Title: Targeted β-catenin Overexpression Mediates Biliary Repair in Murine Model of Intrahepatic Cholestasis

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Abstract:

Introduction: Primary sclerosing cholangitis (PSC) is a chronic progressive cholestatic liver disease with no approved therapies. We and many others have reported the role of β-catenin, a transcriptional coactivator in liver physiology and reprogramming. Hepatocytes (HC) transgenic for Ser-45 mutant β-catenin (TG) show increased expression of biliary markers after short-term exposure to porphyrinogenic biliary toxin 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC), which models some of the phenotypic findings of PSC. However, whether these biliary cell-like hepatocytes fully convert into cholangiocytes (CC) or maintain an intermediate phenotype, remains unclear. Here, we aimed to investigate the role of hepatocyte-specific β-catenin overexpression in mediating hepatocyte-to-biliary cell reprogramming that might help improve intrahepatic cholestasis. **Methods:** Age matched WT control (Con) and TG mice, both containing ROSA26-stop$^{flox/flox}$, were injected with AAV8-TBG-Cre to permanently label hepatocytes with EYFP and then fed 0.1% DDC diet for different time points - 30d, 60d, 90d, 120d, 150d. Before sacrifice, bile flow was measured for 1h to obtain the flow rate. Liver histology and serum biochemistry were analyzed for fibrosis and parameters of injury. Porphyrin measurement was assessed in the liver tissues. Real-time PCR and immunofluorescence staining were performed to determine the expression of bile transporter genes and biliary markers respectively. **Results:** TG mice showed significantly improved bile flow rate as compared to the Con mice after 150d of DDC diet. Liver histology and porphyrin measurement showed significantly less porphyrin accumulation in TG as compared to Con. This was concomitant with decreased hepatic bile load in TG mice than the Con. Analysis of bile homeostasis and transport genes demonstrated comparable levels of expression in TG and Con mice. This is indicative of a plausible mechanism independent of bile metabolizing genes that contributes to the increased bile flow rate observed in TG mice. HC-derived duct-like structures positive for both EYFP and Sox9 were observed more frequently in TG as compared to Con. We also observed increased expression of hypophosphorylated active β-catenin & phosphorylated Tyr654 β-catenin in TG than the Con. Furthermore, increased number of A6-positive HCs in TG as compared to the Con was observed at 120d of DDC diet administration, indicating intermediate HC phenotype undergoing reprogramming to biliary cell type. **Conclusions:** Mice with β-catenin

overexpressing HCs showed significantly improved bile flow rate, reduced porphyrin and decreased hepatic bile load in response to the biliary toxin DDC. Using HC fate-tracing, we observed increased biliary cell markers in TG hepatocytes suggestive of HC-to-biliary cell reprogramming potentially contributing to the enhanced bile flow.