Title: β-catenin Conditional Knockout Elicits Protection Against Lithocholic Acid Induced Cholestatic Injury in Murine Model

Chhavi Goel[1], Rong Zhang[1], Pamela Cornuet[1], Xiaochao Ma[2], Kari Nejak-Bowen[1,3]

[1]Department of Pathology, University of Pittsburgh, School of Medicine, Pittsburgh, Pennsylvania

[2]Center for Pharmacogenetics, Department of Pharmaceutical Sciences, University of Pittsburgh, School of Pharmacy, Pittsburgh, Pennsylvania

[3]Pittsburgh Liver Research Center, University of Pittsburgh, School of Medicine, Pittsburgh, Pennsylvania

Abstract

Introduction: Lithocholic acid (LCA) is a secondary hydrophobic bile acid (BA) with the potential to cause cholestatic liver disease. Our lab recently reported that liver-specific conditional loss or inhibition of β-catenin elicits protection and prevents the development of cholestatic liver injury after bile duct ligation (BDL). BA accumulation is the causal factor for both BDL and LCA-induced cholestatic injury. Therefore, we hypothesized that β-catenin conditional knockout would provide protection from LCA-induced injury as well. **Methods:** Age-matched wild-type control (Con) and β-catenin liver-specific knockout (KO) mice were fed 0.6% LCA diet or normal diet for 7 days and then euthanized. Liver histology and serum biochemistry were analyzed for parameters of cholestatic injury. Immunostaining was performed to analyze ductular reaction and immune response. Relative gene expression was assessed for regulatory bile detoxifying enzymes and transporters. **Results:** The KO mice had fewer and smaller necrotic areas as compared to the Con mice after LCA diet administration. Serum biochemical levels showed a significant decrease in biliary injury in the KO mice. Analyzing the BA homeostasis and transport genes revealed that KO mice had decreased BA uptake transporters, increased apical and basolateral efflux transporters, and increased expression of detoxifying cytochrome P450 enzymes. Surprisingly, the total BA levels in liver and serum were comparable between KO and Con mice. Interestingly, immunostaining for pan-cytokeratin showed increased ductular response in KO mice, which could be a defense mechanism for facilitating enhanced BA clearance. We also found a significant increase in the number of CD45-positive cells in the KO mice after LCA diet, indicative of an increased immune response. **Conclusions:** β-catenin conditional knockout improves the overall outcome and confers protection from LCA-induced cholestatic injury despite insignificant alterations in BA accumulation. Instead, this protection may be accounted to increased number of ducts in the KO mice that expand the bile flow and help alleviate the load of bile accumulation. Alternatively, the increased number of lymphocyte population in KO mice may help clear the necrotic areas.