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The gut–liver axis in hepatocarcinoma: a focus on the nuclear receptor FXR and the enterokine FGF19

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Elevated bile acid (BA) concentrations in the liver is associated with severe disease, including cholestasis and hepatocellular carcinoma. The nuclear Farnesoid X Receptor (FXR) is the master regulator of BAs homeostasis. In the ileum, BA-dependent FXR activation induces the production of the fibroblast growth factor FGF19, a hormone that reaches the liver through the portal system where it represses the expression of CYP7A1, the rate limiting enzyme in the process of hepatic BAs synthesis. This gut–liver FXR–FGF19 dual action is the paradigm of physiological BA regulation and it is currently targeted in the clinical practice for liver disease such as primary cholangitis. At a variance of FXR activation, native FGF19 has strong anti-cholestatic and anti-fibrotic activity in the liver but it retains peculiar pro-tumorigenic actions. Thus, novel analogues have been generated to avoid tumorigenic capacity while maintaining BA metabolic action. Here we present a novel and intriguing view on the putative possibility to target the FXR–FGF19 duo in order to offer a *bona fide* promising therapeutic approach to bile acid promoted hepatocarcinoma.

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Bile acids and hepatocarcinoma (HCC)

Bile acids (BAs) are amphipatic steroids able to facilitate the digestion of dietary lipids and liposoluble vitamins. They represent the major system for cholesterol excretion in which cholesterol is removed from the body, as they are synthesized from cholesterol in the liver [1]. Altered BA signaling in the liver and intestine is associated with severe disease, including the development of inflammation and cholestasis with susceptibility to hepatocarcinoma (HCC). Indeed, despite their beneficial role in solubilizing lipophilic nutrients such as dietary fat, steroids and vitamins, thereby facilitating their intestinal absorption, high levels of BAs causes inflammation, DNA oxidative damage, cell proliferation and inhibits apoptosis, subsequently promoting neoplastic transformation of hepatocytes [2]. Thus, a tight regulation of BA concentration is essential for both cholesterol homeostasis and hepatic health [3,4].

In the liver, BAs are synthesized via a series of enzymatic reactions, initiated by a microsomal cholesterol 7α-hydroxylase (CYP7A1), which converts cholesterol to 7α-hydroxycholesterol, representing the rate-limiting step in the BA synthesis [5]. Before active secretion of BAs into the canalicular lumen, primary BAs are conjugated with taurine or glycine to form less cytotoxic bile salts, readily secretable into bile [6]. After postprandial stimuli, bile salts are released from the gallbladder into the small intestine and at the distal ileum and 95% of BAs are actively absorbed, returned back to the liver through the portal circulation, thus reducing the energy expenditure for *de novo* BA biosynthesis [7]. In the colon, primary BAs are transformed to secondary BAs (lithocholic acid, LCA and deoxycholic acid, DCA) through action of intestinal bacteria by a de-conjugation process and are then are passively absorbed by enterocytes, returned back to the liver where they are re-conjugated. Approximately 5% of the BA pool per day escape intestinal reabsorption and are excreted into the feces. This loss is accurately compensated by *de novo* synthesis in the liver in order to maintain the pool size which represent a major determinant of cholesterol turnover.

Alterations in bile flow, due to defects in the bile formation process or caused by a physical obstruction in bile ducts are responsible for cholestatic liver disorders. Mutations in the ABCB11 gene causes progressive familial intrahepatic cholestasis (PFIC) type 2. The ABCB11 gene encodes for BSEP, the primary canalicular bile salt export pump which mediates the active transport of BAs into the canalicular lumen, generating bile flow [8]. Defective BA export leads to progressive cholestasis and Abcb11−/− mice, as expected, are characterized by progressive accumulation of hepatic BA, leading to liver injury [9]. The elevated BAs in this murine model

induces changes in the metabolic state by disrupting glycolysis and gluconeogenesis and by alterations in the fatty acid oxidation. As a result, intracellular ROS are increased and causes liver inflammation, necrosis and fibrosis [10].

Defects in ABCB4 gene, encoding the multidrug resistance class III (MDR3) protein, cause the PFIC type 3 [11]. MDR3 is expressed in the canalicular membrane of hepatocytes and is mainly involved in phosphatidylcholine excretion in the bile [12]. ABCB4−/− mice are characterized by the absence of MDR3 protein, resulting in low biliary phospholipid levels that promote bile regurgitation into the portal tracts accompanied by spontaneous development of periportal biliary fibrosis and liver injury [13]. After 2–3 weeks of age, Abcb4−/− mice display inflammation, ductural proliferation and fibrosis, resulting in hepatocyte dysplasia at 4–6 months. These mice develop liver tumors in 16 months [14]. Thus, the regulation of these ABC transporters is crucial in order to avoid BAs overload and consequently liver injury and their concentrations require a tight regulation in order to prevent hepatic disease.

FXR–FGF15/19 in the control of BAs synthesis

The nuclear receptor FXR is the master regulator of BAs homeostasis, modulating their synthesis, absorption and uptake [15]. FXR decreases BA *de novo* hepatic biosynthesis by reducing the expression of CYP7A1. At the canalicular membrane, newly-synthesized BAs are conjugated under regulation of FXR through the activation of the BA-CoA-synthase (BACS) and BA-CoA-amino acid N-acetyltransferase (BAAT) enzymes [16]. Conjugated BAs are then secreted into the gallbladder by the bile salt export pump (BSEP/ABCA11) and the multidrug related protein 2 (MRP2/ABCC2). The expression of these genes is under the control of FXR. After postprandial stimuli, BAs are secreted into the intestine. In the ileum, Apical Sodium-dependent Bile Acid Transporter (ASBT) determines the uptake of BAs, while IBABP is responsible for BAs transport from the apical to the basolateral membrane [17–19]. BAs are secreted in the portal blood by the heterodimeric organic solute transporter α/β (OST α/β). Post secretion, BAs are transported back to the liver, where a great majority is reabsorbed by the sodium (Na)-Taurocholate Cotransporter Protein (NTCP) and organic anion transporting polypeptide (OATP), both negatively regulated by FXR, thereby limiting the increase of hepatic BA levels. Finally, BAs are re-secreted into the bile [7], closing up the BAs enterohepatic circulation. Clearly, FXR represents the sensor of intracellular BA concentration in the hepatocytes, being able to transcriptionally regulate both secretion or excretion and their uptake or emittance. Nevertheless, this control by FXR is not limited to the liver. Indeed, FXR is highly expressed in the gut and have been identified as a key regulator of the gut–liver cross talk.

In the ileum, BA-dependent FXR activation induces the production and secretion of the fibroblast growth factor FGF15 (mouse) and 19 (humans) in the portal circulation. FGF19 is an endocrine hormone that is able to repress CYP7A1 expression in the liver and thus reduces BAs synthesis. FGF19 binds the receptor FGFR4 with the coreceptor βklotho, triggering downstream signaling cascades [20–22]. Studies employing chimera of FGF19 protein have revealed that the C-terminus region is responsible for the binding to β-klotho, whereas the N-terminus appears to be important for FGFR activation [23]. The FGF15/19 mechanism of action seems to involve the extracellular signal-related kinase (ERK)1/2/mitogen-activated protein kinase (MAPK) pathway as a mediator of FGF15/19 inhibitory effect on BA synthesis [23] (Figure 1). However, the molecular mechanisms downstream of the FGF15/19–FGFR4–β-klotho complex are not fully understood.

The relative contribution of hepatic and intestinal FXR in mediating CYP7A1 repression was shown through tissue-specific FXRKO murine models, where a more determinant role of intestinal FXR was revealed [24]. This mechanism represents an important crosstalk between intestine and liver for the regulation of BA synthesis.

The protective role of FXR against HCC

HCC is among the most lethal and prevalent human tumors and to date the therapeutic options are limited [25]. Recent findings have clearly indicated that FXR might be implicated in liver tumorigenesis [3,4,26]. Indeed, FXR has been shown to prevent oxidative damage, inflammation and resistance to apoptosis induced by chronic high accumulation of BAs [27].

The important role of FXR in the control of BA metabolism was initially demonstrated in FXR-null mice which are unable to maintain control of BA synthesis and transport. These mice display a deregulation of the CYP7A1 and IBABP genes upon CA-supplemented diet, with subsequently inactivation of the hepatic canalicular secretion, increases of BA hydrophilicity and urinary and fecal BA loss, leading to enlarged BAs pool size [28]. Furthermore, FXR-null mice exhibit high levels of the pro-inflammatory cytokines IL-1β, β-catenin and c-Myc at 3 months of age. The up-regulation of pro-inflammatory cytokines, resistance to apoptosis and cell hyperproliferation induced by increased BA pool size in the FXR-null mice results in spontaneous HCC development between 12 and 15 months of age [26,29–31].

Interestingly, transgenic mice that constitutively express active FXR in the intestine (iVP16FXR) are protected from chemically-induced and genetically-induced cholestasis. This protective effect is attributed to the induction of intestinal FGF15 and repression of hepatic Cyp7a1