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Bile Acid Receptors and Liver Regeneration

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9.1 INTRODUCTION

Liver is one of the few organs that can regenerate itself in response to partial ablation or liver injury. Liver regeneration has been widely studied as a paradigm for regenerative organ regrowth since the introduction of a rodent partial hepatectomy (PHx) model in 1931 [1]. Unlike a typically anatomic regeneration, regeneration of the liver is a compensatory hyperplasia of the remaining tissues and is driven by the functional deficit of the organism. Liver regeneration consists of several well-orchestrated phases, with rapid induction of proliferating factors activating the quiescent hepatocytes and priming their subsequent progression through the cell cycles, followed by re-establishment of original liver size and renewed quiescence [2–4]. Growth factors and cytokines are the important early signals to induce the expression of downstream target genes via activation of several key transcription factors [5]. In addition to growth factors and cytokines, metabolic signals are considered as the third major signals during liver regeneration, which is relatively less studied [6]. Recently, bile acids (BAs) and their receptors were identified as key metabolic signaling pathway during liver regeneration and their roles in promoting liver regeneration have received more and more attention [7,4]. In this review, the roles of BA signaling and BA receptors in liver regeneration will be summarized and discussed.

9.2 METABOLIC SIGNALS AND LIVER REGENERATION

Liver regeneration is an adaptive regrowth response induced by specific stimuli and the subsequently sequential changes in gene expression and morphologic reconstruction.
It is generally accepted that the remaining hepatocytes are the major cell types that replicate to regenerate liver in the models of 70% PHx or liver injury. Only in specific injury models, activation and replication of liver progenitors are observed when the hepatocytes fail to replicate normally. In addition to hepatocytes, other cell types are actively involved in liver regeneration or repair. Recently, several excellent reviews also highlight the roles of liver stellate cells, liver sinusoidal endothelial cells, and liver stem/progenitor cells in liver regeneration and repair [8–11].

Liver regeneration includes a highly complex network of signal transductions. The essential circuitry required for this process is defined mainly by three major networks: cytokine, growth factor, and metabolic signaling [12]. These three networks subsequently activate specific genes and signaling pathways that are essential for liver regeneration. Compared to the cytokine and growth factor networks, little is known about the roles of metabolic signals in liver regeneration. The identification of several nuclear receptors as receptors for liver metabolites provides novel insight into the roles of metabolic signals in liver regeneration. Among them, the farnesoid X receptor (FXR, NR1H4) is identified as a primary BA receptor [13,14]. In addition to FXR, some other nuclear receptors have been implicated in regulating the liver metabolism, including constitutive androstane receptor (CAR, NR1I3), pregnane X receptor (PXR, NR1I2), vitamin D receptor (VDR, NR1I1), and liver X receptor alpha and beta (LXRα, NR1H3; LXRβ, NR1H2). All these receptors bind to DNA either as a monomer or as a heterodimer with a common partner for nuclear receptors, retinoid X receptor (RXR, NR2B1) to regulate the expression of various genes involved in BA, lipid, glucose, and drug metabolism [15]. Their roles in liver regeneration are also under active investigation. For example, upon PHx, liver regeneration is impaired in mice lacking RXRα in hepatocytes [16]. LXR may suppress liver regeneration after PHx through regulating the cholesterol levels in the liver [17]. CAR activation strongly induces hepatomegaly and may contribute to normal liver regeneration after 70% PHx [7,18]. Dai et al. indicated that PXR is required for normal progression of liver regeneration by modulating lipid homeostasis and regulating hepatocyte proliferation [19]. In contrast, PPARγ acts as a negative regulator of hepatocyte proliferation and may be responsible for the inhibition of liver growth in the late phase of liver regeneration [20]. Actually, direct activation of some of these receptors such as PPARα and CAR results in hepatomegaly quickly, which is distinct from the normal liver regeneration [21]. These results indicate that the increase of different endogenous metabolites and hormones during liver regeneration may activate their individual receptor, which help liver regeneration to different extent. In summary, metabolic signaling is an integrated component of normal liver regeneration. Multiple pathways are working in parallel to contribute to the overall process of liver regeneration (Figure 9.1) [22].

Liver is a major organ for metabolism. Therefore, there is an immense metabolic demand during liver regeneration. The requirement of metabolic signals for liver regeneration has been known for a long time. The question is whether all metabolic signals are required for liver regeneration. The answer may be dependent on the levels of endogenous ligands after 70% PH or liver injury. Although all these nuclear receptors have the potential to be activated and promote liver growth, only those whose endogenous ligands are strongly increased, such as BAs and FXR, will play major roles in contribution to normal liver regeneration. Activation of FXR by its ligand has been shown to promote liver regeneration in aged animals [23], suggesting that further understanding of the mechanism by which nuclear receptors stimulate liver regrowth may provide novel approaches to develop drugs for promotion of liver regeneration.
different metabolic signals, BAs are attractive signals for liver regeneration because the levels of BAs are tightly regulated. BAs are intrinsically toxic and cause liver injury if the levels are not controlled properly. As such, liver resection or injury will generate a BA overload in the liver, which is a potential driving force for liver regeneration [7,4].

**FIGURE 9.1** Multiple signaling pathways are engaged in promoting liver regeneration. In addition to cytokines and growth factors, metabolic signals are generated during liver regeneration, which activate the nuclear receptors to modulate the expression of a specific group of genes involved in liver regeneration and repair.

9.3 BA SIGNALING AND LIVER REGENERATION

BAs are liver-specific metabolites. They are end products from cholesterol catabolism and are important for nutrition absorption in the intestine, including cholesterol, lipids, and fat-soluble vitamins. BAs are synthesized in the liver and stored in the gall bladder. They are secreted into the intestine when a meal is ingested, but 95% BAs are reabsorbed and transported back to the liver through the portal vein via enterohepatic circulation. Hepatic BAs comprise less than 5% of the total BA pool, and PHx increases bile influx, which rapidly generates a BA overload in the liver. Consistently with this, there is a sharp repression of Cyp7a1 gene expression after 70% PHx or liver injury [24]. Cyp7a1 is the rate-limiting enzyme required for the BA production from cholesterol catabolism. The importance for a stringent control of BA levels is illustrated by a delicate regulation of Cyp7a1 expression. There are many factors and pathways that can regulate the expression of Cyp7a1 gene. A negative feedback loop is identified to regulate BA levels, in which high levels of BA activate FXR to increase the mRNA levels of SHP, which is a negative regulator of Cyp7a1 gene expression. Moreover, additional regulators of Cyp7a1 expression are identified, including cytokines, growth factors, and nuclear receptors [25–27]. During liver regeneration, in addition to FXR-SHP axis, hepatocyte growth factor and JNK pathways are involved in suppressing Cyp7a1 expression during the acute phases of liver regeneration [24]. Furthermore, during the early phase of liver regeneration, MAPK and other pathways may also participate to suppress the expression of CYP7a1.

The strong suppression of BA synthesis during liver regeneration indicates a BA overload stress in the liver. This also suggests that BAs may participate in the liver regeneration. Indeed, interruption of normal enterohepatic biliary circulation has been previously known to inhibit liver regeneration [28,29]. Moreover, there is also some direct evidence that BAs are able to stimulate hepatocyte proliferation [30–32]. In a study of 70% PHx mouse model, supplementation with a low dose of BAs promotes liver regeneration, while reduction of BA levels by a BA-binding resin delays liver regeneration [7]. Defective BA signaling causing delayed liver regeneration is also demonstrated in other animal models. In the absence of MRP3, a BA transporter, liver regeneration is delayed in mice due to lower BA concentration in the portal blood [33]. Similarly, deletion of Cyp27, an enzyme required for normal BA production and metabolism, results in lower BA pool and defective
liver regeneration in mice [34]. In FXR\(^{-/-}\) mice, the effect of BAs on promoting liver regeneration is lost [7]. Similarly, in MRP3\(^{-/-}\) and Cyp27\(^{-/-}\) mice, the delayed liver regeneration is due to impaired FXR activation, suggesting that FXR is the key player to mediate BA effect on liver regeneration. In conclusion, the identification of a novel role of FXR in liver regeneration is key to understand the molecular mechanism by which BAs promote liver regeneration.

### 9.4 FXR AND LIVER REGENERATION

FXR is highly expressed in the liver, intestine, kidney, and adrenals where the levels of BAs are relatively high [35], but with lower expression in the adipose tissues and heart. FXR is the primary sensor of BAs and both conjugated and unconjugated bile salts are able to activate FXR at physiological concentrations [36,37]. FXR regulates BA homeostasis by regulating genes involved in BA synthesis, secretion, transportation, absorption, conjugation, and detoxification [38-42]. As expected, FXR is also the BA receptor to mediate BA’s effect on liver regeneration [7]. FXR is shown to promote liver regeneration after 70% PHx and stimulate liver repair after CCl\(_4\)-induced liver injury [43]. Interestingly, different from 70% PHx, there is massive cell death in liver injury model. It was shown that FXR activation specifically upregulated ERK pathways and protected liver cells from apoptosis induced by serum deprivation \textit{in vitro} and fasting \textit{in vivo} [44]. During liver repair after injury, this role of FXR in cell survival may be linked to the activation of STAT3, which is a key factor in cell survival [43]. Therefore, FXR is shown to have a dual role in promoting liver regeneration by both stimulating hepatocyte proliferation and protecting the hepatocyte from death [39,44]. However, the exact downstream events after FXR activation to prevent cell apoptosis are still unclear and needs further investigation.

In addition to metabolic genes, Foxm1b is identified as a FXR direct target gene during liver regeneration [7,23]. Foxm1b is a key cell cycle regulator essential for G1/S and G2/M progression [45,46]. Animal studies indicate that Foxm1b is a key transcription factor in liver regeneration. Although liver can fully regenerate itself, aging dramatically reduces this capacity of liver. Previous studies suggest that aging-induced suppression of liver regeneration is mediated by an epigenetic mechanism and this suppression is reversible. Further studies indicate that multiple pathways may work independently to promote liver regeneration [47,48]. The delayed and reduced proliferative response has been attributed to the decreased expression of some key transcription factors, such as c-Myc and Foxm1b and to the failure of aging-liver to diminish the age-specific C/EBP\(\alpha\)-Brm-HDAC1 complex after PHx [49-51]. The complex suppressed Foxm1b induction after PHx through binding to Foxm1b promoter, which results in age-related proliferation defects upon PHx or liver injury [52]. These studies highlight Foxm1b as one of the key regulators in aging-liver regeneration. Loss of Foxm1b function in livers of young mice results in a significant reduction in hepatocyte DNA replication and inhibition of mitosis after PH [49]. More importantly, forced expression of Foxm1b in regenerating livers of old mice is sufficient to restore hepatocyte DNA replication and expression of necessary cell cycle regulatory genes to levels as seen in young animals [51,52]. Defective activation of FXR occurs in aged regenerating livers [23], which may account for the insufficient Foxm1b induction. Compared with young mice, aging mice did not have altered protein levels of FXR and RXR. Therefore, aging may affect the levels of endogenous FXR ligands such as BAs, which could result in defective activation of FXR during liver regeneration. Interestingly, in pregnant mice, loss of FXR results in reduced liver growth, indicating a similar function of FXR in mediating the liver growth during pregnancy [53].
Sirtuin1 (SIRT1) can modulate FXR activity and has an effect on liver regeneration through modulating FXR during liver regeneration [54]. The transgenic mice that overexpress SIRT1 showed increased mortality, enhanced liver injury, and impaired hepatocyte proliferation after PHx. SIRT1 reduces FXR activities through persistent deacetylation and lower FXR expression. In summary, FXR is a key receptor and transcription factor that specifically mediates the effect of BA signaling to promote liver regeneration.

9.5 INTESTINE-FXR AND LIVER REGENERATION

FXR is highly expressed in both the liver and the intestine. Both hepatic- and intestine-FXR are involved in the regulation of BA homeostasis [55]. One critical FXR target gene in the intestine is FGF15. Indeed, several reports suggest that FGF15 secreted from ileum has profound effects on the suppression of Cyp7a1 gene expression and liver metabolism through FGF receptor-mediated signaling pathways in the liver [56–58]. Suppressed Cyp7a1 expression and decreased BA synthesis are known to be beneficial for liver regeneration. Therefore, FGF15 induction after liver damage may also contribute to the normal liver regeneration. Indeed, there is significantly delayed liver regeneration and increased liver injury in intestine-specific FXR knockout (ΔIN-FXR) mice compared to FXR Fl/Fl control mice after either 70% PHx or CCl4 injection [59]. During liver regeneration, FXR also activates the expression of FGF15 in the intestine to suppress Cyp7a1 transcription [59]. There results identify an unexpected role of intestine-FXR in regulating liver regeneration/repair. First, higher levels of BAs in ΔIN-FXR mice after liver injury may hamper the normal liver regeneration/repair. Second, the metabolic and mitogenic activities of FGF15 may contribute to liver regrowth. Third, the hydrophobic BA, deoxycholic acid (DCA), is significantly increased in fecal extracts from ΔIN-FXR mice but not from FXR KO or liver-FXR null mice, and DCA may cause hepatocyte apoptosis [60]. This may also be a protective function of intestine-FXR during liver regeneration/repair. Finally, exogenous delivery of FGF15 rescued the defect of liver repair in ΔIN-FXR and FXR KO mice [59], suggesting a direct role of FGF15 in promoting liver regeneration.

The direct effect of FGF15 on liver regeneration is also examined by comparing liver regeneration between WT and FGF15−/− mice [61]. As expected, in FGF15−/− mice, liver regeneration is delayed, and there is stronger liver injury in FGF15−/− mice. Furthermore, a recent report indicates that selective activation of intestinal FXR or treating mice with FGF19, a human homolog of murine FGF15, could reduce liver necrosis and inflammatory cell infiltration in cholestasis mouse models [62]. Taken together, intestinal FXR and its induction of FGF15 may have important roles in liver protection.

These data show that both liver- and intestine-FXR contribute to liver regeneration. Hepatic FXR directly induces Foxm1b expression and promotes liver regeneration. In contrast, intestine-FXR activates FGF15 expression to promote liver regeneration. Therefore, both the cell-autonomous effect of hepatic FXR and the endocrine FGF15 pathway induced by intestine-FXR are required for normal liver regeneration (Figure 9.2).

9.6 TGR5 AND LIVER REGENERATION

TGR5 is a plasma membrane-bound G-protein-coupled BA receptor, which displays varied levels of expression in different tissues [27,63,64]. Hydrophobic BAs, such as lithocholic acid and DCA, are potent endogenous ligands of TGR5. TGR5 regulates BA homeostasis, glucose homeostasis, energy metabolism as well as inflammation [65–70].
A role of TGR5 in liver regeneration was recently identified [71]. After 70% PHx, TGR5<sup>−/−</sup> mice displayed severe hepatocyte necrosis, prolonged cholestasis, exacerbated inflammatory response, and delayed liver regeneration [71]. TGR5 may primarily protect the BA-overloaded remnant liver by controlling BA hydrophobicity and suppressing inflammatory response. Moreover, TGR5 increases BA efflux in urine through kidney. The regulation of potential BA transporters in kidney needs further investigation. The defective BA clearance in the absence of TGR5 thus leads to exacerbated liver toxicity by BAs. These results highlight a distinct role of TGR5 during liver regeneration.

Interestingly, a recent report indicates that serotonin also helps increase BA secretion through urine and reduces liver toxicity, suggesting that BA excretion through kidney may be an important mechanism for liver protection during liver regeneration [72]. Serotonin is not only a neurotransmitter but also a hormone involved in the initiation of liver regeneration [73,74].

9.7 FXR AND HCC DEVELOPMENT

Hepatocellular carcinoma (HCC) is the most common primary liver cancer and the incidence is rising worldwide largely due to hepatitis B virus and hepatitis C virus infection, alcohol abuse, and the epidemiological obesity-associated NASH [75,76]. FXR deficiency mice not only exhibit delayed LR after 70% PH but also demonstrate defective repair ability in the
damaged liver [7,77]. Without the roles of FXR in promoting liver repair after injury, liver is prone to enter endless cycles of injury/repair that will keep producing inflammatory cytokines and other growth factors that are potential tumor promotors. Moreover, BAs are known to cause DNA damage and induce cell transformation if their levels are not controlled by FXR. Excessive accumulation of BAs has a cytotoxic effect and is considered an important etiology of tumorigenesis [78]. Therefore, FXR’s role in promotion of liver repair could be an intrinsic mechanism to protect liver from carcinogenesis. In addition, FXR was found to participate in regulating hepatic fibrosis [79,80], cholestasis, hepatic inflammation [81,82], and immune response [83,84,43,85]. Studies found that disruption of BA metabolism is the major defect discovered in FXR−/− mice with spontaneous hepatocarcinogenesis [86,87]. Overload of BAs due to the depletion of the FXR gene is the causative factor for injury of liver cells, induction of chronic inflammation, enhancement of the cell proliferation, and development of liver tumor [86–88]. The persistently high levels of BA enhanced the inflammation and bile duct proliferation and led to the downregulation of FXR expression. Those data indicate that during hepatocarcinogenesis, BAs may function as tumor promoters as well as DNA-damaging initiators [86,89]. Therefore, further delineation of the link between FXR’s roles in liver regeneration and hepatocarcinogenesis may provide novel insight into the mechanism of HCC development.

In summary, FXR is also a HCC suppressor. FXR exerts its anti-tumorigenic function via several mechanisms: (1) FXR maintains the normal liver homeostasis and metabolism of BAs, glucose, and lipid; (2) FXR promotes liver regeneration and repair after injury; (3) FXR protects the liver cells from death and enhances cell survival; (4) FXR suppresses hepatic inflammation, thereby preventing inflammatory damage; and (5) FXR can directly increase the expression of some tumor-suppressor genes and repress the transcription of several oncogenes (Figure 9.3) [90].

9.8 CONCLUSIONS AND PERSPECTIVE

BA signaling is now known as an important metabolic signal during liver regeneration. The novel roles of FXR and TGR5 in promoting liver regeneration are consistent with their defending roles against BA toxicity during liver regeneration. Moreover, there is a close relationship between aberrant liver regeneration and HCC in FXR−/− mice [91–93]. Similarly, abnormal BA homeostasis and cell proliferation in SHP−/− mice also result in HCC development [94,95]. Therefore, further studies on FXR and TGR5 in this new area will provide novel insights into the complex mechanism of liver regeneration, HCC, and other liver diseases. Encouragingly, there is already active research in searching for potent FXR and TGR5 ligands.
Thus, both FXR and TGR5 agonist ligands may offer potential approaches to prevent and treat insufficient liver regeneration as well as HCC and other liver diseases.

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