New mouse model of cholangiocarcinoma arising in the setting of progressive biliary injury and fibrosis

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BACKGROUND & AIMS: Cholangiocarcinoma (CCA) is a dreaded complication of primary sclerosing cholangitis (PSC), difficult to diagnose and associated with high mortality. Lack of high-fidelity animal models of CCA that recapitulate the hepatic microenvironment of progressive sclerosing cholangitis precluded basic studies into the underlying mechanisms and development of effective treatment. Here, we report the establishment and characterization of a mouse model of PSC-associated CCA.

METHODS: 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-transposable plasmid system with activated forms of AKT (myr-AKT) and Yap (YapS127A) protooncogenes (SB AKT/YAP1). ALK5 inhibitor (SB-525334, 200 mg/kg in diet) or placebo diet was administered into tumor-bearing mice starting from 1 week post-oncogene transduction to interrogate functional role of TGFβ signaling in our model. Tumor phenotype and burden were analyzed using histological methods. Desmoplasmic stroma of the tumors was characterized and quantified using automatic FibroNest platform (PharmaNest Inc) from Picosiris Red (PSR) 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 (Figure 1A-B). Alternative, hydrodynamic tail vein injection of SB AKT/YAP1 resulted in robust tumorigenesis in fibrotic female 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. 11.03±1.97, p<0.01) with significantly shortened survival were observed in Mdr2-/− mice compared to non-fibrotic controls (Figure 1C-E). Similar to female mice, male Mdr2-/− mice also presented significantly higher tumor burden than WT mice (Figure 1F-H). CCA in Mdr2-/− mice exhibited desmoplasmic reaction and were positive for K67, CK19, Sox9, α-SMA and TGFβ1, but negative for HNF4α and glatitude synthetase, and weak for CD31. Magnification, ×200. Scale bar: 50 μm. (Figure 2). Early pharmacological TGFβ inhibition via ALK5 reduced tumor burden by 2.4 fold (11.03±1.91 vs. 24.80±6.75, n=5, p=0.0481) and desmoplasmic stroma indicated by ensemble collagen area, collagen fiber density of the tumors compared to placebo (Figure 3).

CONCLUSIONS: 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 AK5 in our model suggests that TGFb signaling functionally drives CCA tumorogenesis and promotes desmoplasmic reaction in a complex, stage-specific manner.

Figure 1. Tumorigenesis in wildtype and Mdr2-/− mice with hydrodynamic tail vein injection of AKT/YAP1 SB transposon-transposable. All tumors were diagnosed as cholangiocarcinomas (CCA) characterized by atypical oval forming tubular structures (H&E) with pronounced desmoplasmic reaction (Picosiris Red ). Tumors are uniformly positive for K67, pCK CK19, Sox9, α-SMA, TGFβ1, negative for HNF4α and glatitude synthetase, weak for CD31. ITGB8 is variably expressed in tumor. Magnification, ×200. Scale bar: 50 μm.

Figure 2. Phenotypic characterization of the tumors arising in mice with hydrodynamic tail vein injection of AKT/YAP1: SB transposon-transposable. All tumors were diagnosed as cholangiocarcinomas (CCA) characterized by atypical oval forming tubular structures (H&E) with pronounced desmoplasmic reaction (Picosiris Red ). Tumors are uniformly positive for K67, pCK CK19, Sox9, α-SMA, TGFβ1, negative for HNF4α and glatitude synthetase, weak for CD31. ITGB8 is variably expressed in tumor. Magnification, ×200. Scale bar: 50 μm.

Figure 3. The effect of ALK5 inhibition on tumor progression of SB CCA.Mdr2-/− model at 4 weeks post injection. (A) Representative CK19 staining images of livers (Magnification ×20, scale bar: 500 μm) Arrow indicates viable tumor nodule. (B) Relative liver weight and (C) CK19- tumor number. (D) Representative images of desmoplasmic tumor tissue (Picosiris red, magnification ×20), scale bar: 500 μm (left panel). Color map (middle panel), with higher colors showing higher collagen density and fibrosis phenotype (right panel) (Magnification ×50, scale bar: 200 μm). (E) Histologically collagen architecture was analyzed for collagen fiber density, assembled and fine collagen area (unpaired two-tailed t test).