Recent Advances in Hepatocellular Cancer

Avid Science

Chapter 1

Entero-Hepatic Discord: Consequences for Liver Disease Pathogenesis and the Risk of Hepatocellular Carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is one of the most debilitating malignancies of hepatic origin and does not only contribute significantly to global cancer-related mortality but also global disease burden. Malfunctioning and dysregulation of the entero-hepatic axis culminating from chronic ingestion of dietary and other non-specific food substances have been implicated in liver disease pathogenesis and this has the potential to increase risk of HCC. As a result, there have been increased efforts to advance understanding of the role played by the perturbed gut in liver disease and the key cell and molecular players involved. Luckily, growing evidence from many independent studies seem to lend credence to the phenomenon of bidirectional pathogenesis of entero-hepatic diseases. This has raised hopes of finding more sensitive and specific biomarkers for prognosis and diagnosis of entero-hepatic diseases as well as identification of new therapeutic targets, more specifically for therapy against major risk factors (NAFLD, NASH, liver fibrosis, and cirrhosis) of HCC. This chapter takes a panoramic view of the interactions

between the perturbed gut and the susceptible liver. Specifically, the chapter highlights the consequences of gut dysbiosis for liver disease pathogenesis and the risk of HCC.

Introduction

The functional and structural state of the gut and the liver at any point in time reflect their adaptation to many disruptors including medication, alcohol intake, dietary factors, misuse of antibiotics, alteration of gut microbiome and host immunological and inflammatory responses to these nosae. Indeed, it must be emphasized that the relationship between the gut and the liver is so intimate and delicate that, when the gut coughs the liver picks upa cold and vice versa. In reality, this is so because the variety of substances with their diverse physicochemical properties that frequently enter the gastrointestinal system ultimately reroute the recycling plant of the body (liver) either unchanged or transformed (metabolized) into unstable biochemical molecules which have the potential to cause entero-hepatic injury. Thus the integrity of the liver is dependent not only on what enters the gastrointestinal system, or the functional and structural state of the gut, but also on the responses of the gut to these changes. Further, the anatomical location of the liver in between the gastrointestinal system and the general circulation also presents the liver with high risk of exposure to potentially damaging nosae generated within the gastrointestinal system or from general circulation. Functionally, the unstable biochemical species derived from the gut and the liver conspire at multi-level to short change the liver and the gut by not only weakening host homeostatic balance, but also synchronizing inflammatory and fibrogenic signals emanating from the gut and the liver and this reprograms the core functions of enterocytes, gut wall and hepatic cells making them more supportive of entero-hepatic inflammation and the consequences thereof (Figure 1). Though not too well characterized in pre-clinical and clinical studies, it still remains highly difficult to ignore a possible bidirectional entero-hepatic disease origin at the cellular and subcellular levels. Pioneering studies, have acknowledged this not too well accepted concept of bidirectional entero-hepatic disease pathology [1,2]. Given a strong indication of this concept, Schnabland colleagues have related the bidirectional link between the gut and the liver not only to the activities of pathogen associated molecular patterns (PAMPs) but also to the entire spectrum of secondary metabolites of bacteria translocated products as well as some microbiome-induced metabolic errors [1,3,4]. To contribute, we shed more light on the various interactions at cell and subcellular levels between the gut and the liver, specifically, how gut dysbiosis influence the onset and progression of liver disease to increase risk of HCC.

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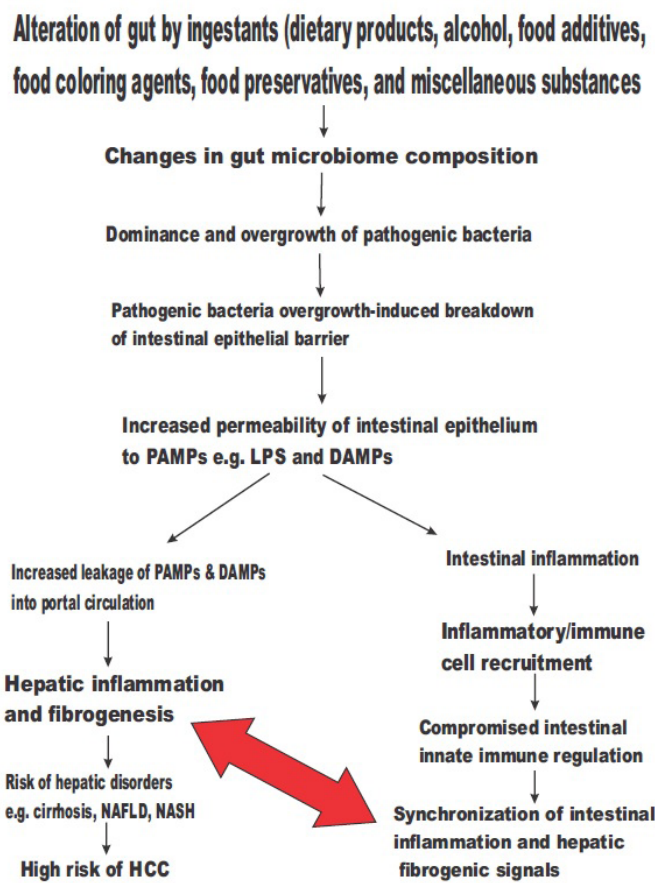

Figure 1: An illustration of the stages involved in gut dysbiosis and its role in entero-hepatic inflammation and fibrogenesis.

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Classical Entero-Hepatic Two-way Interaction

One of the ways the gut and the liver interact directly relates to final digestion of lipids and enteric innate immune regulation. The liver initially regulates the composition of bile by releasing primary bile acids mainly taurine and glycine conjugates into bile. In response to an appropriate signal the primary bile acids as part of bile are released by the gallbladder into the intestine to partake in lipid absorption. To recycle bile acids, some specific gut microbiome modifies theminto secondary bile acids before they are reabsorbed. Bile acids play crucial roles in bacteriostasis [5,6], serving as key endogenous ligands for the activation of certain receptors including TGR5 and farnesoid X receptors (FXR) [7,8] as well as regulating G-protein coupled receptor TGR5 and FXR-mediated signaling leading to production of an giogenin 1, an endogenously derived antimicrobial agent [9]. As noted earlier, to effectively partake in these cellular processes primary bile acids need to be modified into secondary bile acids by a subset of gut microbiome through deconjugation and dehydroxylation metabolic processes. But, these normal functions of the gut microbiome may not be the case, in the event of its alteration secondary to gut dysbiosis. The consequences of bile acid dysregulation and the involvement of FXR and TGR5 in liver disease secondary to gut microbiome changes have been reviewed [7].

Secondly, dendritic cells play key roles in enteric innate immune regulation when activated by an appropriate stimulus such as local intestinal inflammatory signals and liver-dependent signals. Of note, the liver squirts large amounts of retinol in bile and the subsequent release of bile into the intestine does not only induce retinoic acid receptor (RAR)-mediated activity in dendritic cells but also activate dendritic cells to express surface receptors which have the potential to attract gut-specific T cellsto activate entericinnate immune regulation[10]. Dendritic cell-mediated immuno-surveillance becomes impaired in liver disease, especially when retinol releasing capacity of the liver becomes compromised secondary to chronic leakage of damaging nosae such as LPS and bacteria DNA into portal circulation as a result of gut dysbiosis.

Gut Disruptors

Diverse substances have the potential to alter the gut to disrupt entero-hepatic interaction. For ease of explanation we categorize them into endogenous and exogenous factors. The endogenous factors are mainly products of host responses to damage or infection which tend to be deleterious to the host, a scenario akin to self-directed anger. They may include pro-inflammatory cytokines, *de novo* biosynthesis of alcohol and its secondary metabolites, metabolic errors culminating from metabolite depletion or loss of function mutations in some genes for enteric enzymes as well as cell type-specific changes. On

the other hand, the exogenous factors among other things may include dietary factors, alcohol use, antibiotic misuse, polypharmacy, food additives, food coloring agents, food preservatives, some solvents and refined westernized foods. Essentially, the type, nature, and physicochemical composition as well as the frequency and duration of exposure of these exogenous factors to the gut, possibly may alter the gut, most often the gut microbiome. For instance, in humans, it is believed that, intestinal epithelial cell type as well as microbiome composition could be determined in the course of time by long-term reliance on a particular type of diet. For example, over reliance on fatty and protein diets was shown to favor Bacteroides-dominated gut microbiome while reliance on carbohydrate diet favors Prevotella-dominated gut microbiome [9]. Thus, the gut microbiome is the most susceptible to alteration in terms of relative abundance and diversity and this may have far reaching consequences for the health of the host. It has long been suspected that the microgenome inherited from conception could predict not only the risk of entero-hepatic disease but also the health status later in adult life. So, irrespective of agent origin, either endogenous or exogenous, they have the potential tointeract synergistically at multi-level to modify the gut and the extra intestinal organs to increase risk of entero-hepatic disease.

Perturbation of Intestinal Brush Border

On the apical region of enterocytes is a sea of actin-fortified membrane protrusions known as microvilli which form the intestinal brush border. Functionally, the intestinal brush border does not only provide increased absorptive surface [11] for the assimilation of nutrients, but also harbor so called brush border enzymes (glycosidase, phosphatase, lactase, and peptidases) [12-14] as well as provision of niche for some enteric microflora, which play crucial roles in energy metabolism, and host defense [15].

However, perturbation or structural and functional alteration of the intestinal brush border as is the case in gut dysbiosis[16], does not only leads to malabsorption of nutrients, and osmotic diarrhea but also presents far reaching consequences for human health [17], particularly disorders of the gastrointestinal and extra-intestinal organs, of which the liver is integral. For example, loss of the intestinal brush border function was linked to attaching and effacing bacteria (EPEC) infections, microvillus inclusion disease (MVID), celiac disease [17-19], sucrose-isomaltase, lactase, trehalase, enteropeptidase and γ-glutamyl transferase related malabsorption syndromes [20,21]. Similarly, pathogenic bacterial overgrowth, which is a key hallmark of gut dysbiosis was long linked to binding of microbiome-derived lectins to the oligosaccharides

of microvillus membrane of the intestinal brush border [22], while enterotoxins specifically B subunit of the enterotoxin of *Vibrio cholerae* bind to glycolipid receptors on microvillus membrane of the brush border which by a series of cellular events including activation of adenylate-cyclase produce diarrhea just as it occurs with *E. coli* and *Yersia* thermo-stable toxins [23]. Careful assessment of intestinal brush border at the cellular and subcellular levels may provide leads to early detection of entero-hepatic disease.

Derangement of Gut Epithelium

The intestinal epithelium comprises the mucus and epithelial layers. The mucus layer traps and provides niche for useful microbiota as well as minor pathogenic ones. The epithelial layer made up of enterocytes guard against translocation of bacteria, their products as well as luminal antigens [24,25]. Essentially, the completeness of the intestinal epithelium is a function of its ability to serve as a physical interface to selectively permit absorption of some biologically important molecules, while at the same time guard against the entry or leakage of potentially damaging nosae such as viable pathogenic bacteria, bacteria DNA, endotoxins, PAMPs and DAMPs into local enteric sites as well as portal and systemic circulations. To achieve this, the enterocytes are closely held to each other by cell-cell contact complexes including gap junctions (GJs), adherens junctions (AJs), tight junctions (TJs), and desmosomes,

and this kind of anatomical arrangement renders the epithelial layer into an impervious but a selective physical barrier to regulate trafficking of molecules across the intestinal epithelium [26,27]. The major enterocyte-enterocyte junctional transmembrane proteins involved in the gut epithelial barrier function includes but not limited to occludin, zonula occludens (ZO-1), claudin-1, claudin-2, and connexin. Exhaustively, the roles of these junctional proteins as well as the complexes are well explained in relation to various gut-liver related disorders [3,28]. Also, the functions of the gut epithelium depends on local factors e.g. secretory IgA, payer's nodules,intramucosal lymphocytes; reticuloendothelial system, and host-intestinal microflorainteractions [29,30]. Breakdown of the gut epithelium perhaps represents the second most crucial pathological event after pathogenic bacteria overgrowth which underlies gut-related liver disease. Of note, disruption of the gut wall or epithelium creates the opportunity for hitherto, non-absorbable products to gain unregulated entry across the gut barrier into underlying intestinal tissues. Many of such substances including viable bacteria, endotoxins, PAMPs, DAMPs and secondary metabolites of these substances get access to intestinal tissues such as the lamina propria to cause chronic entero-hepatic inflammation. Detection of LPS, a major PAMP and bacteria DNA in portal circulation and in Mesenteric Lymph Nodes (MLNs) as is currently receiving much attention may be good determinants of the extent of bacterial translocation and the risk entero-hepatic disorders. Also, the

expression pattern of cell-cell junctional proteins (occludin, ZO-1, claudin-1, claudin-2, and connexin) could be used as surrogate endpoints or biomarkers for early detection of gut dysbiosis and the risk of liver disease.

Gut Microbiome Alteration

Gut wall dysbiosis entails complete disruption of the functional and structural integrity of the intestinal epithelium so much so that, it does not only affect local functions of the gut but also those of other systems connected to the gut, especially that of the extra-intestinal organs. Under normal physiological conditions, the gut provides an important niche for a diversity of microorganisms. The habit of these microorganisms help to maintain gut integrity in terms of barrier function, absorption of nutrients, biosynthesis of some essential nutrients, energy metabolism, and homeostatic regulation of the host innate and adaptive immunity as well as liver disease [31]. It must be stressed that, these normal functions of the gut reflects the abundance and phylogenetic diversity of the microbiota. For example, symbiotic and commensal bacteria species dominate gut microbiome under normal conditions. However, undue disturbance of the gut milieu in the event of ingestion ofinjurious substances mayalter gut microbiome and this may provide avenue for the proliferation of pathogenic bacteria and the onset of entero-hepatic disorders.For instance, bacteria belonging to *Enterobacteriaceae*, *Enterococcae* and *Bacillaceae* families were reported to

have increased in a gut model of liver disease[3]. Almost all liver disorders especially those related to the gut alter gut microbiome leading to bacteria overgrowth [32-34], but the extent of microbiome change and characteristics are etiology-dependent [3]. Of note, several operational taxanomic unit (OTU)-dependent classificational analysis have shown increased growth of bacteria species at the division taxanomic rank including *Firmicutes* (Genera; Lactobacillus, Dorea, LachaspiraraceaeIncertae, Sedis), *Actinobacteria* (Genus: Coriobacteriaceae) in a chemically induced model of liver disease [3,35]. Similarly, *Firmicutes* increased inversely with *Bacteroidetes* in an obesity-induced model of fatty liver disease in mice [36]; *Gammaproteobacteria* and *Erysipelotrichi* were increased in humans with choline depletion [37]; decreased *Firmicutes* with increase in *Bacteroidetes* and *Verrucomicrobia* in a model of alcoholic steatohepatitis [35]; *Bifidobacteria* decreased while *Enterococcus faecalis* and *Enterobacteriaceae* increased in the feces of hepatitis B infected people. For further reading on division-wide distribution of gut microbiome in relation to etiology-specific liver disease, we suggest that readers consult [2].

Errors in Metabolism Secondary to Gut-Microbiome Changes

Certain metabolic species are crucial for homeostatic interaction between the gut and the liver; however, multi-etiology mediated changes in gut microbiome and

subsequent depletion of these metabolic species have far reaching implications for liver disease pathogenesis. Key among these is choline and its depletion. Depletion of choline secondary to altered gut microbiome dominated by a subset of gut microbes leads to errors in hepatic metabolism of lipids and the reflex accumulation of triglycerides in hepatocytes. Indeed, this has the potential to induce fatty liver disease (FLD). This has been substantiated in some animal and clinical studies. For instance, it was shown that high calorie diet induces gut microbiota changes leading to overgrowth of a specific microbiota which has the potential to exhaust bioavailability of choline (reduction in plasma levels of phosphatidylcholine) via conversion of dietary choline to methylamines [38]. Similarly, it was confirmed in humans with choline deficiency, that indeed choline-deficient diets could induce gut microbiome changes to increase risk of FLD [37]. The mechanism of choline deficiency-induced FLD is possibly mediated by phosphotidylcholine in view of the crucial role it plays in hepatic biosynthesis of very low density lipoproteins [39]. Indeed, the risk of non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) are tied to choline metabolism secondary to gut dysbiosis. Choline depletion or decrease in plasma levels of phosphatidylcholine may therefore be suitable for risk assessment of patients suspected of NAFLD and NASH particularly when there is enough evidence of gut dysbiosis.

Alteration of Intestinal Motility

A well-coordinated intestinal motility is indispensable for normal functioning of the gastrointestinal system as well as its allied extraintestinal organs. Indeed, well-coordinated intestinal motility ensures not only transit of intestinal contents along the gastrointestinal tract within limits, but also it helps mechanical digestion, mixing of intestinal contents with enzymes, absorption and containment of pathogenic bacteria overgrowth. The afore-mentioned may not be the case, in the event of dysbiosis of the intestinal motility by any cause. Dysbiosis of intestinal motility was associated with severity of liver disease in stratified cirrhotic patients [40], and before this observation, Madrid and colleagues had reported that intestinal motility dynamics may be associated with crucial risk factors of HCC including cirrhosis [41]. Also, delayed intestinal transit time was linked to cirrhosis [40,42] and the underlying cause was speculatively linked to disruption of integration of the autonomic and enteric nervous system secondary to liver disease [43]. But there are other studies with contrary reports. For instance, increased intestinal transit time was observed in cirrhotic patients with underlying portal hypertension [44], perhaps to suggest divided opinion on the exact role of perturbed intestinal motility in liver disease.

Nevertheless, some links are apparent, for instance, dysbiosis of intestinal motility may lead to pathogenic

bacteria overgrowth [45] to increase bacterial translocation in the peritoneal cavity which possibly increase risk of spontaneous bacterial peritonitis (SBP) [46]. Given the apparent lack of consensus on the exact role of perturbed intestinal motility in liver disease, it is appropriate that future studies take a critical look at this. But it appears that the role of intestinal motility in entero-hepatic disease may be etiology as well as stage-specific.

Loss of Gut Endogenously Derived Anti-Microbial Capacity

The activities of some specialized gut cells regulate the relative abundance of gut microflora including bacteria, viruses, and fungi within limits [47]. These specialized cells including but not limited to Paneth cells, enterocytes, and neutrophils have the potential to secrete antibiotic-like molecules to create a physical buffer zone between luminal microflora and the intestinal epithelial surface. The antibiotic-like secretions (also referred to as host derived peptides; HDPs) have broad as well as selective antibiotic actions against pathogenic microbial overgrowth. The HDPs also play integral role in the host innate immune regulation. For example, HDPs could normally be expressed by neutrophils in quick response to pathogenic bacteria overgrowth and leakage of LPS [48]. However, damage to these specialized cells (Paneth cells, neutrophils, and enterocytes) culminating from exposure of the gastrointestinal tract to dietary factors as well as host-de-

pendent factors denies the gut of these key protective and regulatory roles setting the stage for pathogenic bacterial overgrowth and the subsequent bacterial translocation, arguably the two pathological events most implicated in gut dysbiosis, endotoxemia and liver inflammation. For instance, loss of HDPs or their secretory cells as a result of gut dysbiosis was linked to bacteria overgrowth, intestinal barrier dysfunction and bacteria translocation in a number of chronic liver diseases [35,49]. Similarly, decrease in regenerating islet-derived 3 (Reg3), a c-type lectin secreted by Paneth cells and enterocytes, was associated with increased bacteria colonization of intestinal epithelial surface [48] just as decrease in Reg3g in mice was linked to suppression by alcohol [35]. Also, low expression of cryptidin 5/7 correlated with diminished antimicrobial activity against ileal commensal bacteria strains[49].

Loss of Gut-Dependent Inflammasome-Mediated Immune Regulation

There are multi-protein complexes within the cytoplasm of enterocytes comprising nucleotide-binding domain and leucine-rich repeat containing proteins (NLRP), also known as inflammasomes. Importantly, inflammasomes constitute an inbuilt mechanism by which the intestinal epithelium detect and respond appropriately to endotoxin-induced intestinal inflammation and microbial invasion. Essentially, inflammasomes are able to sense PAMPs and DAMPs of both endogenous and exogenous

origin and regulate their interaction with pro-inflammatory cytokine effectors such as IL-1β and pro-IL-18 [2] to prevent microbial invasion and intestinal inflammation. Specifically, pro-IL-18 plays key role in inflammasome-dependent surveillance and regulation of microbial invasion as well as anti-inflammatory activities within the intestinal milieu. Expression of inflammasomes and pro-IL-18 has therefore become crucial for assessment of gut functional integrity. In severe intestinal dysbiosis both pro-IL-18 and inflammasomes are degraded to physiological levels insufficient to elicit the right responses for local protection, a pathological event which might in part underlie bacterial overgrowth and intestinal inflammation. Of note, decrease in NLRP3 and NLRP6, two kinds of inflammasomes crucial in regulation of microbial overgrowth within the intestinal microenvironment were linked to intestinal dysbiosis and inflammation of the colon in rodents, with the chemokine CCLS implicated [2].

Entero-Hepatic Inflammation and the onset of Hepatic Fibrosis

LPS and bacteria DNA derived from gut dysbiosis leak into portal circulation to mount chronic stimulation of hepatic cells, particularly hepatic stellate cells (HSCs) and Kupffer cells. LPS/TLR4-and-bacteria DNA/TLR9-dependent signaling induce hepatic inflammation and overproduction of TNF-α. TNF-α in turn induces the release of TGF-β in HSCs and KCs, at the same time

LPS increases the sensitivity of HSCs and KCs to TGF-β stimulation. TGF-β mediates dysregulation of extracellular matrix (ECM) metabolism, which is manifested by increased production and deposition of ECM in hepatic sinusoidal space (Figure 2). This pathological event further provides the signal for the infiltration of many cell types and further release of cytokines, chemokines and transcription factors involved in the transcription of oncogenes.

Entero-Hepatic Inflammation Induces M1/M2 Switch

LPS-and-bacteria DNA-dependent activation of entero-hepatic inflammation does not only chronically activate entero-hepatic cells, but also succeed in reprogramming entero-hepatic cells which in turn switches M1/M2 cytokine profiling leading to compromised host immune regulation, reduced immuno-surveillance, escape of HSCs, and injured hepatocytes leading to the initiation and progression of liver disease. Monocytes upon appropriate stimuli differentiate into various kinds of macrophages. But LPS and Th1 cytokines e.g. IFN-γ can promote M1-type macrophage polarization, while many cytokines over expressed in tumor microenvironment such as IL-10 does not only inhibit M1 polarization but also promote M2-type polarization [50]. Indeed, M2-type macrophages by diverse mechanisms could promote tumor growth, neoplastic cell survival, invasion, and metastasis. Also, tumor

associated macrophages (TAM) can promote suppression of host adaptive immunity. For example, TAM stimulates the release of IL-10, IL-6, and TGF-β, which in turn do not only inhibit maturation of tumor-associated dendritic cells (TADCs) but also their activation. TAM induces CCL18, which attract naïve T cells to TADCs in TAM-rich tumor microenvironment to induce their anergy [51]. Similarly, TAM fails to trigger Th1-mediated immune response but rather induce conversion of $CD4^+$ T cells into T regulatory cells (Treg), which in turn suppresses T cell-mediated anti-tumor activity [52]. TADC, T lymphocytes, and Treg cells abundant in the tumor microenvironment are rendered immature to function properly as antigen presenting cells (APCs).

Chemo kinesare implicated in remodeling of tumor microenvironment by their potential to promote immune cell infiltration, angiogenesis, tumor cell growth, survival, and metastasis [53]. In infection-related inflammation, such as occurs in gut dysbiosis, leucocytes are the most significant infiltrants and the key sources of chemokines. Tumor cells, CAFs, endothelial cells, and leukocytes can produce a variety of chemokines specific to cancer subtype, and this perhaps emphasizes the prognostic value of chemokines in cancer treatment [54,55].

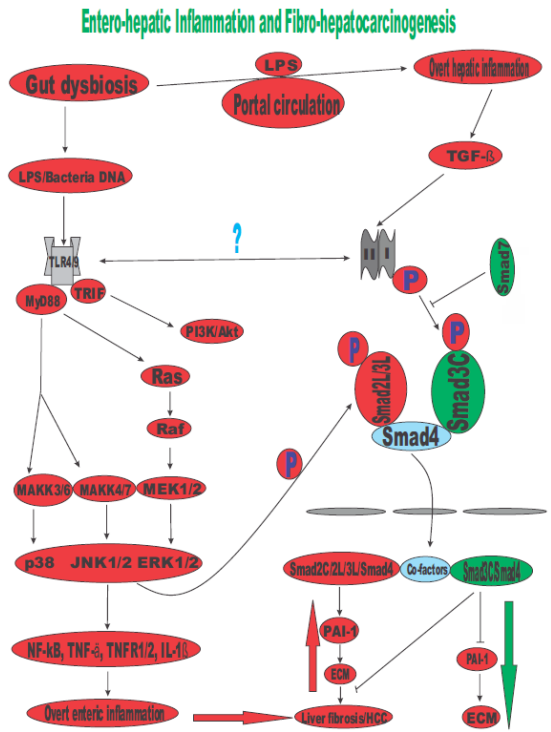

Figure 2: An illustration depicting the interplay of inflammatory and fibrogenic molecular underpinnings in entero-hepatic disease. Intestinal derangement results in the release of LPS and bacteria DNA. LPS and bacteria DNA via portal circulation gain access to hepatic cells. LPS/TLR4 and bacteria DNA/TLR9-dependent signaling induces overt inflammatory response in the entero-hepatic axis, induce recruitment of cells, and also crosstalk with fibrogenic TGF-β/Smad signaling via MAPK-dependent linker phosphorylation of Smad2/3. Upregulation of PAI-1 gene, one out of the many target genes of dysregulatedTGF-β/Smad signaling is the main pathological determinant in liver fibrosis. LPS/TLR4, bacteria DNA/TLR9, TGF-β/Smad, and MAPK signaling cascade mediate synchronization of entero-hepatic inflammation and hepatic fibrogenesis to promote liver fibrosis and risk of hepatocarcinogenesis. DNA (De-oxyribonucleic acid), LPS (Lipopolysaccharide), MAPK (Mitogen activated protein kinase), PAI-1 (Plasminogen activator inhibitor-1), Smad (Small mother against decapentaplegic), TGF-β (Transforming growth factor beta), TLR (Toll-like receptors).

Molecular Underpinnings of Entero-Hepatic Inflammation and Fibrogenesis

The initial signal for intestinal inflammation comes from leakage of LPS and bacteria DNA into local intestinal sites such as MLN and portal vein secondary to pathogenic bacteria overgrowth and increased permeability of intestinal epithelium. Subsequently, activation of LPS/TLR4-and-bacteria DNA/TLR9-dependent signaling generates an inflammatory signal within the intestine. Response to these signals draws other inflammatory and immune cells. Importantly, Toll-like receptors (TLRs) play crucial roles in this initial pathogen-induced inflammatory process. TLRs are first targeted by inflammatory nosae including those from immune cells, bacteria, viruses which lead to activation of a sequence of intracellular signaling to activate NF-kβ [56,57]. Next, TNF-α is released under inflammation-induced conditions via NF-kβ mediation, though it is now apparent that TNF-α and NF-kβ exhibit co-activation, perhaps due to similarity in their biosynthesis. For instance, activation of Rac/Cdc42 and JNK/p38 pathways leads to activation of several transcription factors including NF-kβ, ATF-2, c-jun, CREB, ELK-1 leading to the transcription of TNF-α gene [58]. Also, TNF-α-mediated activation of NF-kβ was reported to exert anti-apoptosis effect via induction of Bcl-2 and superoxide dismutase [58]. Again, TNF-α is produced by a wide range of cells in response to a diversity of stimuli [58]. Thus, it is over expressed in inflammation-induced

tumor cells [59]. Expression of TNF-α also mediate cell recruitment, for example leucocyte infiltration was linked to TNF-α activity. Clearly, TNF-α displays pleiotropic action under varied conditions and this may reflect its binding receptor types [60]. Two of the main binding receptors of TNF-α are (TNF-R) pSS/ TNF-R1 and TNF-Rp75/ TNF-R2 [60]. TNF-Rp55-dependent activation leads to recruitment of intracellular adaptor proteins which activate many signal transduction pathways such as FADD/ Caspase8/Caspase3/MAPK/AP-1, and NF-kβ [61]. Whiles TNF-Rp75 activation leads to stimulation of MAPK, JNK/ AP-1, NF-kβ, but not FADD/Caspase8/Caspase3 [61]. MAPK/JNK/AP-1 and NF-kβ-dependent activation leads to expressions of many genes including those of IL-1, IL-6, chemokines, and adhesion molecules. Further, TNF-α induces the release of other cytokines (COX-2, IL-1, IL-6), stromal cell-derived factor (SDF-1/CXCL12) [62]. Out of the TNF-α-induced inflammatory cytokines, particularly IL-6 plays key role in entero-hepatic inflammation mainly through recruitment of other cells. For example, IL-6 induces expression of ICAM-1, which is involved in the recruitment of neutrophils, and granulocytes under inflammatory conditions and also activation of gut epithelial cells [63,64]. The switch from intestinal inflammation to hepatic inflammation and fibrogenesis, which is pivotal in hepatocarcinogenesis may possibly be governed by LPS/TLR4/TNF-α/TNF-Rp75 and the MAPK-regulated TGF-β signaling, in view of the many reported roles

of TGF-β and MAPK pathways in hepato carcinogenesis [65,66].

TGF-β/Smad Signaling: A Complex Link Between Enteric Inflammation and Hepatic Fibrogenesis

TGF-β is a promiscuous cytokine with complex functional diversity. It partakes in almost all cellular processes in metazoans including growth, developmental timing, homeostasis, and wound healing as well as disease pathogenesis [66-68]. Many cells including HSCs, immune cells, Kupffer cells and inflammatory cells may release TGF-β under an appropriate inflammatory and fibrogenic stimuli. Initially, TGF-β is produced in an inactive form under the regulation of many factors including thrombospondin-1 and MMPs [68]. Biosynthesis and spatio-temporal release of TGF-β among other factors may be determined in part by cell/tissue/organ type and species type. Interaction between TGF-β and pro-inflammatory signals is perhaps evident in the expression pattern of TGF-β pseudo receptor BMP and activin membrane bound inhibitor (BAMBI) in response to inflammatory signals. For example, down-regulation of BAMBI was shown to be secondary to LPS and TNF-α activity [69]. Natural killer (NK) cell-dependent HSC apoptosis was disrupted by TGF-β activity [70] and this could lead to survival and proliferation of HSCs. IL-1β induces upregulation of TIMP-1 in HSCs, while it down regulates BAMBI [71]. TNF-α activ-

ity upregulates TIMP-1 expression while down regulating BAMBI leading to dysregulated HSC apoptosis [72,73] and their escape. In HSCs TGF-β decoy receptor BAMBI is downregulated by TLR4-dependent signaling leading to enhanced TGF-β-dependent activation of HSCsand the mechanism was linked to TNF-α-and TLR4-dependent down regulation of BAMBI [32,74].

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