Mini Review

The Multitasking Behavior of Cholangiocytes in the Reaction to Liver Damage

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Abstract

Cholangiocytes are the epithelial cells lining the bile ducts. Within the wide functional heterogeneity displayed by cholangiocytes through the different portions of the biliary tree, ductular cells of the finest peripheral ramifications, in strict contact with the canals of Hering, possess a reactive phenotype. This phenotype is essential for generating the hepatic reparative system, a multicellular complex including epithelial, mesenchymal and inflammatory cells, whereby they extensively communicate by mutually exchanging a variety of paracrine signals. Herein, we review the most recent insights on the molecular mechanisms underlying the ability of reactive cholangiocytes to orchestrate the development of this sort of ‘multiethnic society’, whose persistent activation is a critical determinant of liver disease progression.

INTRODUCTION

Cholangiocytes are the epithelial cells that line the biliary tree, a complex system of ducts divided into an intrahepatic and an extrahepatic tract, connecting the liver to the intestine. After the hepatocytes, the other liver epithelial cell, cholangiocytes represent the second most common cell type in the liver (about 5% of the total liver cell mass). Similar to hepatocytes and their ‘functional regionalization’ through the Rappaport’s acinus zones, cholangiocytes display a wide range of functions, which markedly vary upon their topographic localization within the biliary system [1,2]. In Table 1 the current nomenclature of cholangiocytes lining different bile ductal structures in normal and diseased conditions is outlined. While cholangiocytes lining the larger ducts are essentially involved in absorptive and secretory activities by which they modify the primary bile secreted by hepatocytes, cholangiocytes of the smallest bile ductules, abutting the canals of Hering (terminal ductules), possess peculiar and sophisticated biological properties, such as plasticity, reactivity and stemness, enabling them to orchestrating the reaction to liver damage. Plasticity is the ability to modify the native epithelial phenotype by acquiring some traits typically expressed by mesenchymal cells, reactivity is the propensity to set in motion the inflammatory response elicited by liver damage, whilst stemness is the attitude to behave as progenitor cells with the potential ability to differentiate bidirectionally into both hepatocyte and cholangiocyte lineages [3,4]. All of these features can be variably activated in response to liver damage, regardless of its etiology, and lead to the ductular reaction, which is closely associated with the activation and proliferation, at different degrees, of the hepatic progenitor cell (HPC) compartment, by which it is directed.

The hepatic reparative system

Ductular reaction is a stereotyped histological lesion common to different forms of chronic liver injury underpinning the generation of the ‘hepatic reparative system’ (Figure 1). This...
is dynamic, multicellular, morpho-functional complex, where ductular epithelial cells, developing as irregular strings along
the margins of the portal tract, acquire a ‘reactive’ phenotype characterized by the de novo expression of a variety of cytokines,
chemokines, growth factors and angiogenic factors coupled with a rich receptor equipment [1,3,5]. These phenotypic changes enable ductular reactive cells (DRC) to establish intense paracrine communications with multiple stromal cell types, including myofibroblasts, inflammatory cells and endothelial cells. Ductular reaction is the mirror of the severity of liver disease, since several studies indicate a close correlation between the expansion of DRC and the extent of hepatocellular and/or biliary injury. In fact, the correlation between the expansion of the DRC and HPC population and the stage of portal fibrosis has been demonstrated in several liver pathologies such as Non-alcoholic fatty liver disease (NAFLD) [6], chronic liver diseases [7], chronic viral hepatitis [8], and genetic cholangiopathies [9].

Epithelial cell elements engaged in the hepatic reparative system

As previously stated, HPC and DRC are the main effector cells driving the response to liver damage in both chronic and acute disease conditions. HPC are small cells, single or in small clumps, with an oval shape and scant cytoplasm, confined to the periportal niche, where they lay in close contact with the canals of Hering, the boundary line between hepatocytes and cholangiocytes. HPC are bipotential cells, able to differentiate into either the biliary or the hepatocyte lineage [10]. In humans, differentiation towards hepatocytes occurs via intermediate hepato-biliary cells (IHBC), whereas differentiation towards the biliary lineage leads to the formation of DRC. DRC are epithelial cells organized into irregularly shaped, tube-like structures, tightly anastomosed in the formation of DRC. DRC are epithelial cells organized into irregularly shaped, tube-like structures, tightly anastomosed each other, often without a recognizable lumen. HPC, IHBC and DRC can be identified by a specific immunophenotype (Table 1). In addition to the stem cell markers c-Kit and CD34, HPC express neuroendocrine features, together with cholangiocyte and hepatocyte markers. In contrast, DRC display the expression of the typical biliary lineage markers, [10-13], in conjunction with the de novo expression of several neural markers [14,10,13], whilst the epithelial membrane antigen (EMA), a marker of mature biliary epithelium, is down-regulated [13].

Molecular factors regulating the development of the hepatic reparative system

According to the classical view, the HPC compartment is activated only when the proliferative ability of mature hepatocytes is compromised because of the severity of the necro-inflammatory liver damage, which precludes them to replace the parenchymal loss [15]. However, this is true only in experimental conditions, since in human liver diseases, HPC activation may occur even in milder forms of liver damage, regardless of the level of inhibition of the hepatocyte replication. The factors triggering the HPC activation and then regulating the expansion of the DRC compartment are an area of deep and continuously evolving investigation. Interactions with the extracellular matrix (ECM) components are likely pivotal determinants of these mechanisms [16]. This process depends upon a complex interplay between paracrine signals released by HPC and DRC leading to the activation of a fibrogenic program and changes in the ECM composition aimed at favoring reciprocal cell-cell interactions. In fact, generation of the hepatic reparative complex first requires the detachment of HPC from the periportal niche and subsequently, their spreading as DRC along the boundaries with the lobular parenchyma to reach areas whereby they can critically interact with the other cooperating cell elements, mostly mesenchymal and inflammatory cells. During this process, several factors are emerging to play a crucial function amenable to targeted intervention.

Neural cell adhesion molecule (NCAM)/Polysialic Acid: The neo-expression of the neuroendocrine marker NCAM and its post-transcriptional modification is a mechanism of particular relevance for HPC spreading and DRC generation. NCAM is a surface glycoprotein mediating homo (cell-cell) and heterotypic (cell-matrix) interactions during the development of several epithelial tissues, including the nervous system, lung and gut. In the liver, NCAM is transiently expressed during fetal development by the embryonic structures from which the intrahepatic bile ducts originate, i.e. the ductal plates. NCAM could also act as a Polysialic acid (PolySia) carrier, given its ability to bind up to 100 PolySia residues. Following conjugation with PolySia, NCAM changes its adhesive properties to anti-adhesive due to the size of the multiple PolySia chains and their high hydrophilic content and may behave as a sort of “lube oil” [17,18]. Therefore, once bound to PolySia, the anti-adhesive NCAM allows HPC to acquire migratory properties, by which they leave the periportal niche and reach the margins of the portal tract, where, as DRC, they can cross talk with other NCAM-expressing cell types, mainly fibroblasts, to mount the hepatic reparative response. The cooperation among the different cell types populating the portal microenvironment depends on the interplay between ligands and receptors reciprocally expressed by DRC and mesenchymal-derived cell types. As mentioned above, DRC may secrete a wide range of soluble factors, including growth factors, such as angiopoietin-1, connective tissue growth factor (CTGF), endothelin-1, platelet-derived growth factor (PDGF)-BB, PDGF-DD, and vascular endothelial growth factor (VEGF)-A [19-22], cytokines and chemokines (i.e. CINC, IL-6, IL-8, MCP-1, Osteopontin (OPN), TGFβ2, TNFα) [3,5], and other pro-inflammatory molecules (nitric oxide and reactive oxygen species) [5, 23-25]. Thanks to these huge secretory activities, DRC orchestrate the recruitment, activation and proliferation of multiple cell types (myofibroblasts, macrophages, endothelial cells, immune cells), with which they build the complex machinery responsible for liver repair and regeneration.

Osteopontin (OPN): In the extensive cross talk centered on DRC, OPN is a mediator attracting increasing interest. OPN is a pleiotropic factor acting as either cytokine or ECM protein able to direct cell motility and adhesion, as well as the response of T-lymphocytes and macrophages in inflammation and immunity. OPN was originally found to be frequently over-expressed in hepatocellular carcinoma, where it appears to be involved in the modulation of tumoral cell growth, invasion and metastasis. Two recent studies demonstrated OPN over-expression by DRC in different mouse models of chronic liver injury with evolution to fibrosis, Opn-/- mice treated with thioacetamide or bile duct ligation [26], and C57BL/6 feed with 3,5-Diethoxycarbonyl-1,4-
dihydrocollidine or Methionine-choline deficient diets [27]. In these experimental models, OPN is a critical player promoting the activation of the HPC compartment and its expansion leading to the development of ductular reaction. This mechanism is relevant for the progression of liver fibrosis; in fact, OPN neutralization obtained with OPN-specific aptamers resulted in a milder fibrotic response associated with a reduced extent of HPC and DRC.

Connective tissue growth factor (CTGF)/CCN2 and CCN1: Another driving factor recently emerged in ductular reaction is CTGF. CTGF is a member of the cysteine-rich, secreted, heparin-binding CCN protein family (also called CCN2) able to modulate external signal transduction into cells by interacting with ECM components (fibronectin), cell surface glycoproteins (integrins), and growth factors. This cytokine is fundamental for the modulation of the fibrogenic response occurring during the hepatic reparative response thanks to its ability to synergize with TGFβ1. Moreover, CTGF may promote proliferation, migration and adhesion of several inflammatory cell types, macrophages in particular. In a recent study [22] performed in a CTGF-KO mouse model (Ctgfk/k), CTGF was found to stimulate activation of the HPC niche by interacting with αvβ6 integrin. αvβ6 integrin is a surface glycoprotein expressed by several epithelial cell types in response to CTGF.

<table>
<thead>
<tr>
<th>Structures</th>
<th>Immunophenotype</th>
<th>Morphology (K7)</th>
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<tbody>
<tr>
<td>Normal Liver</td>
<td></td>
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<tr>
<td>Canals of Hering</td>
<td>K7, K8, K18, K19, EpCAM (HEA125), EMA, OV-6, HNF1β, HNF6</td>
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<tr>
<td>Bile Ducts</td>
<td>K7, K8, K18, K19, EpCAM (HEA125), EMA, OV-6, HNF1β, HNF6</td>
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<td>Diseased Liver</td>
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<td>Hepatic Progenitor Cells (HPC)</td>
<td>Biliary markers: K7, K19, EpCAM (HEA125), OV-6, α-fetoprotein</td>
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<td></td>
<td>Hepatocyte markers: K8, K18, Hepar-1, and</td>
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<td>Stem cell markers: CD34, c-Kit</td>
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<td>Neural markers: Chrom-A, NCAM</td>
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<td>Ductular Reactive Cells (DRC)</td>
<td>Biliary markers: K7, K19, EpCAM (HEA125), OV-6, α-fetoprotein</td>
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<td>Hepatocyte markers: K8, K18</td>
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<td>Stem cell markers: c-Kit</td>
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<td>Neural markers: muscarinic acetylcholine receptors</td>
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<td>M1-3, and M5, Chrom-A, NCAM</td>
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<td>Intermediate Hepato-Biliary Cells (IHBC)</td>
<td>Biliary markers: K7, EpCAM (HEA125), OV-6</td>
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<td>Hepatocyte markers: α-1-antitrypsin, albumin, K8; K18</td>
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<td>Neural markers: Chrom-A, NCAM</td>
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to injury, which locally activates the latent form of TGFβ1. Down-regulation of CTGF led to a reduced number of HPC and consequently, to a decreased fibrosis deposition following liver injury. Similar to CTGF/CCN2, another member of the CCN family, CCN1, was recently found to be involved in promoting ductular reaction. CCN1 is a matricellular protein able to modulate several cellular activities, such as proliferation, senescence, apoptosis, migration and adhesion. CCN1 stimulated Jag1 expression and activated the Jag/Notch signaling in cholangiocytes through its interaction with the αvβ3/αvβ5 integrins. This led to an increased cholangiocyte proliferation in vitro, a response prevented by either treatment with specific siRNA against CCN1, or by using different α or β integrin sub-units. Furthermore, using mouse models in which CCN1 expression was deleted, or the CCN1 gene harbored inactivating mutations in the binding site to integrins, the ductular reaction induced by bile duct ligation resulted markedly impaired [28].

Functional effects on cholangiocytes exerted by the inflammatory microenvironment: insights from experimental models of biliary injury

The brisk pro-inflammatory response mounted by ductular reaction is pivotal to not only promoting liver fibrosis and evolution to cirrhosis, but also the further progression to liver malignancies, such as hepatocellular carcinoma or cholangiocarcinoma. Once recruited, a mutual exchange of paracrine and autocrine signals between DRC and the recruited inflammatory cells and fibroblasts sustain and expand the ductular reaction. Among the pro-inflammatory and pro-fibrotic mediators released within the portal microenvironment, IL-6 is a prevalent cytokine, which strongly stimulates cholangiocyte proliferation [29]. The interaction between IL-6 and its specific receptor IL6R expressed by DRC in conjunction with the co-receptor gp130, activates the p42/44 MAPK signaling, the main pro-proliferative pathway in cholangiocytes, in concert with the phosphorylation of JAK1 and STAT3 and consequently the pro-proliferative pathway in cholangiocytes, in concert with proliferation [29]. The interaction between IL-6 and its specific receptor IL6R expressed by DRC in conjunction with the co-receptor gp130, activates the p42/44 MAPK signaling, the main pro-proliferative pathway in cholangiocytes, in concert with the phosphorylation of JAK1 and STAT3 and consequently the activation of the PI3K/AKT pathway regulating the escape from cell death [30,31]. The cooperation between these two pathways is a fundamental mechanism sustaining to the growth of DRC.

Another molecular factor activated by pro-inflammatory cytokines with relevant effects on DRC functions is nitric oxide (NO). The elevated concentrations in the portal area of IL-1β, IL-6, TNFα, and IFNγ may activate the inducible nitric oxide synthase (iNOS) isoform, the enzyme mainly responsible for the transformation of L-arginine in NO, in the colangiocytes. A pathogenic effect of NO on cholangiocyte functions is dual. On the one side, it induces cholestasis by reducing the secretion of Cl- and HC03- mediated by CFTR and AE2, through inhibition of the adenyl cyclase activity, which decreases the cyclic adenosine monophosphate (cAMP) intracellular levels [24,32,33]. On the other side, NO may stimulate neoplastic transformation of the biliary epithelium by inducing DNA damages or inhibiting the controlling functions of DNA repair proteins, in particular of the 8-oxodeoxiguanine DNA glycosylase 1 [34]. This mechanism might be relevant in primary sclerosing cholangitis, a biliary disease at high risk of developing cholangiocarcinoma, where iNOS up-regulation in cholangiocytes is particularly pronounced [24,35].

The harmful effects on cholangiocytes of the pro-inflammatory cytokines mediating the cross talk within the ductular reaction have been recently pinpointed in a mouse model of biliary atresia (BALB/c mice infected with rhesus rotavirus), a disease with a marked hyperplasia of the bile ducts. The treatment of these mice with IL-33, a cytokine predominantly secreted in biliary atresia with profound mitogen effects on different epithelial cell types, stimulated the hepatic accumulation of non-cytotoxic innate lymphoid cells type 2 (ILC2), the subtype of immune cells responsible for a Th2 response [36], IL-33-recruited ILC2 secreted in the portal environment high amounts of IL13, which in turn, promoted and sustained the proliferation of cholangiocytes and the activation of the repair mechanisms through the activation of the YAP and AKT pathways [37]. Therefore, in this biliary injury model, the IL-33/ILC2/IL-13 axis promoted epithelial repair. However, induction of this circuit in mice with constitutive activation of AKT and YAP in bile ducts was oncogenic since it induced cholangiocarcinoma with liver metastases [37].

Congenital hepatic fibrosis (CHF) and the cystic fibrosis liver disease (CFLD) are liver disease conditions characterized by an evolving scarring course, which is driven by the inflammatory microenvironment generated by a ‘reactive’ biliary phenotype caused by genetic defects. In CHF, the genetic mutation in fibrocystin (FPC), a ciliary protein expressed by cholangiocytes, biliary cysts progressively enlarge in association with a dense and worsening fibrosis and a smolder portal inflammatory infiltrate. In a mouse model of CHF, harboring a genetic inactivation of the gene encoding for FPC (Pkhd1(−/−)) [38], inflammatory cells, mostly macrophages, were recruited by FPC-defective cholangiocytes due to the secretion of the chemokines CXCL1, CXCL10, CXCL12 induced by an over-activation of β-catenin, a signalling system emerging as a novel regulator of inflammation [39,40]. In turn, cholangiocytes responded to macrophages-derived cytokines (TNFα and TGF-β) by up-regulating the αvβ6 integrin, which locally activated TGFβ1. Targeting macrophage recruitment in vivo by clodronate was clinically relevant as it reduced the extent of fibrosis, the development of portal hypertension and also the growth of the biliary cysts [41]. These data indicate that in CHF, recruited macrophages stimulate fibrosis along with the expansion of the biliary cysts [41]. In a mouse model of CFLD (CF-KO mice), defects in the cystic fibrosis transmembrane conductance regulator (CFTR), a protein originally known as driver of the Cl-transport across ductal epithelial cell membranes, led in cholangiocytes to an aberrant secretion of several pro-inflammatory cytokines and chemokines (CXCL1, G-CSF, IL-1α, IL-6, LIX, MCP-1, and MIP-2). In CFLD, the reactive, hypersecretory phenotype displayed by cholangiocytes is induced by the activation of the Toll-like receptor (TLR)4/-NF-κB signalling. In addition to stimulating biliary proliferation (IL-1α, IL-6), these mediators promoted the recruitment and activation of both neutrophils (CXCL1, G-CSF, MIP-2 and LIX) and macrophages (MCP-1). Interestingly, in CF-KO mice the interference with the TLR4/NF-κB axis led in vivo, to a significant reduction in the extent of the ductular reaction [42].

CONCLUSIONS

Following acute and chronic injury, the liver stereotypically reacts mounting a reparative response driven by HPC activation.
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