Review

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Implications of microbiota and bile acid in liver injury and regeneration

Hui-Xin Liu, Ryan Keane, Lili Sheng, Yu-Jui Yvonne Wan*

Department of Medical Pathology and Laboratory Medicine, University of California, Sacramento, CA, USA

Summary

Studies examining the mechanisms by which the liver incurs injury and then regenerates usually focus on factors and pathways directly within the liver, neglecting the signaling derived from the gut-liver axis. The intestinal content is rich in microorganisms as well as metabolites generated from both the host and colonizing bacteria. Through the gut-liver axis, this complex "soup" exerts an immense impact on liver integrity and function. This review article summarizes data published in the past 30 years demonstrating the signaling derived from the gut-liver axis in relation to liver injury and regeneration. Due to the intricate networks of implicated pathways as well as scarcity of available mechanistic data, it seems that nutrigenomic, metabolomics, and microbiota profiling approaches are warranted to provide a better understanding regarding the interplay and impact between nutrition, bacteria, and host response in influencing liver function and healing. Therefore elucidating the possible molecular mechanisms that link microbiota alteration to host physiological response and vice versa.
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Introduction

A unique feature of the liver is its extraordinary regenerative ability. Liver regeneration is crucial for restoration of function following injury and an understanding of the underlying mechanisms would be of therapeutic value in liver disease treatment and transplantation. Liver regeneration is an orchestrated biological process that includes sequential changes in gene expression, growth factor production, and tissue remodeling. Following liver resection, hepatocytes, which are not terminally differentiated, exhibit substantial proliferative capacity. Many mitogens, cytokines, and growth factors, which are involved in liver regeneration, have been identified and extensively reviewed [1–16]. In addition to the presence of growth factors and mitogens, active metabolism is required to generate the energy and precursors for biosynthesis of macromolecules necessary for cell proliferation and tissue remodeling during liver regeneration. Because nuclear receptors play a crucial role in hepatic metabolism, their actions in liver regeneration have been extensively studied in recent years as well [17–27]. However, liver regeneration research has typically focused on signaling pathways intrinsic to the liver, overlooking those derived from the gut. The current review details the signaling within the gut-liver axis and summarizes the interactions between microbiota and bile acids (BAs) in maintaining gastrointestinal (GI) health and impacting liver injury and regeneration.

The relationship between gut microbes, liver injury, and liver regeneration

The gut microbiota refers to the 100 trillion bacteria that reside in the human GI tract, and is now often referred to as its own organ [28]. Over the past decade, an exponential amount of research into the human microbiome, termed "the forgotten organ", has shifted our perspective on the influence of the host-microbiome relationship in the pathogenesis of human diseases [29]. In addition, gut microbiota affects intestinal signaling and enterohepatic circulation of BAs, a growing body of evidence supports that the gut microbiota may promote liver regeneration and health.

Bacterial endotoxin and liver regeneration

Endotoxin lipopolysaccharides (LPS) are glycolipids present in the outer membrane of Gram-negative bacteria such as *Neisseria* spp. and *Haemophilus* spp. LPS have three components: O-antigen, core oligosaccharide, and lipid A. O-antigen is exposed

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* Corresponding author. Address: Department of Medical Pathology and Laboratory Medicine, University of California, Davis Health Systems, Ph.D. Room 3400B, Research Building III, 4645 2nd Ave, Sacramento, CA 95817, USA. Tel.: +1 916 734 4293.
E-mail address: yjywan@ucdavis.edu (Y.-J.Y. Wan).
Abbreviations: Abcg5, ATP-binding cassette transporters G5; BAs, bile acids; GI, gastrointestinal tract; LPS, lipopolysaccharide; PHx, partial hepatectomy; HGF, hepatocyte growth factor; NKT, natural kill T; IL, interleukin; I/R, ischemia/reperfusion; TGR5, G protein-coupled membrane receptor; FXR, farnesoid x receptor; KO, knockout; *Mdr2*, multidrug resistance 2; SHP, small heterodimer partner; FGF15, fibroblast growth factor 15; CYP7A1, cholesterol 7α-hydroxylase; CYP8B1, sterol 12α-hydroxylase; TNFα, tumor necrosis factor α; CA, cholic acid; CDCA, chenodeoxycholic acid; MCA, muricholic acid; DCA, deoxycholic acid; LCA, lithocholic acid; T-β-MCA, tauro-β-muricholic acid; TCA, tauro-cholic acid; PPARγ, peroxisome proliferator-activated receptor gamma.

Key points

- Microbiota and bile acids within the gut-liver axis are crucial in regulating metabolism and inflammatory processes, and thus are important for liver injury and liver regeneration.
- There exists a "gut-liver axis" that facilitates bidirectional communication between intestinal microbes and hepatic bile acid metabolism. In one direction, the gut microbiota plays a pivotal role in regulating bile acid homeostasis while on the other end, bile acids influence gut microbiota composition.
- Because hepatic regeneration is dependent on signaling mediators derived from the gastrointestinal tract, diseases or pathologies that disturb the normal intestinal environment, particularly the gut microbiota, interfere with liver regeneration.
- Despite the exponential growth in marketing of synbiotics and probiotic products, there is a lack of established mechanistic links between gut microbiota alterations and physiological responses from the host. The summarized data provide promising evidence that bile acids and microbiota jointly regulate nutrient absorption, hepatic metabolism, and inflammatory processes thus maintain the health of gut and liver.

on the outer surface of the bacterial and recognized by host antibodies. In contrast, the lipid A is conserved, and those hydrophobic fatty acid chains anchor the LPS into the bacterial membrane. Through Toll-like receptor 4, the receptor of LPS, lipid A activates the mammalian immune system with production of inflammatory mediators that lead to septic shock [30]. Chemically, LPS do not have O-antigens and only have the lipid A and oligosaccharide core, and LPS administration is frequently used to induce liver injury for *in vivo* study of hepatic regeneration and function. While this would initially appear to indicate that bacteria negatively influence liver regeneration, evidence indicates that endotoxin is necessary for liver regeneration. Gut-derived endotoxin administered both before and after partial hepatectomy (PHx) induced hepatic DNA synthesis and release of several hepatotrophic factors such as insulin [31]. Conversely, hepatic DNA synthesis in mice was impaired when gut-derived endotoxin was prevented from reaching the liver [32]. In addition, conditions that eliminate bacteria or reduce endotoxin could inhibit DNA synthesis following 67% liver resection. Those conditions include gut sterilization using neomycin and cefazolin, reduction of endotoxin and BAs using cholestyramine, and neutralization of the lipid A portion of circulating endotoxin by polymyxin B [32]. Endotoxin tolerant rats as well as Gram-negative bacteria deficient rats all showed impaired DNA synthesis in response to PHx [32]. Furthermore, LPS could rescue both germ-free and LPS-resistant mice from delayed liver regeneration [33]. The observed LPS-induced hepatocyte proliferation may result from augmentation of hepatocyte growth factor (HGF) activity. Treatment of rats with a combination of LPS and HGF increased JNK and AP-1 DNA binding, possibly through c-JUN and STAT3 upregulation [34]. LPS-HGF modulation of hepatocyte proliferation indicates a potential contribution from the gut microbiota to the liver regeneration program.

Although endotoxin has been shown to induce hepatocyte proliferation, it is important to note that not all endotoxin-releasing bacteria are beneficial for liver regeneration. In mice, orthotopic liver transplantation was associated with increased hepatic inflammation and increased portal endotoxin levels after surgery, often leading to liver injury and rejection [32]. However, when *Bifidobacterium*, *Lactobacillus*, *Bacteroides*, and *Eubacterium* was increased and *Enterobacteriaceae* was reduced, portal LPS levels and Kupffer cell activation decreased, which was beneficial for preventing liver injury found in rats after orthotopic liver transplantation [35]. These findings suggest differential effects of specific bacteria on liver regeneration. This is also supported by experiments using antibiotic treatment. It has been shown that norfloxacin treatment did not affect DNA synthesis and hepatic ornithine decarboxylase activity 24 h after 70% liver resection in a rat model. Thus, selective bowel decontamination with norfloxacin does not seem to change hepatocyte proliferation [36]. A recent study showed that ampicillin-sensitive bacteria were associated with normal liver regeneration [37]. The number of CD1d-dependent natural killer T (NKT) cells was increased in antibiotic-treated hepatectomized mice, and these NKT cells were overly activated to produce elevated interferon-γ. NKT cells deficiency or antibody blockade of the CD1d-NKT interaction increased hepatocyte proliferation, which improved liver regeneration. Moreover, increased Kupffer cells were observed in antibiotic-treated mice, which had elevated interleukin 12 (IL-12) to activate hepatic NKT cells. IL-12p40 deficiency or treatment with anti-IL-12 antibody reduced NKT cell activation and restored liver regeneration in antibiotic-treated mice [37]. Together, mild bacterial translocation with specific bacteria and subsequent endotoxin release is essential to stimulate liver regeneration, but sustained dysbiosis has a negative impact on liver regeneration.

Probiotics

Emerging evidence indicates that the presence of several key bacterial species, mainly *Lactobacillus*, *Bifidobacterium*, and *Bacteroides* species, influences liver injury and regeneration. Carbon tetrachloride-induced cirrhosis was linked to a decreased microbial diversity [38]. In addition, a high proportion of *Bifidobacterium animalis* was also positively correlated with elevated IL-10 expression, which reinforces the hepatoprotective effects of *Bifidobacterium* species [38]. Additionally, *Bifidobacterium infantis* has been implicated in modulating colonic microbial diversity and reducing fecal endotoxin levels [39]. Decreased abundance of these species, particularly *Bifidobacterium* species, can exacerbate hepatic injury and impede regeneration [40]. Hepatic ischemia/reperfusion (I/R)-induced injury resulted in reduced density of *Lactobacillus, Bifidobacterium*, and *Bacteroides* and increased density of *Enterococcus* and *Enterobacteriaceae* [41]. Probiotic treatment reduces liver injury and examples are listed below. *Lactobacillus rhamnosus* treatment improved liver function and reduced inflammation in alcohol-induced liver injury in mice [42,43]. A combination of *Bifidobacterium infantis, Lactobacillus gasseri,* and *Lactobacillus plantarum* relieved colorectal inflammation and tumor-associated hepatic injury [44]. This probiotic treatment in combination with blueberry