared to passive delivery. Using the refined protocol fluorescent nanoparticles could be pushed greater than 200 µm into the tumor stroma within the extravascular compartment (**Figure 1**). Such high penetration depth has not been reported in any study till date. Based on these results we hypothesized that therapeutic efficacy of

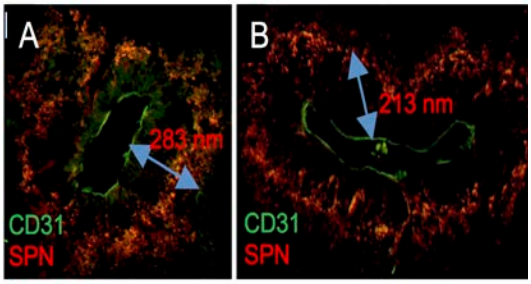

Figure 1. Our optimized ultrasound parameters resulted in greater than 200 nm penetration depth of fluorescent nanoparticles (SPN) into tumor stroma as measured from blood vessels (CD 31-stained).

the miRNA/antimiR genetic reprogramming therapy can be further improved using our refined ultrasound parameters. To that end, we have further extended our studies to assess the longitudinal treatment effects of our therapeutic strategy on HCC drug resistance and proliferation using optimized ultrasound parameters developed in the last 6 months (**Figure 2**). Our initial results show that combined miRNA-122 and antimiR-21 delivery into HCC using our optimized ultrasound protocol can result in significant decrease in HCC tumor proliferation compared to control tumors treated without ultrasound. As an alternative genetic manipulation approach, we have also established the feasibility of using PLGA nanoparticles containing co-encapsulated gemcitabine and antimiR-21 for the therapy of HCC. Gemcitabine and antimiR-21 co-encapsulated nanoparticles increased treatment efficacy in HCC, compared to cells treated with either antimiR-21 or gemcitabine-loaded nanoparticles alone at equal concentration, indicating that down-regulation of endogenous miRNA-21 function can reduce HCC cell viability and proliferation in response to gemcitabine treatment.

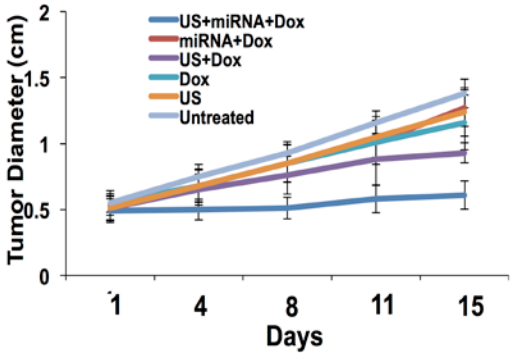

Figure 2. Our optimized ultrasound parameters combined with novel genetic manipulation therapy resulted in greater inhibition of HCC proliferation compared to controls treated without ultrasound only.

Future Plans:

In the next 6 months, we will continue optimizing our ultrasound-guided drug delivery platform to successfully scale up the localized and spatially confined delivery of miRNA loaded PLGA nanoparticles in to HCC tumors in large animals (rabbits). In the last 6 months, we have gained significant experience in ultrasound guided cancer treatment of HCC in murine models and have been successful in substantially decreasing HCC proliferation by delivery of significant amounts of PLGA nanoparticles loaded with miRNA's into the tumor parenchyma. Furthermore, we have shown feasibility of a new model of human HCC (using HepG2 cells) in rabbits; in the next 6 months we will further optimize this model. We also plan to expand our efforts of testing our optimized ultrasound-guided drug delivery protocol to explore the efficacy of delivering complementary miRNA's into human HCC tumors in this rabbit model.

Presentations:

Mullick Chowdhury S, Wang TY, Bachawal S, Devulapally R, Paulmurugan R, Willmann JK. Ultrasound Guided Therapeutic Modulation of Hepatocellular Carcinoma Using Complementary MicroRNAs. Radiological Society of North America (RSNA), Chicago, Illinois, November 28- December 2, 2016