Research Article

JOURNAL OF HEPATOLOGY

Functional analysis of the relationship between intestinal microbiota and the expression of hepatic genes and pathways during the course of liver regeneration

Hui-Xin Liu[1], Clarissa Santos Rocha[2], Satya Dandekar[2], Yu-Jui Yvonne Wan[1,*]

[1]*Department of Medical Pathology and Laboratory Medicine, University of California, Davis, Sacramento, CA, USA;* [2]*Department of Medical Microbiology and Immunology, University of California, Davis, Davis, CA, USA*

Background & Aims: The pathways regulating liver regeneration have been extensively studied within the liver. However, the signaling contribution derived from the gut microbiota to liver regeneration is poorly understood.

Methods: Microbiota and expression of hepatic genes in regenerating livers obtained from mice at 0 h to 9 days post 2/3 partial hepatectomy were temporally profiled to establish their interactive relationships.

Results: Partial hepatectomy led to rapid changes in gut microbiota that was reflected in an increased abundance of Bacteroidetes *S24-7* and *Rikenellaceae* and decreased abundance of Firmicutes *Clostridiales*, *Lachnospiraceae*, and *Ruminococcaceae*. Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) was used to infer biological functional changes of the shifted microbiota. RNA-sequencing data revealed 6125 genes with more than a 2-fold difference in their expression levels during regeneration. By analyzing their expression pattern, six uniquely expressed patterns were observed. In addition, there were significant correlations between hepatic gene expression profiles and shifted bacterial populations during regeneration. Moreover, hepatic metabolism and immune function were closely associated with the abundance of *Ruminococcacea*, *Lachnospiraceae*, and *S24-7*. Bile acid profile was analyzed because bacterial enzymes produce bile acids that significantly impact hepatocyte proliferation. The data revealed that specific bacteria were closely associated with the concentration of certain bile acids and expression of hepatic genes.

Conclusions: The presented data established, for the first time, an intimate relationship between intestinal microbiota and the expression of hepatic genes in regenerating livers.

Introduction

Commensal bacteria are implicated in digestive tract health and disease. It is known that intestinal microbiota plays a role in regulating host cell proliferation and tissue repair [1,2]. For example, germ-free mice have reduced intestinal epithelial cell turnover due to reduced proliferation, apoptosis, and crypt-to-tip cellular migration [3]. Germ-free mice also exhibit increased cancer incidence compared to conventional mice [4]. In addition, increased bacterial load and dysbiosis are found in colonic biopsies of patients with colorectal adenoma or cancer [5]. Moreover, Gram-negative bacteria-generated lipopolysaccharide (LPS) stimulates liver regeneration and tissue repair through Toll-like receptor 4 (TLR4) signaling [6]. Gut microbiota also affects metabolic phenotype of the mammalian host and participates in microbial-host co-metabolism [7]. Alterations in gut bacterial communities are associated with metabolic disorders [8], metabolic syndrome [9], obesity [10–12], and non-alcoholic steatohepatitis [13]. There is an intrinsic link between proliferation and metabolism. Cell proliferation elevates metabolic demands to generate the energy and precursors for biosynthesis of macromolecules, and yet metabolic disorder dampens proliferation. Thus, through the gut-liver axis, intestinal microbiota, which is implicated in both proliferation and metabolism, may significantly regulate liver regeneration.

The liver is a major organ for host metabolism that can remarkably regenerate itself in response to partial resection or

Keywords: Bile acid; Partial hepatectomy; Metabolism; Immune response; Gut-liver axis.

Received 10 August 2015; received in revised form 8 September 2015; accepted 19 September 2015; available online 12 January 2016

* Corresponding author. Address: Room 3400B, Research Building III, 4645 2nd Ave, Sacramento, Department of Medical Pathology and Laboratory Medicine, University of California, Davis Health Systems, CA 95817, USA. Tel.: +1 916 734 4293; fax: +1 916 734 3787.

E-mail address: yjywan@ucdavis.edu (Y.-J. Yvonne Wan).

Abbreviations: LPS, lipopolysaccharide; TLR4, toll-like receptor 4; BA, bile acid; FXR, farnesoid x receptor; PHx, partial hepatectomy; PCoA, Principal Coordinates Analysis; PICRUSt, Phylogenetic Investigation of Communities by Reconstruction of Unobserved States; STEM, Short Time-series Expression Miner; GO, Gene Ontology; *Fgfr1*, fibroblast growth factor receptor 1; *Shp*, small heterodimer partner; *Cyp7a1*, cholesterol 7 alpha-hydroxylase; *Nceh1*, neutral cholesterol ester hydrolase 1; *Adcy7*, adenylate cyclase; *Ephx1*, epoxide hydrolase 1; *Hmgcr*, 3-hydroxy-3-methylglutaryl-CoA reductase; *Abcg5*, ATP-binding cassette, subfamily G, member 5; *Kcnn2*, potassium intermediate/small conductance calcium-activated channel, subfamily N, member 2; *Ntcp*, solute carrier family 10, member 1; *Oatp2*, sodium taurocholate cotransport peptide; *Aqp4*, aquaporin 4; *Bsep*, bile salt export pump; *Mdr1*, multidrug resistance gene; CDCA, chenodeoxycholic acid; β-MCA, beta-muricholic acid; DCA, deoxycholic acid; UDCA, ursodeoxycholic acid; CA, cholic acid; NKT, natural killer T cell; SCFA, short chain fatty acid.

injury [14]. Liver regeneration requires activation of an array of genes and networks of signal transducers. Bile acids (BAs) have been identified as key metabolic signals during liver regeneration, and BA levels are tightly regulated by both host and microbiota [15]. There exists a "gut-liver axis" that facilitates bidirectional communication between intestinal microbes and BAs [1]. In one direction, the gut microbiota plays a pivotal role in regulating BA homeostasis. On the other end, BAs influence the gut microbiota profile. Although the bidirectional relationship of BAs and microbiota in the gut-liver axis has been investigated in humans and mice, whether it is linked to the regenerative process after liver resection remains largely unclear [16].

Previous studies have demonstrated the significance of BAs and its receptor farnesoid X receptor (FXR) in regulating liver regeneration [15]. However, the interplay between BAs, gut microbiota, and hepatic gene profiles during liver regeneration has not been defined. This is the first study to demonstrate the dynamic shift of hepatic transcripts and pathways in relation to gut microbiota as well as BA profiles in partial hepatectomy (PHx)-induced liver regeneration.

Materials and methods

Animal experiments and sample collection

See Supplementary material and methods for sources of materials and methodological details.

Statistical analysis

Data are given as mean ± SD. Statistical analysis was performed using Student's *t* test or one-way analysis of variance. Significance was defined by $p < 0.05$.

Results

PHx-induced liver regeneration

After 2/3 liver resection, liver mass was restored to its original size at 7 to 9 days, consistent with previously reported findings (Supplementary Fig. 1A) [17–19]. Ki67 immunostaining of liver sections revealed that cell proliferation started 1 day after PHx, peaked on day 2, and ceased on day 9 (Supplementary Fig.1B, C).

Alteration in microbial communities during liver regeneration

To characterize changes in the intestinal microbiota associated with regeneration, we constructed and sequenced 16S rRNA amplicon libraries from cecal contents. Mice receiving PHx followed by wound closure and immediate killing (0 time point) were used as controls. Sham operation (Sham) followed by wound closure and immediate killing was also performed. Distinct changes in microbiota composition were noted during the course of regeneration (1 h to 9 days) as compared to controls based on principal coordinates analysis of taxon abundance data (Fig. 1A). The most abundant phyla consisted of Bacteroidetes and Firmicutes, which accounted for >95% of all sequences (Fig. 1B). Interestingly, Bacteroidetes abundance steadily increased while Firmicutes reciprocally decreased during liver regeneration (Fig. 1B). At lower taxonomic levels, *Clostridiales*, *Lachnospiraceae*, *Ruminococcaceae*, *Ruminococcus*, *Oscillospira*, and *Coprococcus* were the most abundant taxa within the Firmicutes phylum. Members of the families *S24-7* and *Rikenellaceae* were the most abundant representatives of the Bacteroidetes phylum (Fig. 1C). Overall, Firmicutes contraction was linked to decreased *Clostridiaceae* (44.9% to 25.9%, $p = 0.07$), *Lachnospiraceae* (21.7% to 6.1%, $p < 0.001$), and *Ruminococcaceae* (19.3% to 10.3%, $p < 0.01$), while Bacteroidetes expansion was linked to *S24-7* (11.1% to 47.7%, $p < 0.001$) and *Rikenellaceae* (0% to 5.8%, $p < 0.001$) enrichment during the course of liver regeneration (Fig. 1D). Gut microbiota of sham-operated mice was compared with that of controls, and there was no significant difference for the aforementioned five families between the two groups (Supplementary Fig. 2).

To study the potential function of gut microbiota at each studied time, linear discriminative analysis effect size (LEfSe) was applied to the relative abundance of KEGG pathways predicted by Phylogenetic Investigation of Communities by Reconstruction of Unobserved States PICRUSt [20]. Fig. 1E showed the biomarkers found at pathway level: 67 at 1 h and 22 in controls. In controls, the pathways with the highest three discriminative power were "bacterial chemotaxis", "bacterial motility proteins", and "flagellar assembly" under the cell motility category, followed by pathways under the membrane transport category, including "ABC transporters", "secretion system", and "transporters". Eight metabolic pathways were found in this group under carbohydrate metabolism, enzyme families, lipid metabolism, metabolism of cofactors and vitamins, and xenobiotics biodegradation and metabolism categories. At 1 h, the pathway with the highest discriminative power was the "DNA repair and recombination proteins" under replication and repair category. In addition, "mismatch repair" and "DNA replication proteins" as well as "DNA replication" were also noted. Under cellular processes and signaling category, the "cell cycle" and "cell division" pathways also had significant discriminative power. Other biomarkers with significant discriminative power were "lipopolysaccharide biosynthesis proteins" and "lipopolysaccharide biosynthesis" pathways. Most strikingly, functional biomarkers in one hour post-surgery were mainly involved in the metabolism pathways (61%, 41 out of 67 pathways) including "energy metabolism", "nucleotide metabolism", and "carbohydrate metabolism".

Day 2 data, when hepatocyte proliferation peaked, was also applied to LEfSe relative to controls. There were 24 and 64 pathways found in controls and day 2 samples, respectively (Fig. 1F). In controls, the pathways with the highest two discriminative power were the "transporters" and "ABC transporters" pathways under membrane transport category, followed by the "bacterial motility proteins" and "bacterial chemotaxis" pathways under cell Motility category. Eleven metabolic pathways were found in controls, and they were carbohydrate metabolism, metabolism of cofactors and vitamins, xenobiotics biodegradation and metabolism, metabolism of terpenoids and polyketides, and lipid metabolism categories. For the day 2 group, the highest discriminative power pathway was the "DNA repair and recombination proteins" under replication and repair category. In addition, "homologous recombination", "chromosome", "mismatch repair", "nucleotide excision repair", "DNA replication", and "base excision repair", were also found in this group. Under cellular processes and signaling category, the "membrane and intracellular structural molecules", "pores ion channels", "cell cycle", "lysosome", "peroxisome", and "cell division" pathways were identified in this group. Again, functional biomarkers in day 2 group were also mainly involved in metabolism pathways (61%, 39 out of 64 pathways) (Fig. 1F).