Biliary-derived hepatocytes in chronic liver injury: Bringing new troops to the battlefield?

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The adult liver represents a paradigm of *bona fide* regeneration whereby, after damage, it can fully restore its architecture and function. Following mild and moderate injury, this regeneration occurs via hepatocyte proliferation.[1] However, in severe chronic liver disease, the regenerative capacity of hepatocytes becomes impaired,[2,3] leading to failure of the hepatocyte compartment to fully restore normal structure and function. The question of whether cells of a non-hepatocyte origin, such as biliary cells, can contribute to parenchymal repair in such a setting cannot yet be determined from human tissue samples. Animal models of liver disease, which enable cell fate tracing, have therefore been used to try and answer this question. However, these studies have produced conflicting and contradictory results.

Hepatocyte self-duplication has been considered the sole mechanism of parenchymal regeneration. Yanger *et al.*[4] and Schaub *et al.*[5] showed independently, through labelling of multiple cells types within the liver, that regeneration after injury is mediated via hepatocyte proliferation rather than from extra-hepatic sources. Font-Burgada *et al.*[6] further identified a population of regeneratively-proficient hepatocytes within the periportal region. These cells express a combination of hepatocyte and biliary genes and respond to injury with extensive proliferation that can repopulate the liver following injury.[6]

Hepatic progenitor cells (HPCs) have also been proposed as a source of hepatocyte regeneration. However, there has been controversy regarding their nature. HPCs have been described as cells of biliary origin that reside in the canals of Hering in a quiescent state. During severe injury they proliferate and expand into the parenchyma in atypical structures, termed ductular reactions (DRs).[7] Biliary-derived cells contribute to parenchymal restoration, although minimally, following chronic carbon tetrachloride (CCL_4) treatment and partial hepatectomy.[8] However, these aforementioned injury models fail to reproduce a key characteristic feature of chronic liver disease in humans – that of significant hepatocyte senescence. Other rodent models, described below, have attempted to induce widespread hepatocyte senescence or inhibit hepatocyte prolif-

eration to recapitulate the replicative arrest observed in hepatocytes in human disease.

Transplanted mouse HPCs engraft and expand as hepatocytes in a mouse model in which deletion of the negative regulator of p53 – Mdm2 – leads to widespread hepatocyte senescence and parenchymal injury, thus creating a non-competitive repopulation environment.[9] Two recent studies investigated the relationship between a defect in native hepatocyte replication and the contribution of biliary-derived hepatocytes to parenchymal regeneration. Raven and Lu[10] explored multiple models combining inhibition of hepatocyte proliferation, either via *Itgb1* deletion or p21 overexpression, and diet-induced liver injury models (3,5-diethoxycarbonyl-1,4-dihydrocollidine [DDC], methionine-choline deficient diet, thioacetamide [TAA]) to show that biliary-derived hepatocytes can contribute to restore a proportion of the liver parenchyma, but only when the native hepatocytes are unable to proliferate. Russel *et al.*[11] explored a model where native hepatocyte regeneration is impaired via β-catenin deletion and observed robust HPC-to-hepatocyte differentiation during the recovery phase following choline deficient, ethionine-supplemented diet-induced liver injury. Another study by Deng *et al.*[12] used long-term injury models (up to 52 weeks TAA and up to 24 weeks DDC), where there was a time-dependent induction of native hepatocyte senescence, combined with lineage tracing, to show that biliary-derived hepatocytes could regenerate liver parenchyma.

The present study[13] adds to the evidence for the role of biliary-derived hepatocytes in restoration of the liver parenchyma following chronic liver injury. The authors rely on mice with an inducible Osteopontin-iCreER[T2] allele to label biliary cells with several different marker genes – YFP, mT/mG or *confetti*. They used this technique to trace biliary cells and their derivatives to define their role in liver regeneration following chronic liver injury induced by repeated CCl_4 injection. It should be noted that there is some evidence in the injured liver that OPN expression is not strictly restricted to the biliary compartment but is also present in other cell types, including damaged hepatocytes.[14] This could affect whether the genetic label is strictly related to biliary cells or could be non-specifically expressed in hepatocytes. The authors therefore performed stringent control experiments to minimise the possibility that the emergence of biliary-derived hepatocytes in their model is an artefact of
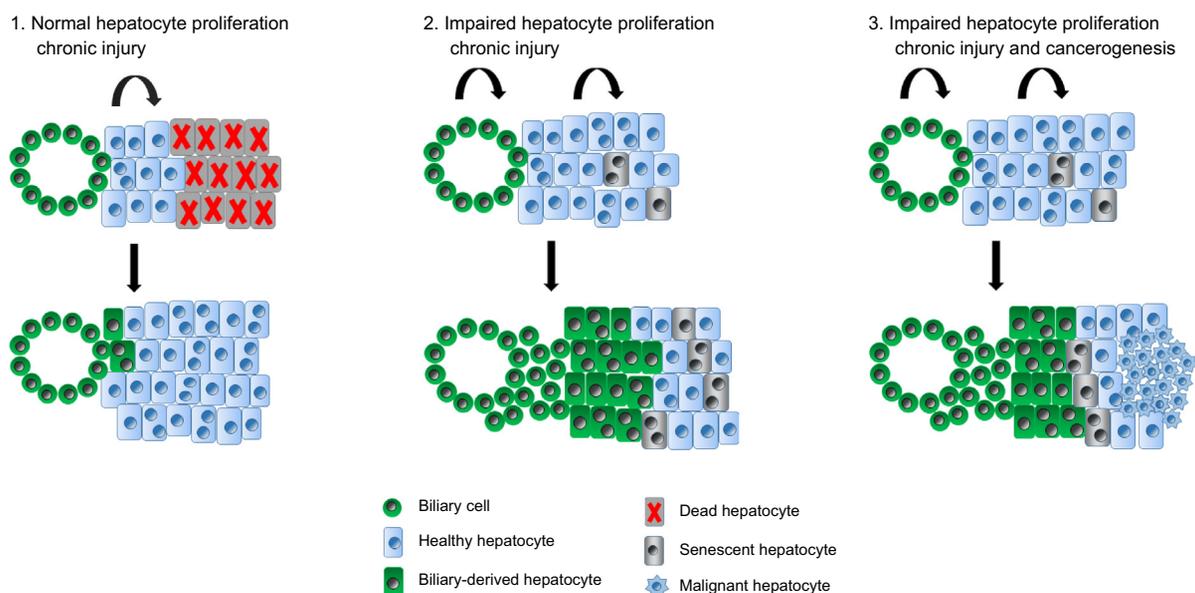
Fig. 1. Rodent models of chronic liver disease, in which the biliary-derived hepatocytes contribute to parenchymal regeneration. In chronic injury, when the native hepatocytes are able to proliferate normally, the biliary-derived hepatocytes contribute minimally to parenchymal restoration even in injury scenarios with widespread hepatocyte loss (1.). In chronic injury, when the capacity of the native hepatocytes to proliferate is impaired, the biliary-derived hepatocytes contribute significantly to parenchymal regeneration (2.). In prolonged chronic injury and compromised native hepatocyte proliferation, the biliary-derived hepatocytes contribute to parenchymal restoration. Importantly, the damaged, native hepatocytes are susceptible to malignant transformation (3.).

the labelling system. The authors use a 3 times-a-week CCl_4 injection regime for up to 24 weeks that they describe as "severe". This results in discrete and transient DR formation, progressive central fibrosis and gradual failure of regeneration by the hepatocyte compartment. In agreement with Raven and Lu[10] and Russel,[11] the authors emphasise the requirement for inhibition of native hepatocyte proliferation to observe a significant, biliary-mediated regenerative response. Furthermore, emergence of biliary-derived hepatocytes in this model coincided with decreased proliferative activity in native hepatocytes. The authors provide data suggesting that the drop in proliferative index in native hepatocytes is due to the acquisition of a senescent phenotype. Senescent cells are known to secrete a cocktail of soluble proteins, termed senescence-associated secretory phenotype (SASP), through which they can influence the fate of surrounding tissue in a paracrine manner.[15] It is therefore tempting to hypothesise that SASP factors, secreted by senescent native hepatocytes influence the differentiation of biliary cells towards a hepatocyte phenotype. However, one cannot exclude other factors being relevant, for example proliferative hepatocytes may themselves inhibit biliary differentiation. Further studies are therefore required to determine the signals controlling the biliary-to-hepatocyte fate change.

In patients with chronic liver disease, the development of fibrosis is correlated with widespread hepatocyte senescence and persistent DR.[16,3] The chronic CCl_4 injury regime described here recapitulates the first 2 features; the authors report a mild and transient DR response at onset of injury that disappears by the time significant numbers of biliary-derived hepatocytes are seen. Therefore, the authors hypothesise that the biliary-derived hepatocyte patches observed in this model are a result of clonal expansion of rare differentiation events and not multiple biliary cells differentiating simultaneously. Interestingly, the experiments performed using the OPN-Cre; Rosa26R[Confetti] mouse suggest this holds true as no mosaicism was observed within the

biliary-derived hepatocyte patches – in other words the biliary-derived hepatocyte patches were clonal. However, caution must be exercised when translating such findings to other models of liver disease, where injury is accompanied by florid DR and the activation of the biliary compartment diminishes only after the hepatotoxic agent is removed.

The authors investigate whether the damage response elicited by CCl_4 administration differs between native and biliary-derived hepatocytes. Although both native populations exhibit similar levels of DNA damage during injury, the biliary-derived hepatocytes are shown to have less activation of pathways associated with apoptosis and cellular senescence, as well as lower levels of nuclear polyploidy, which is linked to cellular stress. During the injury recovery phase, the biliary-derived hepatocytes have a proliferative advantage over the native hepatocytes, and are able to resolve previously accumulated DNA damage, as indicated by elimination of nuclear γ-H2A foci. Therefore, the increase in number of biliary-derived hepatocytes during recovery is, again, attributed to clonal expansion and not *de novo* differentiation. Of note, the native hepatocytes are exposed to CCl_4 for substantially longer than biliary-derived hepatocytes (which appear from week 6 of CCl_4 treatment onwards), thus the longer exposure to the hepatotoxic agent can cause the native hepatocytes to be more damaged, and more likely to enter apoptosis or senescence.

The proliferative advantage of the biliary-derived hepatocytes could be considered a double-edged sword. Whereas this might be beneficial for regeneration, uncontrolled proliferation can also lead to development of cancer. Importantly, to investigate whether the biliary-derived hepatocytes possess a malignant predisposition, the authors subjected mice to 24-week CCl_4 treatment to model the pre-neoplastic nodules of cirrhosis. Although a fraction of the cirrhotic nodules were composed of YFP+ biliary-derived hepatocytes or were mosaics between YFP+/YFP- cells, the majority of nodules were composed of

YFP⁻ native hepatocytes. Exposure to the hepatocarcinogen diethylnitrosamine led to the development of hepatocellular carcinoma (HCC) nodules that were composed of native, YFP⁻ hepatocytes. This is in agreement with a recent study from Tummala *et al.*[17] where HCCs are shown to originate from damaged hepatocytes.

The present study contributes significant extra information to the knowledge of biliary-mediated hepatocyte regeneration (Fig. 1). Considering that the only definitive treatment option for patients with end-stage chronic liver disease is transplantation and demand for donor organs far outweighs availability, describing the endogenous regenerative mechanism including biliary-mediated regeneration is important. It may be that if we can understand the molecular pathways governing the replication failure of hepatocytes and biliary-to-hepatocyte differentiation we can target these for clinical benefit. However, for this to be realised clinically, a rigorous understanding of the pathways concerned is required to guarantee safety of such approaches.

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Conflicts of interest

The authors declare no competing personal or financial interests.

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Supplementary data

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Author names in bold designate shared co-first authorship.

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