Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Pharmacology

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

Marilidia Piglionica[1,2,4], Marica Cariello[1,4] and Antonio Moschetta[1,3]

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.

Addresses
[1] Department of Interdisciplinary Medicine, 'Aldo Moro' University of Bari, Italy
[2] INBB, National Institute for Biostructures and Biosystems, Rome, Italy
[3] National Cancer Center, IRCCS Istituto Tumori 'Giovanni Paolo II', Bari, Italy

Corresponding author:
Moschetta, Antonio (antonio.moschetta@uniba.it)
[4] These authors equally contributed to the work.

Current Opinion in Pharmacology 2018, **43**:93–98

This review comes from a themed issue on **Endocrine & metabolic diseases**

Edited by **James Bowe** and **Shanta Persaud**

https://doi.org/10.1016/j.coph.2018.08.005

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.

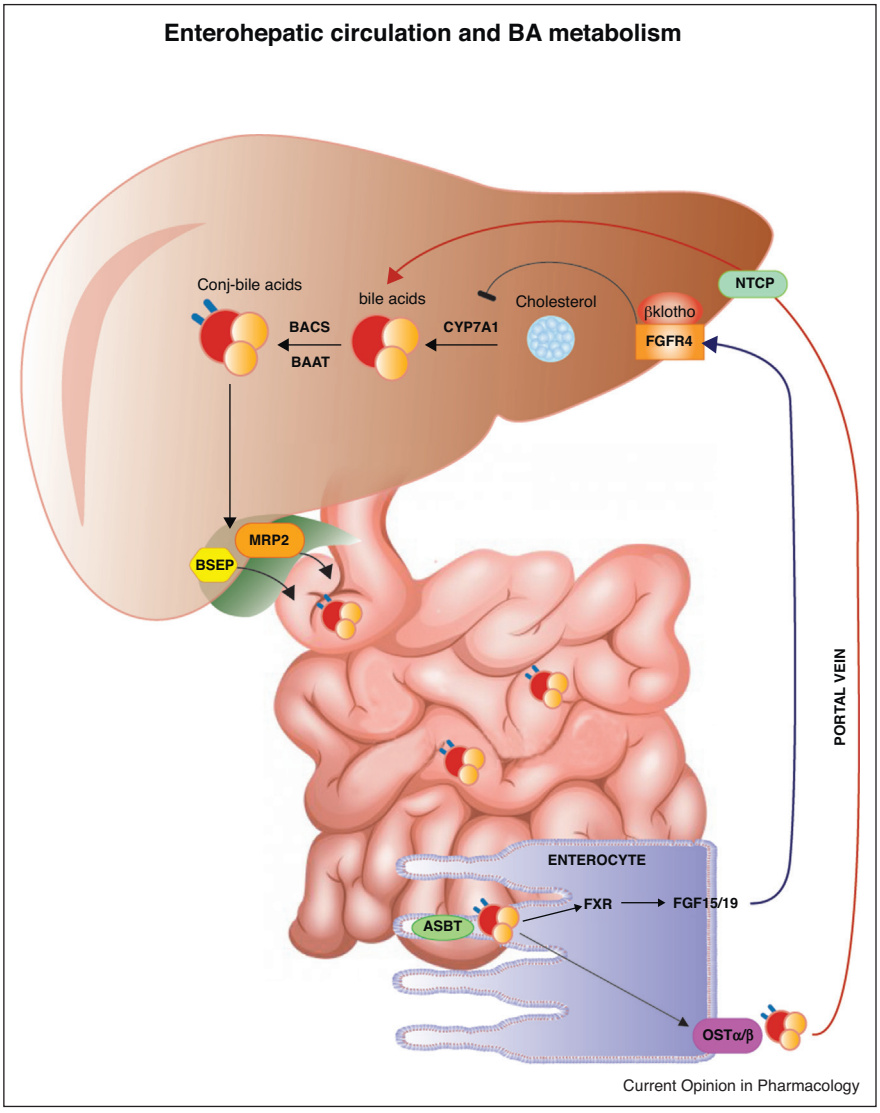
The protective role of FXR against HCC

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

Figure 1



The enterohepatic circulation. At the canalicular membrane, BAs are conjugated and secreted in the gallbladder by BSEP/ABCA11 and MRP2/ABCC2. After postprandial stimuli, BAs are secreted into the intestine and they are actively transported into the enterocytes by ASBT, while IBABP is responsible for BAs movement from the apical to the basolateral membrane. BAs are then secreted in the portal circulation by OST α/β and, in the liver, they are reabsorbed by NTCP and OATP. BA-dependent FXR activation induces the secretion in the portal circulation of the FGF15/19 that reaches the liver and binds the receptor FGFR4 with the co receptor β klotho, repressing CYP7A1 expression and thus reducing BAs synthesis.

expression, downregulation of BA synthesis and upregulation of intestinal BA disposal [32]. De Girolamo *et al.* demonstrated that intestinal FXR activation protects from hepatocarcinogenesis through a tight control of BA synthesis via the Fgf15/Fgfr4 enterohepatic signaling axis. Moreover, iVP16FXR mice displayed a reduction of liver inflammation, hyperproliferation and collagen deposition and the activation of intestinal FXR protected mice from spontaneous HCC formation even in absence of hepatic FXR [33•]. Taken together, these data strongly indicates that intestinal FXR is a negative modulator of hepatic inflammation and cellular proliferation induced by hepatic toxic bile acid accumulation.

In this scenario, semisynthetic and synthetic FXR agonists have been designed to create selective and potent FXR activators that could be beneficial in patients with metabolic disease and HCC. Obeticholic acid (OCA), also known as INT-747, is a selective FXR agonist that was described for the first time in 2002 and is currently used for the treatment of primary biliary cholangitis. The FLINT study demonstrated that long-term OCA

administration ameliorates liver fibrosis, steatosis and lobular inflammation, preventing progression and eventually 'treating' non-alcoholic steatohepatitis (NASH) [34••]. Long term studies are definitively required to define the eventual role of INT-747 in the prevention of HCC in patients at risk and/or as a treatment option of HCC.

Intriguingly, a dual FXR and membrane bile acid receptor TGR5 agonist named INT-767, a novel semi-synthetic 23-sulfate derivative of obeticholic acid, improves the cholestasis phenotype with a reduction of biliary BA output [35]. In db/db mice, INT-767 ameliorates hepatic histological features and decreases the production of pro-inflammatory cytokines [35]. Furthermore, in Abcb4−/− mice FXR activation by long term-administration of INT-767 stimulates Fgf15 at the transcriptional level, thereby repressing hepatic Cyp7a1 expression, triggering a synergistic gut–liver signaling pathway that leads to total serum BAs reduction and HCC prevention [36•]. Thus, INT-767 prevents from spontaneous HCC development via FXR activation by regulating BA synthesis, supporting the eventual therapeutic exploitation of FXR activation in the clinical management of HCC [36•].

The role of FGF19 in HCC development: friend or foe?

The discovery of the role of the enterokine FGF15/19 in the feedback regulation of BA synthesis emphasizes the importance of the crosstalk between the liver and intestine for BA homeostasis [21]. Indeed, FGF15/19 has a key role in the regulation of BA homeostasis through the FXR-mediated Cyp7a1 inhibition [21]. In addition, FGF15/19 is able to reduce glucose synthesis and lowers triglycerides levels, inducing fatty acid oxidation and glycogen and protein synthesis. In mice, the administration of FGF19 protects from diet-induced obesity [37,38].

Elevated FGF19 plasma levels were found in patients with extrahepatic cholestasis, insulin resistance and non-alcoholic fatty liver disease (NAFLD) [39] suggesting that FGF19 modulation might offer a promising therapeutic potential in several metabolic disease. Modica *et al.* demonstrated, that intestinal FXR overexpression induces FGF15 gene expression and protects liver from cholestasis reducing BA pool size in mice [32]. However, this hormone has been also involved in the development of HCC. Indeed, the expression of FGF19 in transgenic mice induces HCC development between 10 and 12 months of age [40]. In HCC patients, FGF19 expression is up-regulated and is correlated with poor prognosis [41]. The FGF19 tumorigenic activity has been attributed to a cross-talk between FGFR4 and β-catenin [42]. Accordingly, it has been shown that in mice with ectopic FGF19 expression, the use of FGFR4-neutralizing antibody reduced tumor growth, opening to novel therapeutic strategies to limit hepatocyte proliferation [42,43]. Recently, it has been demonstrated that hepatocytes-specific deletion of stat3 and genetic or pharmacological ablation of IL6 are able to inhibit FGF19 induced HCC, with unchanged FGF19 functions on BA, glucose and energy metabolism [44•].

To obviate the mitogenic FGF19 activity, several engineered FGF19 variants have been generated aiming to maintain the metabolic activity excluding the protumorigenic ones. One variant, M70, that differs from wild-type FGF19 by 5-amino acids deletion at the N-terminus and the substitution of 3-amino acids [44•,45], fully retains BA regulatory activity but is devoid of pro-tumoral activity in mouse models [45]. In Abcb4−/− mice, M70 reduces hepatic inflammation and biliary fibrosis through CYP7A1 inhibition decreasing BA pool size [46]. Moreover, in a mouse model of NASH, M70 decreases liver injury and ameliorates histological features of NASH, glucose and lipid metabolism representing a promising approach to treat this pathology [46]. A phase II clinical trial has tested the safety and efficacy of NGM282 (M70) for the treatment of NASH. In these patients, the administration of 3 mg or 6 mg of NGM282 was well tolerated and reduced liver fat content as well as liver inflammation and fibrosis [47••]. Further studies are needed to evaluate the impact of M70 or others FGF19 engineered variants in the management of gut–liver axis diseases.

In conclusion, the gut–liver FXR–FGF19 duo represents the paradigm of physiological BA negative feedback regulation of their synthesis. The activation of this pathway could be a putative antifibrotic therapy in NASH. Here we presented a novel and intriguing view on the possibility to target this FXR–FGF19 duo in order to offer a *bona fide* promising therapeutic approach to bile acid promoted hepatocarcinoma. Future studies are needed to prove this hypothesis correct.

Grant support

Moschetta is funded by Italian Association for Cancer Research (AIRC, IG 18987), NR-NET FP7 Marie Curie ITN, Italian Ministry of Health (Young Researchers Grant GR-2010-2314703).

Conflict of interest statement

The authors received in the years 2014-2016 grants for scientific projects from Intercept Pharmaceuticals (San Diego CA) and NGM Biopharma (San Francisco CA).

Acknowledgments

The authors are indebted to Dr R. Le Donne for the artwork.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as

- • of special interest
- •• of outstanding interest

1. Calkin AC, Tontonoz P: **Transcriptional integration of metabolism by the nuclear sterol-activated receptors LXR and FXR**. *Nat Rev Mol Cell Biol* 2012, **13**:213-224.
2. Perez MJ, Briz O: **Bile-acid-induced cell injury and protection**. *World J Gastroenterol* 2009, **15**:1677-1689.
3. Modica S, Gadaleta RM, Moschetta A: **Deciphering the nuclear bile acid receptor FXR paradigm**. *Nucl Recept Signal* 2010, **8**: e005.
4. Modica S, Moschetta A: **Nuclear bile acid receptor FXR as pharmacological target: are we there yet?** *FEBS Lett* 2006, **580**:5492-5499.
5. Myant NB, Mitropoulos KA: **Cholesterol 7 alpha-hydroxylase**. *J Lipid Res* 1977, **18**:135-153.
6. Falany CN, Johnson MR, Barnes S, Diasio RB: **Glycine and taurine conjugation of bile acids by a single enzyme. Molecular cloning and expression of human liver bile acid CoA:amino acid N-acyltransferase**. *J Biol Chem* 1994, **269**:19375-19379.
7. Love MW, Dawson PA: **New insights into bile acid transport**. *Curr Opin Lipidol* 1998, **9**:225-229.
8. Hayashi H, Takada T, Suzuki H, Akita H, Sugiyama Y: **Two common PFIC2 mutations are associated with the impaired membrane trafficking of BSEP/ABCB11**. *Hepatology* 2005, **41**:916-924.
9. Perwaiz S, Forrest D, Mignault D, Tuchweber B, Phillip MJ, Wang R, Ling V, Yousef IM: **Appearance of atypical 3 alpha,6 beta,7 beta,12 alpha-tetrahydroxy-5 beta-cholan-24-oic acid in spgp knockout mice**. *J Lipid Res* 2003, **44**:494-502.
10. Zhang Y, Li F, Patterson AD, Wang Y, Krausz KW, Neale G, Thomas S, Nachagari D, Vogel P, Vore M *et al.*: **Abcb11 deficiency induces cholestasis coupled to impaired beta-fatty acid oxidation in mice**. *J Biol Chem* 2012, **287**:24784-24794.
11. Jacquemin E, De Vree JM, Cresteil D, Sokal EM, Sturm E, Dumont M, Scheffer GL, Paul M, Burdelski M, Bosma PJ *et al.*: **The wide spectrum of multidrug resistance 3 deficiency: from neonatal cholestasis to cirrhosis of adulthood**. *Gastroenterology* 2001, **120**:1448-1458.
12. Morita SY, Kobayashi A, Takanezawa Y, Kioka N, Handa T, Arai H, Matsuo M, Ueda K: **Bile salt-dependent efflux of cellular phospholipids mediated by ATP binding cassette protein B4**. *Hepatology* 2007, **46**:188-199.
13. Fickert P, Fuchsbichler A, Wagner M, Zollner G, Kaser A, Tilg H, Krause R, Lammert F, Langner C, Zatloukal K *et al.*: **Regurgitation of bile acids from leaky bile ducts causes sclerosing cholangitis in Mdr2 (Abcb4) knockout mice**. *Gastroenterology* 2004, **127**:261-274.
14. Mauad TH, van Nieuwkerk CM, Dingemans KP, Smit JJ, Schinkel AH, Notenboom RG, van den Bergh Weerman MA, Verkruisen RP, Groen AK, Oude Elferink RP: **Mice with homozygous disruption of the mdr2 P-glycoprotein gene. A novel animal model for studies of nonsuppurative inflammatory cholangitis and hepatocarcinogenesis**. *Am J Pathol* 1994, **145**:1237-1245.
15. Makishima M, Okamoto AY, Repa JJ, Tu H, Learned RM, Luk A, Hull MV, Lustig KD, Mangelsdorf DJ, Shan B: **Identification of a nuclear receptor for bile acids**. *Science* 1999, **284**:1362-1365.
16. Solaas K, Ulvestad A, Soreide O, Kase BF: **Subcellular organization of bile acid amidation in human liver: a key issue in regulating the biosynthesis of bile salts**. *J Lipid Res* 2000, **41**:1154-1162.
17. Dawson PA, Hubbert M, Haywood J, Craddock AL, Zerangue N, Christian WV, Ballatori N: **The heteromeric organic solute transporter alpha-beta, Ostalpha-Ostbeta, is an ileal basolateral bile acid transporter**. *J Biol Chem* 2005, **280**:6960-6968.
18. Gong YZ, Everett ET, Schwartz DA, Norris JS, Wilson FA: **Molecular cloning, tissue distribution, and expression of a 14-kDa bile acid-binding protein from rat ileal cytosol**. *Proc Natl Acad Sci U S A* 1994, **91**:4741-4745.
19. Meier PJ, Stieger B: **Bile salt transporters**. *Annu Rev Physiol* 2002, **64**:635-661.
20. Cicione C, Degirolamo C, Moschetta A: **Emerging role of fibroblast growth factors 15/19 and 21 as metabolic integrators in the liver**. *Hepatology* 2012, **56**:2404-2411.
21. Degirolamo C, Sabba C, Moschetta A: **Therapeutic potential of the endocrine fibroblast growth factors FGF19, FGF21 and FGF23**. *Nat Rev Drug Discov* 2016, **15**:51-69.
22. Lin BC, Wang M, Blackmore C, Desnoyers LR: **Liver-specific activities of FGF19 require Klotho beta**. *J Biol Chem* 2007, **282**:27277-27284.
23. Wu X, Lemon B, Li X, Gupte J, Weiszmann J, Stevens J, Hawkins N, Shen W, Lindberg R, Chen JL *et al.*: **C-terminal tail of FGF19 determines its specificity toward Klotho co-receptors**. *J Biol Chem* 2008, **283**:33304-33309.
24. Gadaleta RM, Cariello M, Sabba C, Moschetta A: **Tissue-specific actions of FXR in metabolism and cancer**. *Biochim Biophys Acta* 2015, **1851**:30-39.
25. Fattovich G, Stroffolini T, Zagni I, Donato F: **Hepatocellular carcinoma in cirrhosis: incidence and risk factors**. *Gastroenterology* 2004, **127**:S35-S50.
26. Kim I, Morimura K, Shah Y, Yang Q, Ward JM, Gonzalez FJ: **Spontaneous hepatocarcinogenesis in farnesoid X receptor-null mice**. *Carcinogenesis* 2007, **28**:940-946.
27. Verhaag EM, Buist-Homan M, Koehorst M, Groen AK, Moshage H, Faber KN: **Hormesis in cholestatic liver disease; preconditioning with low bile acid concentrations protects against bile acid-induced toxicity**. *PLoS One* 2016, **11**: e0149782.
28. Sinal CJ, Tohkin M, Miyata M, Ward JM, Lambert G, Gonzalez FJ: **Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis**. *Cell* 2000, **102**:731-744.
29. Yang F, Huang X, Yi T, Yen Y, Moore DD, Huang W: **Spontaneous development of liver tumors in the absence of the bile acid receptor farnesoid X receptor**. *Cancer Res* 2007, **67**:863-867.
30. Meng Z, Wang X, Gan Y, Zhang Y, Zhou H, Ness CV, Wu J, Lou G, Yu H, He C, Xu R, Huang W: **Deletion of IFNgamma enhances hepatocarcinogenesis in FXR knockout mice**. *J Hepatol* 2012, **57**:1004-1012.
31. Li G, Kong B, Zhu Y, Zhan L, Williams JA, Tawfik O, Kassel KM, Luyendyk JP, Wang L, Guo GL: **Small heterodimer partner overexpression partially protects against liver tumor development in farnesoid X receptor knockout mice**. *Toxicol Appl Pharmacol* 2013, **272**:299-305.
32. Modica S, Petruzzelli M, Bellafante E, Murzilli S, Salvatore L, Celli N, Di TG, Palasciano G, Moustafa T, Halilbasic E *et al.*: **Selective activation of nuclear bile acid receptor FXR in the intestine protects mice against cholestasis**. *Gastroenterology* 2012, **142**:355-365.
33. • Degirolamo C, Modica S, Vacca M, Di TG, Morgano A, D'Orazio A, Kannisto K, Parini P, Moschetta A: **Prevention of spontaneous hepatocarcinogenesis in farnesoid X receptor-null mice by intestinal-specific farnesoid X receptor reactivation**. *Hepatology* 2015, **61**:161-170.
This study demonostrates that intestinal FXR in mice is sufficient to restore BA homeostasis through the FGF15 axis and prevent progression of liver damage to HCC even in the absence of hepatic FXR.
34. •• Neuschwander-Tetri BA, Loomba R, Sanyal AJ, Lavine JE, Van Natta ML, Abdelmalek MF, Chalasani N, Dasarathy S, Diehl AM, Hameed B *et al.*: **Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic**

steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. *Lancet* 2015, **385**:956-965.
This is the first study providing the efficacy of obeticholic acid in reducing liver fibrosis and histology in adult patients with non-alcoholic steatohepatitis.

35. Baghdasaryan A, Claudel T, Gumhold J, Silbert D, Adorini L, Roda A, Vecchiotti S, Gonzalez FJ, Schoonjans K, Strazzabosco M *et al.*: **Dual farnesoid X receptor/TGR5 agonist INT-767 reduces liver injury in the Mdr2−/− (Abcb4−/−) mouse cholangiopathy model by promoting biliary HCO(−)(3) output**. *Hepatology* 2011, **54**:1303-1312.

36. • Cariello M, Peres C, Zerlotin R, Porru E, Sabba C, Roda A, Moschetta A: **Long-term administration of nuclear bile acid receptor FXR agonist prevents spontaneous hepatocarcinogenesis in Abcb4(−/−) mice**. *Sci Rep* 2017, **7**:11203.
This study demonstrates that long term administration of INT-767 prevents spontaneous hepatocarcinogenesis in Abcb4−/− mice via reduction of hepatic BA synthesis.

37. Fu L, John LM, Adams SH, Yu XX, Tomlinson E, Renz M, Williams PM, Soriano R, Corpuz R, Moffat B *et al.*: **Fibroblast growth factor 19 increases metabolic rate and reverses dietary and leptin-deficient diabetes**. *Endocrinology* 2004, **145**:2594-2603.

38. Benoit B, Meugnier E, Castelli M, Chanon S, Vieille-Marchiset A, Durand C, Bendridi N, Pesenti S, Monternier PA, Durieux AC *et al.*: **Fibroblast growth factor 19 regulates skeletal muscle mass and ameliorates muscle wasting in mice**. *Nat Med* 2017, **23**:990-996.

39. Schaap FG, van der Gaag NA, Gouma DJ, Jansen PL: **High expression of the bile salt-homeostatic hormone fibroblast growth factor 19 in the liver of patients with extrahepatic cholestasis**. *Hepatology* 2009, **49**:1228-1235.

40. Nicholes K, Guillet S, Tomlinson E, Hillan K, Wright B, Frantz GD, Pham TA, Dillard-Telm L, Tsai SP, Stephan JP *et al.*: **A mouse model of hepatocellular carcinoma: ectopic expression of fibroblast growth factor 19 in skeletal muscle of transgenic mice**. *Am J Pathol* 2002, **160**:2295-2307.

41. Miura S, Mitsuhashi N, Shimizu H, Kimura F, Yoshidome H, Otsuka M, Kato A, Shida T, Okamura D, Miyazaki M: **Fibroblast growth factor 19 expression correlates with tumor progression and poorer prognosis of hepatocellular carcinoma**. *BMC Cancer* 2012, **12**:56.

42. Pai R, Dunlap D, Qing J, Mohtashemi I, Hotzel K, French DM: **Inhibition of fibroblast growth factor 19 reduces tumor growth by modulating beta-catenin signaling**. *Cancer Res* 2008, **68**:5086-5095.

43. French DM, Lin BC, Wang M, Adams C, Shek T, Hotzel K, Bolon B, Ferrando R, Blackmore C, Schroeder K *et al.*: **Targeting FGFR4 inhibits hepatocellular carcinoma in preclinical mouse models**. *PLoS One* 2012, **7**:e36713.

44. • Zhou M, Yang H, Learned RM, Tian H, Ling L: **Non-cell-autonomous activation of IL-6/STAT3 signaling mediates FGF19-driven hepatocarcinogenesis**. *Nat Commun* 2017, **8**:15433.
This work reveals the key role for the IL-6/STAT3 axis in driving the native FGF19 induction of HCC in mice.

45. Zhou M, Wang X, Phung V, Lindhout DA, Mondal K, Hsu JY, Yang H, Humphrey M, Ding X, Arora T *et al.*: **Separating tumorigenicity from bile acid regulatory activity for endocrine hormone FGF19**. *Cancer Res* 2014, **74**:3306-3316.

46. Zhou M, Learned RM, Rossi SJ, DePaoli AM, Tian H, Ling L: **Engineered fibroblast growth factor 19 reduces liver injury and resolves sclerosing cholangitis in Mdr2-deficient mice**. *Hepatology* 2016, **63**:914-929.

47. •• Harrison SA, Rinella ME, Abdelmalek MF, Trotter JF, Paredes AH, Arnold HL, Kugelmas M, Bashir MR, Jaros MJ, Ling L *et al.*: **NGM282 for treatment of non-alcoholic steatohepatitis: a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial**. *Lancet* 2018, **391**:1174-1185.
This is the first study assessing the safety and efficacy of NGM282 in patients with non-alcoholic steatohepatitis.