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Bile Acids Activate YAP to Promote Liver Carcinogenesis

Sayeepriyadarshini Anakk,[1,5,*] Manoj Bhosale,[5] Valentina A. Schmidt,[4] Randy L. Johnson,[3] Milton J. Finegold,[2] and David D. Moore[1,*]
[1]Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX 77030, USA
[2]Department of Pathology, Baylor College of Medicine, Houston, TX 77030, USA
[3]Department of Biochemistry and Molecular Biology, MD Anderson Cancer Center, Houston, TX 77030, USA
[4]Department of Medicine, Stony Brook University, Stony Brook, NY 11794, USA
[5]Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign, IL 61801, USA
*Correspondence: anakk@illinois.edu (S.A.), moore@bcm.edu (D.D.M.)
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SUMMARY

Elevated bile acid levels increase hepatocellular carcinoma by unknown mechanisms. Here, we show that mice with a severe defect in bile acid homeostasis due to the loss of the nuclear receptors FXR and SHP have enlarged livers, progenitor cell proliferation, and Yes-associated protein (YAP) activation and develop spontaneous liver tumorigenesis. This phenotype mirrors mice with loss of *hippo* kinases or overexpression of their downstream target, YAP. Bile acids act as upstream regulators of YAP via a pathway dependent on the induction of the scaffold protein IQGAP1. Patients with diverse biliary dysfunctions exhibit enhanced IQGAP1 and nuclear YAP expression. Our findings reveal an unexpected mechanism for bile acid regulation of liver growth and tumorigenesis via the *Hippo* pathway.

INTRODUCTION

Hepatocellular carcinoma (HCC) is a leading cause of cancer mortality, with poor prognosis and very few effective chemotherapy options. Several lines of evidence indicate that bile acids (BAs) are promoters of hepatocarcinogenesis (Kitazawa et al., 1990; Tsuda et al., 1988; Yang et al., 2007). BAs are produced in the liver to facilitate the absorption of lipids and lipid-soluble nutrients from the intestine (Hofmann, 1999; Russell, 2003). BAs also function as signaling molecules and play important roles in liver regeneration (Huang et al., 2006) as well as tumor promotion (Kim et al., 2007). As detergents, they are potentially cytotoxic, and their concentrations are tightly regulated at several levels, including a negative feedback loop involving the nuclear receptors farnesoid X receptor (FXR) and small heterodimer partner (SHP) (Goodwin et al., 2000). Targeted deletion of both of these receptors, but not either individually, leads to marked elevation in hepatic BA levels and liver injury (Anakk et al., 2011). The development of spontaneous liver tumors in our $Fxr^{-/-}$ $Shp^{-/-}$ double-knockout (DKO) mouse model in which mice have chronically elevated BA levels enabled us to study the mechanisms that underlie BA-dependent tumor promotion.

The mammalian *Hippo* pathway includes the serine/threonine kinase Mst1/2, which phosphorylates and activates the downstream kinase Lats1/2, and their regulators, Mob1A/B and Sav1. Lats1/2 phosphorylation of the transcriptional coactivators Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ) causes them to be retained in the cytoplasm, inhibiting their ability to drive proliferation. Hippo signaling is critical in regulating liver size (Cai et al., 2010; Camargo et al., 2007; Dong et al., 2007; Lee et al., 2010; Song et al., 2010) and intestinal regeneration (Cai et al., 2010; Karpowicz et al., 2010; Staley and Irvine, 2010). Notably, downregulation of Mst1/2 or overexpression of YAP in mouse liver results in hepatocellular carcinoma (HCC) (Cai et al., 2010; Dong et al., 2007). While the core components of this pathway are well defined, its upstream regulators are still being sought after. Cell-cell contact suppresses the pathway via factors such as atypical cadherins (Hamaratoglu et al., 2006), α-catenin (Schlegelmilch et al., 2011), and the apical adaptor proteins *Kibra-Expanded* and *Merlin* (Cai et al., 2010; Genevet et al., 2010; Grusche et al., 2010; Hamaratoglu et al., 2006). Decreased cell density or increased extracellular matrix stiffness was shown to increase nuclear localization of YAP and TAZ (Dupont et al., 2011; Schlegelmilch et al., 2011). Recently, G protein coupled receptors have been suggested as upstream regulators of the Hippo pathway in mammalian cells (Mo et al., 2012; Yu et al., 2012).

The increased liver size, hepatocyte proliferation, and subsequent development of spontaneous HCC in DKO mice strongly resembled the phenotype of mammalian Hippo pathway Mst1/2 liver-specific knockouts. (Anakk et al., 2011; Lee et al., 2010; Lu et al., 2010; Song et al., 2010). Consistent with this overlap, we found that elevated BA levels are sufficient to activate YAP in both livers and isolated hepatocytes, and we identified induction of the scaffolding protein IQGAP1 as a key intermediate in this process.

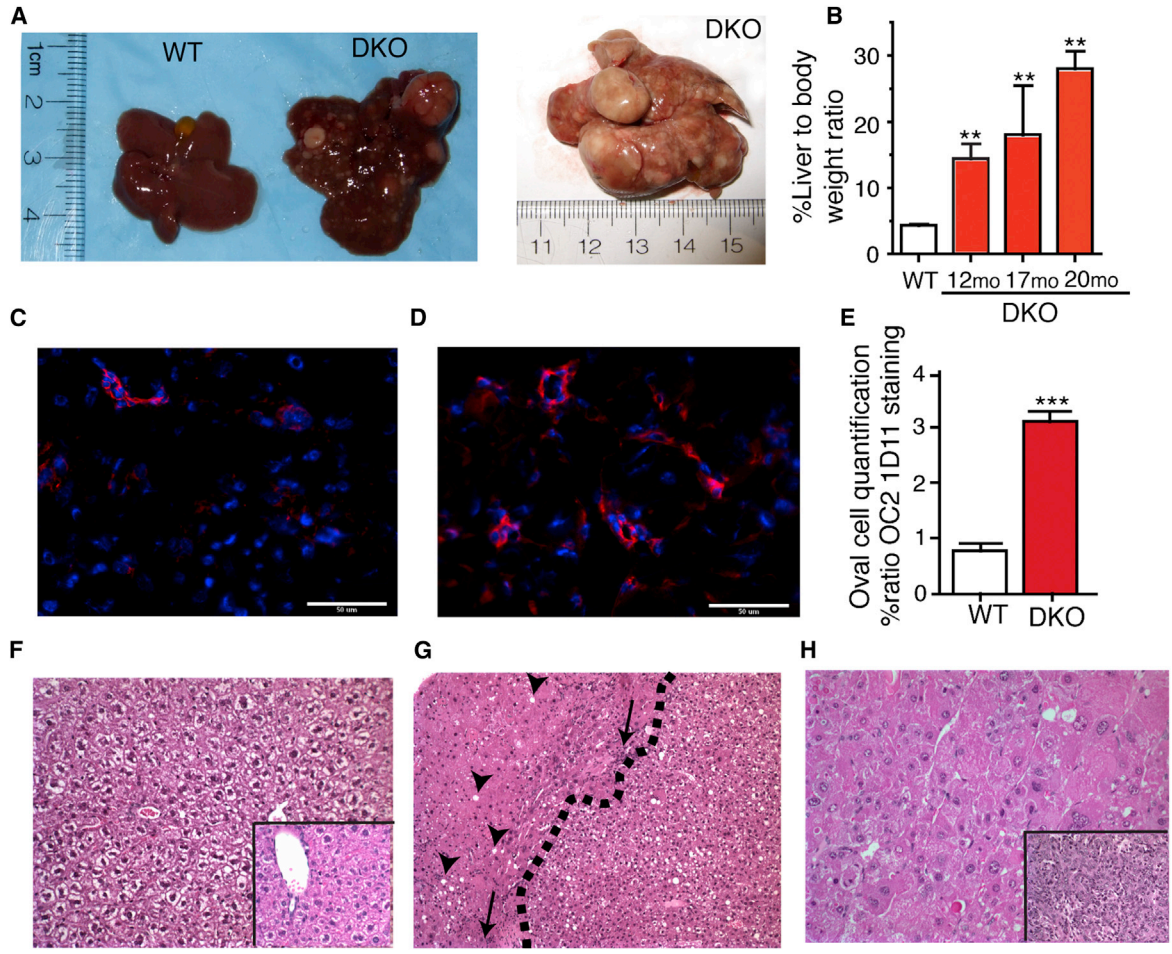

Figure 1. *Fxr*$^{-/-}$*Shp*$^{-/-}$ Mice Develop Spontaneous Hepatocellular Carcinoma and Phenocopy Mst1$^{-/-}$/Mst2$^{-/-}$ Mice

(A) Gross liver examination shows the presence of multiple tumor nodes with 100% penetrance compared to tumor-free WT mice (n = 20).

(B) Progressive tumor growth is evident from increasing liver to body weight ratio of DKO mice with age.

(C–E) Hepatic oval cell marker, OC21D11 (red), shows increased staining in 8- to 10-week-old DKO (D) compared to WT (C) mice and its quantification is shown in (E). DAPI (blue) stains the nuclei (n = 4–6).

(F–H) Hematoxylin and eosin (H&E) staining of a year-old normal liver WT (F) and a DKO liver (G and H). DKO mice develop adenomas, whose growth rate exceeds the adjacent host liver (G), and HCC, with marked nuclear atypia and mitotic activity (H); the inset shows cholangiofibrosis. Arrowheads show fat accumulation, arrows point to focal inflammation, and the dotted line demarcates the adenoma boundary from the injured liver tissue. Magnification is 120×, and insets are 250×.

*p < 0.05, **p < 0.001 compared to WT.

RESULTS

Fxr$^{-/-}$*Shp*$^{-/-}$ Mice Phenocopy Mst1/Mst2 Liver-Specific Knockouts

Combined loss of FXR and SHP results in early-onset cholestasis (Anakk et al., 2011). In accord with the tumor-promoting effects of BAs, we observed spontaneous hepatic tumorigenesis in year-old DKO mice, which are maintained on the tumor-resistant C57/B6 mouse background (Figure 1A). DKO mice develop tumor nodules as early as 9 months, and by 12 months we can see well-developed adenomas that spread through the entire liver between 15 and 17 months of age (Figure 1B). Additionally, OC2-1D11, a marker for a hepatic progenitor/oval cell population, is dramatically increased in DKO mice compared to wild-type (WT) mice as early as 8–10 weeks of age (Figures 1C–1E). Histological analysis of DKO tumors revealed heterogeneous hepatocyte populations with injury, cholangiofibrosis, and multiple adenomas that can progress to hepatocellular carcinoma (Figures 1F–1H) consistent with what is observed in HCC patients (Llovet et al., 2003).

These results revealed a striking overlap of DKO and Mst1/2 knockout phenotypes, including an increase in the hepatic progenitor/oval cell population and aggressive liver tumors (Lu et al., 2010; Song et al., 2010). This overlap was reinforced by the observation that Mst1/2 knockout mice also accumulated hepatic BAs (Figures S1A–S1C).