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PII: S2352-345X(22)00240-5
DOI: <https://doi.org/10.1016/j.jcmgh.2022.11.007>
Reference: JCMGH 1113

To appear in: *Cellular and Molecular Gastroenterology and Hepatology*
Accepted Date: 18 November 2022

Please cite this article as: Monga SP, Nejak-Bowen K, DUCTULAR REACTION AND LIVER REGENERATION: FULFILLING THE PROPHECY OF PROMETHEUS!, *Cellular and Molecular Gastroenterology and Hepatology* (2022), doi: <https://doi.org/10.1016/j.jcmgh.2022.11.007>.

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DUCTULAR REACTION AND LIVER REGENERATION: FULFILLING THE PROPHECY OF PROMETHEUS!

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Funding: This work was supported by NIH grants 1R01DK62277, 1R01DK116993, and Endowed Chair for Experimental Pathology to SPM, and R01DK119435 and R01DK103775 to KNB

Summary Word Count: 140

Word Count: 1053

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Many acute and chronic liver injuries exhibit histologically as proliferating cholangiocytes, commonly referred to as ductular reaction. The origin of the cells comprising the ductular reaction is dependent on the injury (hepatocyte versus cholangiocyte) as well as capability of these two-liver epithelial or 'hepithelial' cells to divide and replace injured cells. What is the role of ductular reaction in hepatobiliary injury versus repair remains debated. While ductular reaction has been shown to be source of pro-inflammatory and profibrogenic factors, it has also been shown to contribute towards maintaining hepatobiliary function during injury. In fact, reducing ductular reaction has been shown to exacerbate while promoting it could alleviate liver injury. We discuss some of the studies that highlight various mechanisms through which ductular reaction plays a beneficial role in modulating hepatobiliary health during injury and eventually discuss how regulating such process may have a therapeutic benefit.

Ductular reaction is a common hallmark of many liver pathologies including cholangiopathies, viral hepatitis, and alcoholic and non-alcoholic fatty liver diseases^{1, 2}. Although variable in its histological appearance, ductular reaction is caused usually by proliferation of cholangiocytes or due to transdifferentiation of hepatocytes into cholangiocytes. The ductular reaction can manifest as appearance of multiple small ducts with well- to ill-defined lumen, appearance of multilayered ductular structures or as isolated small cells with biliary markers interspersed in the hepatic parenchyma beyond the portal mesenchyme, referred to as invasive ductular reaction³. Preclinically, such variability in ductular reaction can be seen in response to bile duct ligation, diets that are choline-deficient ethionine-supplemented (CDE), or containing 3,5-Diethoxycarbonyl-1, 4-Dihydrocollidine, among other protocols. Such variable ductular reaction can also be seen in patients with alcoholic hepatitis, primary sclerosing cholangitis, primary biliary cholangitis, viral hepatitis and even non-alcoholic steatohepatitis cases, and the overall significance remains debated and not completely clear^{1, 2}.

Why would ductular reaction occur in such variable hepatic pathologies in the first place? Liver is unique in its ability to regenerate and intriguingly, all constituent cells of the liver participate in the process⁴. Hence a simple explanation for the ductular reaction in many preclinical models as well as in clinical scenarios is that ductular reaction is a reparative response to cholangiocyte injury. When cholangiocytes are the target of an insult that may be genetic, immunological, iatrogenic, infectious, obstructive, or idiopathic, some cholangiocytes die, while the others proliferate and expand in an attempt to repair the injured bile ducts. Thus, in a subset of pathologies, ductular

reaction may be a valuable component of the reparative response to replace dying cholangiocytes. It is conceivable that such repair may be transient and non-perpetuating, especially if an injury is self-limited or is treated. However, if injury to the cholangiocytes persists, ductular reaction lingers to continually replace dying cholangiocytes and may manifest as persistent, variable and atypical ductular reaction. Such forms of ductular reaction may be an attempt to generate *de novo* collaterals to drain bile, or merely generate 'ectopic' cholangiocytes, to compensate for lost cholangiocyte mass and perform some functions as discussed below.

Cholangiocytes line the intrahepatic bile ducts, which serve as a conduit to transport bile from the biliary canaliculi to the extrahepatic biliary tree and small intestines. In this manner, bile, which contains relatively toxic bile acids among other components, stays sequestered from other cells within the hepatic parenchyma. Cholangiocytes also regulate the composition and flow of bile through absorption and secretion of ions especially HCO_3^- , water, glucose and others, into the bile⁵. Unconjugated bile acids can passively diffuse into cholangiocytes and to the hepatocytes via cholehepatic shunting, while conjugated bile acids can be absorbed by Apical Na^+ -dependent bile acid transporter (ASBT). Cholangiocytes express several xenobiotic metabolism enzymes like CYP2E1 and CYP1A2, and also participate in the cholesterol metabolism among many other functions^{6, 7}.

Other than providing cells to replace dead cholangiocytes and 'patch' leaking bile ducts, the ductular reaction could promote repair in an injury setting in three different ways. First, in the setting of intrahepatic cholestasis, a ductular reaction which is observed as number of small duct-like structures that are mostly lined by a single layer

of cholangiocytes and contain lumen, may really serve as collaterals to rid the liver of bile. It is likely that these *de novo* mini ductular structures serve as channels to drain bile into existing intact remnant intrahepatic bile ducts that may be relatively spared by an insult. Indeed, Miyajima and colleagues have shown that when animals were fed diets like CDE, the depth and complexity of the intrahepatic tree increases drastically, with terminal ducts now almost encroaching the hepatic parenchyma in the pericentral zone⁸. As hepatocytes succumb to diets like CDE and there is an ensuing collapse of hepatocyte-lined biliary canaliculi, such ductular reaction may be the only way to collect bile produced by remnant hepatocytes so that it can be eventually transported into the intestines. Second, the increase in cholangiocyte mass irrespective of the presence of a functional lumen, may contribute to some of the static functions of cholangiocytes such as in xenobiotic or drug metabolism as well as cholesterol metabolism. Thus, as bile ducts succumb to pathologic insults, the ductular reaction, even without a patent lumen, may assist in maintaining homeostasis by helping regulate the content and toxicity of bile acids produced in hepatocytes and performing limited but important metabolic functions^{2, 6}. A third way that ductular reaction may contribute to repair during chronic liver injury is the possibility that a subset of cholangiocytes within these structures could give rise to *de novo* hepatocytes. Hepatocytes, which are the major functional units of a liver, are often the main cell type afflicted by a multitude of insults. Normally, such injury results merely in death of some hepatocytes, allowing other hepatocytes to proliferate and replace the dying cells. However, if the injury is chronic and if hepatocyte proliferation is compromised due to senescence, epigenetic or signaling aberrations, or other mechanisms, hepatocyte markers begin to appear in a subset of cholangiocytes

within a ductular reaction, demonstrating the likelihood of reprogramming and eventually transdifferentiation of such cholangiocytes into hepatocytes⁹. This stochastic event has been shown to yield small hepatocytes that eventually proliferate, expand and mature, to eventually contribute to hepatocyte repair as has been shown by careful fate-tracing studies⁹⁻¹¹. When hepatocyte-specific β -catenin knockout mice which are incapable of normal hepatocyte proliferation, but with intact cholangiocyte β -catenin expression, have been administered CDE diet for 2 weeks followed by recovery on normal diet, a very small subset of β -catenin-positive cholangiocytes have been shown to give rise to β -catenin-positive small hepatocytes, which then proliferate and repopulate the liver (Figure 1)^{10, 12}. Such cell repopulation has been followed for several months after cessation of injury and has been found to be both durable and non-transforming¹². While heterogeneity in cholangiocytes is well documented, it is presently unclear whether only a small subset or all cholangiocytes possess comparable capability to transdifferentiate into hepatocytes, since both of these 'hepithelial cells' are derived from a common progenitor during development¹³⁻¹⁵. Since ductular reaction, is often rampant in many clinical pathologies which are primarily hepatocyte-directed such as NASH and alcoholic hepatitis, strategies to promote cholangiocyte-to-hepatocyte transdifferentiation through such mechanisms as epigenetic or signaling modulation, may have notable clinical benefits^{13, 16}.

In conclusion, in a subset of liver pathologies, ductular reaction may have many beneficial effects, thus promoting the resilience and repair of this Promethean organ. Facilitating the process of ductular reaction-to-hepatocyte transdifferentiation through

modulation of key signaling pathways may have notable therapeutic benefit in patients with hepatocyte insufficiency but presence of ample ductular reaction.

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Figure and Figure Legend

Figure 1

β -Catenin Immunohistochemistry

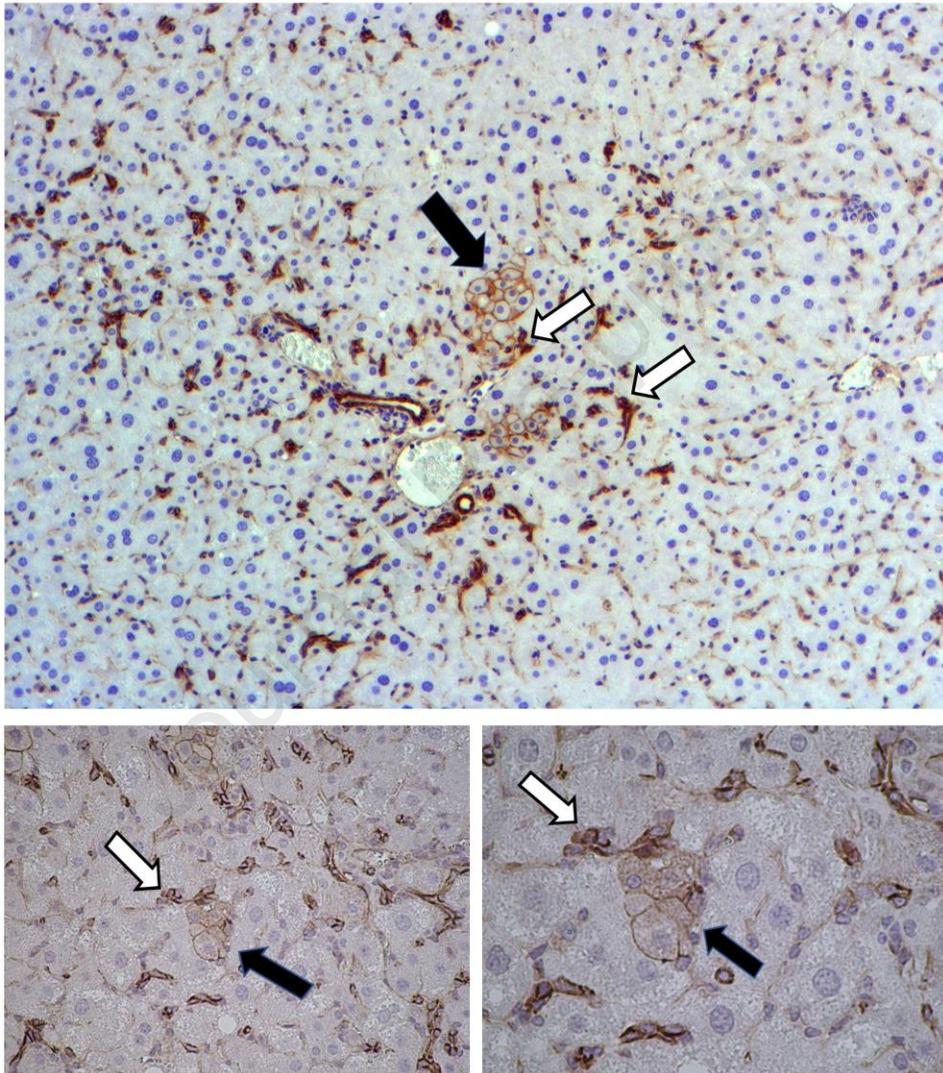


Figure 1. Transdifferentiation of a subset of cholangiocytes within a ductular reaction into hepatocytes. Immunohistochemistry for β -catenin shows appearance of β -catenin-positive small hepatocytes (closed arrows) in close proximity to β -catenin-

positive ductular reaction (open arrows) in the hepatocyte-specific β -catenin knockout mice after they were fed CDE diet for 2 weeks and switched to normal diet for 7 days for recovery. Image magnification: 50x (top), 100x (bottom left) and 200x (bottom right)

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