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The FXR-FGF19 Gut–Liver Axis as a Novel "Hepatostat"

See "Fibroblast growth factor signaling controls liver size in mice with humanized livers," by Naugler WE, Tarlow BD, Fedorov LM, et al, on page 728.

The liver possesses an extraordinary regenerative capacity that is triggered upon death of parenchymal cells or after partial liver resection. This fundamental response likely evolved to protect the organ and the organism from endogenous and exogenous toxins and thus preserve systemic metabolic homeostasis.[1] A number of mediators involved in the onset and termination of liver regeneration have been identified over the years, and these where categorized by the late Nelson Fausto into 3 types of interconnected pathways known as the cytokine, growth factor, and metabolic networks.[2] Most of this knowledge has been acquired from partial hepatectomy (PH), hepatocyte transplantation, and liver transplantation experiments performed in animal models.[3] These studies revealed the tremendous proliferative ability of the hepatocyte, with an almost unlimited clonogenic potential. However, they also demonstrated that the regenerative response was proportional to the liver mass removed in the case of PHs, and that the size of the transplanted liver adapts (ie, grows or shrinks) in relation to the size of the recipient body.[3] Such findings indicate the existence of mechanisms that tightly control the onset and termination of adult liver growth, namely, a "hepatostat," or specific sensors that maintain the proper liver size.[1] Given the fundamental role of the liver in systemic metabolism, it was likely that this hepatostat would reside at least in part within the metabolic network. In this context, bile acids (BA) are increasingly recognized as key players in the regulation of liver regeneration and constitute attractive candidates to modulate the hepatostat. Indeed, systemic and intrahepatic BA levels increase shortly after PH both in rodents and humans, and the modulation of BA enterohepatic circulation strongly influences liver regeneration.[4] Early experimental reports also showed that feeding BA-enriched diets elicited hepatocyte proliferation and liver growth.[4,5] In contrast, BA levels need to be finely tuned to avoid their excess and hepatic toxicity. The nuclear receptor farnesoid X receptor (FXR) is a central transcriptional sensor of BA metabolic cascades, as was originally demonstrated in FXR-null mice undergoing PH.[6] FXR is highly expressed in the liver and in the enterocytes.[7] The main FXR target gene in the gut is fibroblast growth factor 15 (FGF15; FGF19 in humans), which is an enterokine secreted into the portal blood upon BA stimulation. FGF15/19 reaches the liver where it activates the duo FGF receptor 4 (FGFR4)/beta KLOTHO on the hepatocyte basolateral membrane triggering intracellular pathways that repress cholesterol 7-α-hydroxylase (CYP7A1), which is the rate limiting enzyme in BA synthesis.[8] The down-regulation of BA synthesis via intestinal FXR/FGF15 activation is known to protect from cholestatic injury even when FXR is ablated in the liver.[9] Indeed, FGF15/19 inhibits hepatocellular CYP7A1 expression in a complex and not completely understood manner. As occurs for FXR, FGF15/19 action also depends on the transcriptional repressor small heterodimer partner (SHP). However, FGF15/19 does not change SHP protein levels or the position of this repressor on the *CYP7A1* promoter, suggesting the involvement of additional factors that interact with the SHP complex.[10] In the liver, FXR seems to promote directly hepatocellular proliferation by the induction of the transcription factor FoxM1b.[6] In contrast, when FGF15 binds to the FGFR4/beta KLOTHO complex, there is a net reduction of BA overload and injury after PH, and a putative contribution to liver regeneration through the up-regulation of FoxM1b, among other proliferative genes, suggesting that FoxM1b can be also activated in an FXR-independent manner.[11,12] Together, these findings point to an important role for the BA–FXR–FGF15 axis in the regulation of liver growth. Interestingly, FXR expression is down-regulated upon acute BA accumulation in the liver,[11] as well as in certain cholestatic conditions,[13] and this response may represent an adaptive mechanism evolved to prevent excessive liver growth. In this issue of *Gastroenterology*, Naugler et al,[14] working in an elegant experimental model of mice with humanized livers, make a strong case for BAs as key players in the regulation of the hepatostat.

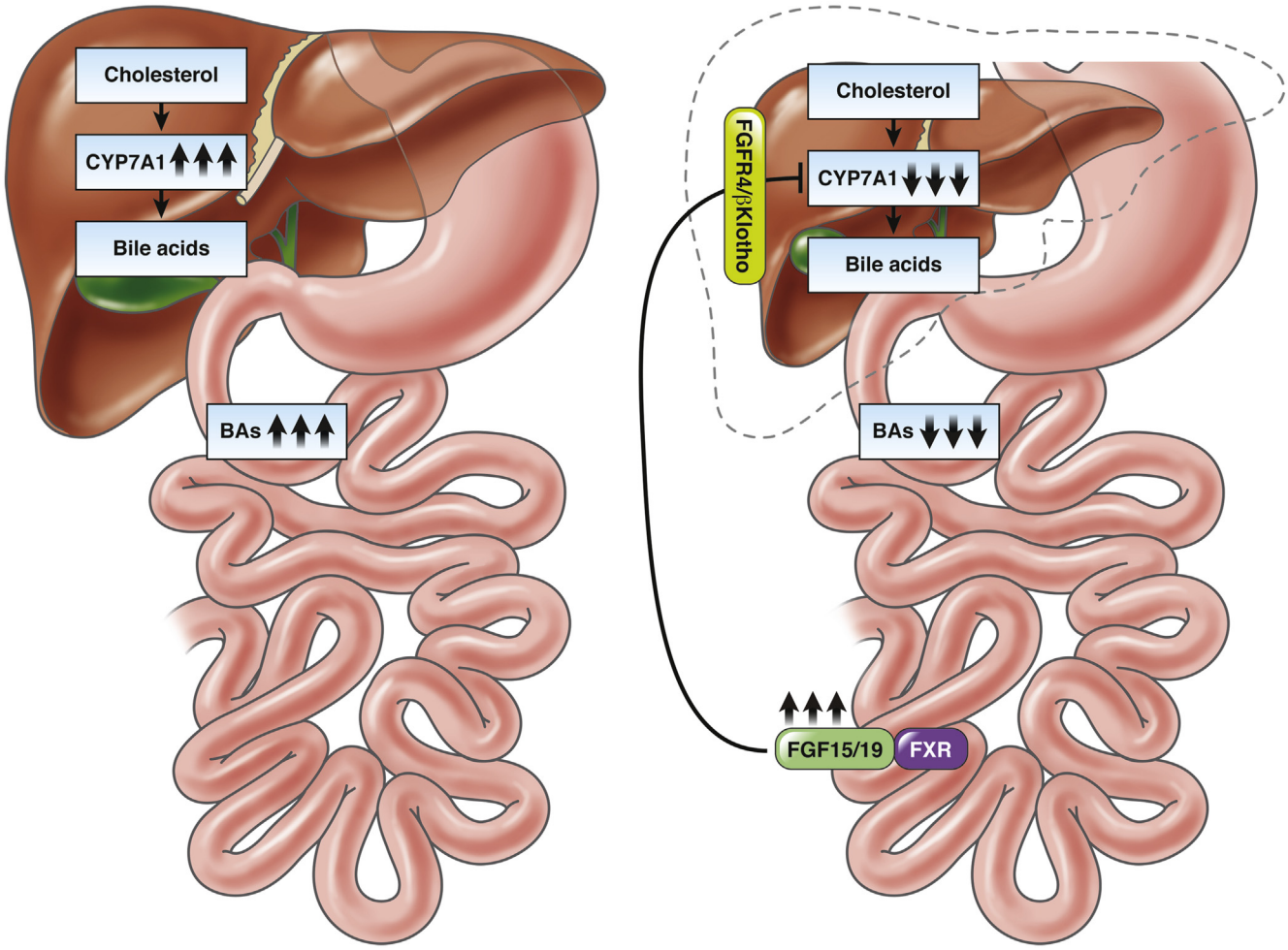

Figure 1. The bile acid–farnesoid X receptor (FXR)–fibroblast growth factor (FGF)19 hepatostat. Increased hepatocyte mass and liver size is observed in conditions with an increased circulating bile acid pool owing to up-regulation of hepatic bile acid synthesis via CYP7A1. When the gut–liver FXR–FGF19 axis is working normally, intestinal bile acids induce the enterokine FGF19 that, via activation of the FGFR4/beta KLOTHO complex on the hepatocyte basolateral membrane, represses CYP7A1 expression and reduces the circulating bile acid pool, hepatocyte mass, and liver size.

Mice with humanized livers are chimeric models in which the recipient animals are transplanted with human hepatocytes that extensively repopulate the liver parenchyma.[15] In one of these models, the immune deficient Fah$^{-/-}$, Rag2$^{-/-}$, Il2r$^{-/-}$, NOD mice, or FRGN mice, in which human hepatocyte repopulation is favored by an inducible murine suicidal hepatocyte genetic defect, Markus Grompe and his team[16] previously described the overexpression of *CYP7A1* in the transplanted, proliferating human hepatocytes along with a marked elevation in the BA pool. This situation was reversed upon administration of recombinant FGF19, suggesting that transplanted human hepatocytes were not sensitive to murine FGF15 (which is elevated in this model), but retained the ability to respond to the human enterokine.[16] These findings, together with the increased liver size observed in these chimeric mice, provided Naugler et al with an excellent model in which to further test the role of BA in liver growth, in the absence of the potentially confounding signals elicited during PH. The authors then restored the physiologic regulation of the BA pool in these mice by generating a transgenic strain of FRGN mice in which the FGF19 gene, along with its regulatory region, was introduced.[14] Human hepatocytes were then transplanted into these FRGN19^{+} mice and the control FRGN animals, and 4 months later liver repopulation by human hepatocytes was complete. FRGN19^{+} mice showed very low levels of FGF19 messenger RNA in the intestine and FGF19 protein was undetectable in sera under normal conditions. However, BA infusion led to marked elevations in FGF19 expression, indicating that these mice responded to BA signaling as expected. It was shown previously that in patients with cholestasis hepatic specific expression of FGF19 was up-regulated,[13] whereas under normal conditions FGF19 is expressed only in the intestine. In the present study, the authors performed bile duct ligation and induced cholestasis. Intriguingly, the humanized FRGN19^{+} mice began transcribing FGF19 in the liver. Notably, the immunolocalization revealed a

nonparenchymal expression pattern of the enterokine, indicating that human hepatocytes do not express FGF19, even in cholestatic conditions.

In agreement with previous findings in FGF19–treated FRGN mice,[16] hyperexpression of hepatic CYP7A1 was corrected in FRGN19$^+$ animals, and this response was accompanied by the restoration of a normal (decreased) total BA pool size. This finding is consistent with observations in other mouse models demonstrating that FGF19 or FGF15 administration decreased hepatic CYP7A1 expression and BA levels, and confirms the central role played by this enterokine in the regulation of BA metabolism.[9,11] Interestingly, the size of the livers repopulated with human hepatocytes in FRGN19$^+$ mice was almost 3 times smaller than that found in FRGN animals, and this was not owing to differences in the percentage of repopulation between the 2 strains. Taken together, these findings provide additional support to the hypothesis of liver growth being regulated by the size of the BA pool (Figure 1). Nevertheless, to further substantiate the conclusions drawn in this experimental model, it will be interesting to test the effect on liver growth of reducing the BA pool in transplanted FRGN mice by an alternative method, such as feeding these animals a BA-sequestering resin.[11]

In accordance with their increased size, livers from transplanted FRGN mice showed a significantly higher hepatocyte proliferation than those of FRGN19$^+$ mice. Consistently, transcriptome analyses revealed enhanced expression of genes involved in DNA synthesis and cell cycle in repopulated FRGN livers. One critical regulator of liver growth is the Hippo-YAP pathway.[17] In the quiescent liver, the transcriptional coactivator YAP is phosphorylated and retained in the cytosol unable to drive cell proliferation. Upstream signals activating this pathway may emanate from a decreased cell density and alterations in the extracellular milieu, but their nature has not been fully established yet.[18] Interestingly, in their study Naugler et al[14] also identified a gene signature consistent with the activation of the Hippo-YAP pathway in transplanted FRGN livers. This remarkable finding is in agreement with the recently reported activation of YAP in response to elevated BA levels in the liver,[19] and highlights the interaction between a metabolic signal and a critical growth regulatory pathway that may work in concert in the regulation of the hepatostat. In line with these findings, it has been shown recently that the reactivation of the enterohepatic FXR-FGF15 axis reduces circulating BA levels to normal and prevents spontaneous proliferation and hepatocarcinoma of FXR-null mice.[20] Thus, the present study highlights the FXR-FGF19 gut–liver axis as a novel hepatostat and opens new therapeutic avenues based on the physiologic regulators of this hormonal gut–liver axis.

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