Gene Expression, Vol. 20, pp. 19–23
Printed in the USA.

1052-2166/20 $90.00 + .00
DOI: https://doi.org/10.3727/105221619X15664105014956
E-ISSN 1555-3884
www.cognizantcommunication.com

Thinking Out Loud

If It Looks Like a Duct and Acts Like a Duct: On the Role of Reprogrammed Hepatocytes in Cholangiopathies

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Cholangiopathies are chronic, progressive diseases of the biliary tree, and can be either acquired or genetic. The primary target is the cholangiocyte (CC), the cell type lining the bile duct that is responsible for bile modification and transport. Despite advances in our understanding and diagnosis of these diseases in recent years, there are no proven therapeutic treatments for the majority of the cholangiopathies, and liver transplantation is the only life-extending treatment option for patients with end-stage cholestatic liver disease. One potential therapeutic strategy is to facilitate endogenous repair of the biliary system, which may alleviate intrahepatic cholestasis caused by these diseases. During biliary injury, hepatocytes (HC) are known to alter their phenotype and acquire CC-like features, a process known as cellular reprogramming. This brief review discusses the potential ways in which reprogrammed HC may contribute to biliary repair, thereby restoring bile flow and reducing the severity of cholangiopathies. Some of these include modifying bile to reduce toxicity, serving as a source of de novo CC to repair the biliary epithelium, or creating new channels to facilitate bile flow.

Key words: Hepatocyte; Cholangiocyte; Reprogramming; Transdifferentiation; Cholestasis; Cholangiopathies; Bile ducts

INTRODUCTION

Cholangiocytes (CC) are the epithelial cells that line the biliary tree and are responsible for modulation and movement of bile in the liver. The term cholangiopathies encompasses a wide range of diseases of CC origin with diverse etiologies, including genetic, viral, immune-mediated, inflammatory, infectious, ischemic, and idiopathic; these diseases can occur in infancy, childhood, or adulthood[1]. Despite this heterogeneity, all cholangiopathies share some common mechanisms. Injury to CC results in a multitude of cellular responses, including inflammation, proliferation, differentiation, and repair. If the insult persists or is perpetuated, the above responses may result in abnormal ductular reaction, fibrosis, and/or malignancy[2]. Chronic injury to CC also leads to cholestasis due to impaired bile flow, which can then progress to cirrhosis, hepatocellular insufficiency, and liver failure[3]. Cholangiopathies are associated with high morbidly and mortality and accounted for 47% of pediatric liver transplants and 8% of adult transplants in 2012[4]. Because there are few effective medical therapies to halt disease progression, cholangiopathies represent a major unmet need in clinical hepatology.

PHYSIOLOGY AND FUNCTION OF CC

When bile enters the biliary tree through the canaliculi, it is subjected to both secretory and reabsorptive processes by both small and large CC that result in significant modification of the volume and composition of bile[5]. Secretin, which is released into the circulation after a meal, induces secretion of HCO_3^- from CC through the cAMP-dependent opening of cystic fibrosis transmembrane conductance regulator (CFTR), which in turn induces activation of the Cl^-/HCO_3^- anion exchanger 2 (AE2); alternatively, TMEM16A, a Ca^{2+}-activated Cl^- channel, can regulate HCO_3^- efflux[6–9]. ATP release from CC also stimulates

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both activation of Cl^- channels and increases in $[Ca^{2+}]_I$[10,11]. HCO_3^- drives efflux of water from CC through aquaporin (AQ) channels in the apical membrane, which results in increased biliary volume and enhanced choleresis[12,13]. Maintenance of an alkaline pH in the bile may also protect CC against the accumulation of toxic bile acids that is a hallmark of many cholangiopathies[14–16]. On the other hand, CC also contribute to bile modification through absorption of ions, bile acids, glucose, and other molecules. Unconjugated bile acids passively diffuse into CC and return to hepatocytes (HC) in a process known as cholehepatic shunting, while conjugated bile acids are taken up from bile by the Na^+-dependent transporter ASBT[17,18]. CC also express MRP3 and MRP4, which efflux organic ions from the basolateral membrane, and Mdr1a, which excretes them into the bile[5]. Thus, CC play an essential role in the modification of bile composition and flow.

DUCTULAR PROLIFERATION IN CHOLESTASIS

CC proliferation after biliary injury can be grouped into three major types: typical, atypical, and oval cell. "Typical" CC proliferation results in an increased number of intrahepatic ducts and is confined to portal areas; this type of proliferation is observed after bile duct ligation or feeding of either -naphthylisothiocyanate or lithocholic acid in animal models, as well as in the early stages of chronic cholestasis and after acute obstructive cholestasis in patients[19,20]. "Atypical" proliferation, on the other hand, occurs after chronic exposure to xenobiotics or chemicals such as 3,5-diethoxycarboncyl-1,4-dihydrocollidine (DDC) in animal models and is also commonly seen in patients with prolonged cholestatic liver diseases such as primary sclerosing cholangitis (PSC) or primary biliary cholangitis (PBC)[21,22]. This process is characterized by irregular proliferation of CC that spread into the periportal and parenchymal regions, resulting in ducts that lack a well-defined lumen and are often functionally inefficient[19,20]. Atypical proliferation can also involve an oval cell response, characterized by hyperplasia of bipotential cells with characteristics of both HC and CC[23–27]. The role of these atypical ductules in cholangiopathy is controversial. On the one hand, these atypically proliferating CC may protect against biliary insult or contribute to hepatobiliary repair[2]. However, over time, proliferating atypical ductules can release proinflammatory and profibrotic mediators, which cause activation of cells responsible for extracellular matrix deposition[28–30]. Recent findings indicate that this secretory CC phenotype is a result of cellular senescence, which is induced by injury and stress and results in proliferative arrest[31,32].

HC REPROGRAMMING

HC and CC arise from the same common progenitor in liver development[33,34]. This may in part explain why HC exhibit remarkable plasticity and are capable of acquiring a CC-like phenotype in models where biliary injury is the predominant insult to the liver. Early studies utilizing in vitro organoid culture systems demonstrated that isolated HC exposed to a defined culture medium organize into a distinct histological architecture, with CC covering the surface of the tissue exposed to media[35,36]. Additional studies employing strain-tagged rat HC demonstrated that the CC were derived from HC that have undergone cellular reprogramming and that this phenomenon can be recapitulated in vivo under conditions in which the biliary epithelium is incapable of repair due to toxic injury[37,38]. More recent work utilizing genetic mouse models and lineage tracing has confirmed that HC are indeed capable of converting to a biliary lineage under conditions that induce chronic liver injury and biliary toxicity[39–42]. Importantly, HC reprogramming also has been reported in various cholangiopathies, as evidenced by HC expression of (1) biliary transcription factors[40,43], (2) the ductal marker OV-6[44,45], and (3) CC-specific cytokeratins[46–48]. Some studies have also suggested that the number of HC-expressing biliary markers increases over time during biliary injury[49–51]. Thus, one could hypothesize that as cholestasis progresses, more and more HC are "recruited" to compensate for damage to or loss of the biliary epithelium and that induction of HC reprogramming may be of significance in promoting repair in diseases such as PSC.

FATE OF CC-LIKE HC

Previous studies have shown that HC can transdifferentiate into CC that incorporate into biliary ductules[39–41,52]. This incorporation may occur in two ways: the first is that HC immediately surrounding the dying or damaged CC phenotypically convert to replace the injured epithelium. If that is the case, ducts would be composed of a mosaic of native CC and HC-derived CC that transdifferentiate to maintain tissue continuity. The second possibility is that HC transdifferentiate to form de novo branches of the biliary tree in order to expedite removal of bile from the parenchyma. If that is the case, entire intrahepatic branches would be composed exclusively of HC-derived CC. Studies that evaluate the distribution of HC-derived CC to determine the pattern of incorporation will provide unique insights into the formation and repair of biliary structures during injury.

Similarly, although previous studies have compared the proliferation, clonogenicity, and plasticity of HC-derived CC[41,53–56], the functional capacity of these cells has never been directly compared to native CC. It is likely that once incorporated into ductules, HC-derived CC will perform most functions of native CC, albeit potentially less efficiently due to lack of selective pressure to irreversibly switch to CC. Indeed, when injury is reversed, these

reprogrammed cells can revert back to HC, which is more consistent with metaplasia than transdifferentiation[41]. However, when there is sufficient selective pressure, such as in bile duct paucity, HC will transdifferentiate stably into functional CC. A recent publication has demonstrated that transplanted mouse HC can build a biliary system in vivo by permanently transdifferentiating into mature CC that form functional bile ducts[52].

Of note, some HC-expressing CC markers may never fully convert into CC[55,57]. The role of these biphenotypic, intermediate cells is unclear, although a recent study has indicated that this phenotype endows HC with competence to respond to injury-induced signals[58]. One possibility is that intermediate HC may be able to perform some functions of CC—such as modifying bile and/or forming intermediate pseudochannels, thereby preventing injury progression—while evading CC-directed immune injury. Alternatively, reprogrammed HC may provide prosurvival or proproliferative signals to maintain native CC function, similar to the way that HC can direct the formation of a prometastatic niche by producing myeloid chemoattractants, thus altering the immune microenvironment[59]. Studies characterizing the phenotype and function of these intermediate HC will yield valuable insights into the role, if any, that these cells play in ameliorating biliary injury.

FUTURE DIRECTIONS

Treatment for many types of cholangiopathies is limited and often focused on symptomatic relief and palliative care. The reestablishment of bile flow in diseases such as PSC, PBC, Alagille syndrome, and biliary atresia, either through creation of de novo biliary channels or through adaptation of surrounding HC to perform CC-specific bile modification, may help alleviate the complications associated with cholangiopathies. However, given the plasticity of HC, I propose that it is not an either/or scenario and that reprogrammed HC can have a multifunctional role during cholestasis. For example, reprogrammed HC may do any or all of the following: (1) phenotypically convert to replace injured or senescent CC, (2) transdifferentiate en masse to form de novo biliary branches, and (3) retain their intermediate status and contribute to modification of bile flow/composition or maintenance of CC function in addition to functioning as a reservoir for replacement of CC (Fig. 1).

The last 15 years' worth of research has provided us with a wealth of knowledge on the role and function of HC reprogramming in biliary injury. However, there is much we still do not know—not the least of which is, "why"? With the exception of diseases where a functioning biliary system is absent, CC proliferate robustly in response to biliary injury, at least before the onset of replicative senescence. And yet, HC begin to acquire biliary

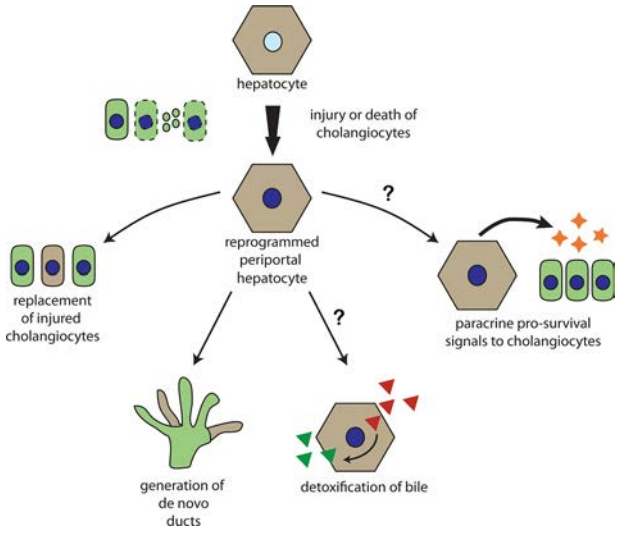

Figure 1. Some possible mechanisms by which reprogrammed hepatocytes (HC) may contribute to biliary repair in cholangiopathies. Question marks indicate that these pathways are hypothesized but not confirmed.

markers very early after induction of cholestasis[39,60]. If CC are for the most part capable of proliferation (and by implication, self-repair), what drives HC to sacrifice their identity to aid neighboring CC? One possibility is that a HC-derived CC make for a better CC than a sick or injured native CC. Generation of de novo healthy CC and ducts from an alternative cell source like HC could potentially increase the number of functional CC and reduce the deleterious effects of atypically proliferating or senescent CC. Another possibility is that a HC-derived CC may be able to evade the immune system. This would be especially relevant in diseases such as PBC, an autoimmune disease that is caused by loss of tolerance to mitochondrial antigens in intrahepatic biliary cells[61]. Still other possibilities include the still-unknown functions of "intermediate" HC, those that express both markers of fully differentiated HC and primitive CC. Could these cells produce regenerative or survival signals for nearby CC? Alternatively, can CC-like HC themselves modify bile through expression of CC-restricted markers like CFTR and AQ that enhance choleresis? Analysis of these and other possibilities will hopefully become the basis for future experimental work.

As is clear from the dearth of established medical treatments for cholangiopathies, studies investigating the mechanisms of biliary repair are desperately needed. Although HC plasticity has become a widely accepted phenomenon in the field, the role and regulation of specific signaling pathways in this process are still largely unknown. A detailed investigation into the mechanisms of reprogramming is essential for developing potential therapeutic targets to improve bile stasis in cholangiopathies.

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