A novel mouse model of primary sclerosing cholangitis associated cholangiocarcinoma and TGFβ signaling involved in tumorigenesis

Pinzhu Huang¹, Guangyan Wei¹, Shuangshuang Zhao¹, Disha Badlani¹, Kahini Vaid¹, Li Chen², Mathieu Petitjean², Xin Chen³, Gregory Gores⁴, Yury Popov¹

¹Division of Gastroenterology and Hepatology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, ²PharmaNest, Inc, NJ, ³University of California, san Francisco, CA and ⁴Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN

Background and Aims: Cholangiocarcinoma (CCA) is a dreaded complication of primary sclerosing cholangitis (PSC), difficult to diagnose and associated with high mortality. Currently, lack of animal models of CCA recapitulating the hepatic microenvironment of progressive sclerosing cholangitis precludes tumor treatment. Thus, we sought to develop a PSC associated CCA animal model and test the therapeutic value of TGFβ signalling via targeting ALK5.

Method: Ten weeks old Mdr2-/- mice with congenital PSC-like progressive biliary disease, and healthy wild-type littermates (WT) mice were subjected to either modified retrograde biliary instillation (Yamada, et al. Hepatology 2015, without concomitant IL-33 or bile duct ligation) or hydrodynamic tail vein injection (Zhang, et al. J Hepatol 2017) of sleeping beauty transposon-transposase plasmid system with activated forms of AKT (myr-AKT) and Yap (YapS127A) protooncogenes (SB AKT/YAP1). ALK5 inhibitor (SB-525334, 300 mg/kg in diet) or placebo diet was administered into tumor-bearing mice in 1-4 weeks post injection to interrogate functional role of TGFβ signalling in our model. Tumor phenotype and burden were analysed using histological methods. Desmoplastic stroma of the tumors was quantified using automated FibroNest platform (PharmaNest Inc) from Sirius Red (SR) staining.

Results: While SB AKT/YAP1 plasmids via retrograde biliary injection caused tumors in all Mdr2-/- but not in healthy wildtype mice (n = 10), only 26.67% (4/15) of these tumors were CCA and this approach was deemed unsuccessful. Alternative, hydrodynamic tail vein injection of SB AKT/YAP1 resulted in robust tumorigenesis in fibrotic Mdr2-/- mice (n = 10), with 100% incidence and high CCA burden after 6 weeks. In contrast, only 6 out of 9 healthy wildtype mice (66.67% incidence) developed tumors. Higher CCA numbers (52.60 ± 6.81 vs. 1.11 ± 0.51, p < 0.01) with profound desmoplastic reaction and significantly shortened survival were observed in Mdr2-/- mice compared to non-fibrotic controls.

Other than the cholangiocyte markers CK19 and pan-CK, the expression of p-smad2, TGFβ1 and integrin β8 were also found to be increased in the tumors. Pharmacological TGFβ inhibition via Alk5 reduced tumor burden by 2.4 fold (11.00 ± 1.41 vs. 24.80 ± 5.75, n=5, p = 0.0481) and desmoplastic stroma of the tumors by 2 fold (tumor collagen area %, 7.36 ± 0.57 vs. 13.96 ± 1.92, n = 5, p = 0.01), respectively, compared to placebo.

Conclusion: We established a new high-fidelity cholangiocarcinoma model in mouse, termed SB CCA.Mdr2-/. It recapitulates the increased susceptibility to CCA in the setting of progressive biliary injury and fibrosis observed in PSC, and enables mechanistic research and formal testing of new therapies for this devastating disease. Furthermore, pharmacological targeting of Alk5 in our model suggests that TGFβ signalling functionally drives CCA tumorigenesis and promotes desmoplastic reaction.

Abstract accepted for Poster Presentation at AASLD “the Liver Meeting” 2021. November 12-15 2021