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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 have therefore been used which allow cell fate tracing to try and answer this question. However, these studies have produced conflicting and contradictory results.

Hepatocyte self-duplication has been considered to be 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 and can repopulate the liver following injury [6].

Hepatic progenitor cells (HPCs) have also been proposed as 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 and 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 CCL₄ 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

1 hepatocyte senescence or inhibit hepatocyte proliferation to recapitulate the replicative arrest
2 observed in hepatocytes in human disease.

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

17
18 The present study [13] adds to the evidence for the role of biliary-derived hepatocytes in restoration of
19 the liver parenchyma following chronic liver injury. The authors rely on mice with an inducible
20 Osteopontin-iCreER^{T2} allele to label biliary cells with several different marker genes- YFP, mT/mG or
21 *confetti*. They used this technique to trace biliary cells and their derivatives to define their role in liver
22 regeneration following chronic liver injury induced by repeated CCl₄ injection. It should be noted that
23 there is some evidence in the injured liver, that OPN expression is not strictly restricted to the biliary
24 compartment but is also present in other cell types, including damaged hepatocytes [14]. This could
25 affect whether the genetic label is strictly related to biliary cells or could be non-specifically expressed
26 in hepatocytes. The authors therefore performed stringent control experiments to minimise the
27 possibility that the emergence of biliary-derived hepatocytes in their model is an artefact of the
28 labelling system. The authors use a three times-a-week CCl₄ injection regime for up to 24 weeks that
29 the authors describe as “severe”. This results in discrete and transient DR formation, progressive
30 central fibrosis and gradual failure of regeneration by the hepatocyte compartment. In agreement with
31 Raven and Lu [10] and Russel [11], the authors emphasise the requirement for inhibition of native
32 hepatocyte proliferation to observe a significant, biliary-mediated regenerative response. Furthermore,
33 emergence of biliary-derived hepatocytes in this model coincided with decreased proliferative activity
34 in native hepatocytes. The authors provide data suggesting that the drop in proliferative index in
35 native hepatocytes is due to the acquisition of a senescent phenotype. Senescent cells are known to
36 secrete a cocktail of soluble proteins, termed senescence-associated secretory phenotype (SASP),
37 through which they can influence the fate of surrounding tissue in a paracrine manner [15]. It is
38 therefore tempting to hypothesise that SASP factors, secreted by senescent native hepatocytes
39 influence the differentiation of biliary cells towards a hepatocyte phenotype. However, one cannot
40 exclude other factors being relevant, for example proliferative hepatocytes may themselves inhibit
41 biliary differentiation. Further studies are therefore required to determine the signals controlling the
42 biliary-to-hepatocyte fate change.

43
44 In patients with chronic liver disease, the development of fibrosis is correlated with widespread
45 hepatocyte senescence and persistent DR [16] [3]. The chronic CCl₄ injury regime described here
46 recapitulates the first two features; the authors report a mild and transient DR response at onset of
47 injury that disappears by the time significant numbers of biliary-derived hepatocytes are seen.
48 Therefore, the authors hypothesise that the biliary-derived hepatocyte patches observed in this model
49 are a result of clonal expansion of rare differentiation events and not multiple biliary cells
50 differentiating simultaneously. Interestingly, the experiments performed using the OPN-Cre;
51 Rosa26R^{Confetti} mouse suggest this holds true as no mosaicism was observed within the biliary-derived
52 hepatocyte patches- in other words the biliary derived hepatocyte patches were clonal. However,
53 caution must be exercised when translating such findings to other models of liver disease, where
54 injury is accompanied by florid DR and the activation of the biliary compartment diminishes only after
55 the hepatotoxic agent is removed.

1 The authors investigate whether the damage response elicited by CCl₄ administration differs between
2 native and biliary-derived hepatocytes. Although both native populations exhibit similar levels of DNA
3 damage during injury, the biliary-derived hepatocytes are shown to have less activation of pathways
4 associated with apoptosis and cellular senescence, as well as lower levels of nuclear polyploidy,
5 which is linked to cellular stress. During the injury recovery phase, the biliary-derived hepatocytes
6 have a proliferative advantage over the native hepatocytes, and are able to resolve previously
7 accumulated DNA damage, as indicated by elimination of nuclear γ -H2A foci. Therefore, the increase
8 in number of biliary-derived hepatocytes during recovery is, again, attributed to clonal expansion and
9 not *de novo* differentiation. Of note, the native hepatocytes are exposed to CCl₄ for substantially
10 longer than biliary-derived hepatocytes (which appear from week 6 of CCl₄ treatment onwards), thus
11 the longer exposure to the hepatotoxic agent can cause the native hepatocytes to be more damaged,
12 and more likely to enter apoptosis or senescence.

13 The proliferative advantage of the biliary-derived hepatocytes could be considered a double-edged
14 sword. Whereas this might be beneficial for regeneration, uncontrolled proliferation can also lead to
15 development of cancer. Importantly, to investigate whether the biliary-derived hepatocytes possess a
16 malignant predisposition, the authors subjected mice to 24-week CCl₄ treatment to model the pre-
17 neoplastic nodules of cirrhosis. Although a fraction of the cirrhotic nodules were composed of YFP⁺
18 biliary-derived hepatocytes or were mosaics between YFP⁺/YFP⁻ cells, the majority of nodules were
19 composed of YFP⁻ native hepatocytes. Exposure to the hepatocarcinogen DEN led to the
20 development of hepatocellular carcinoma (HCC) nodules that were composed of native, YFP⁻
21 hepatocytes. This is in agreement with a recent study from Tummala et al. [17], where HCCs are
22 shown to originate from damaged hepatocytes.

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

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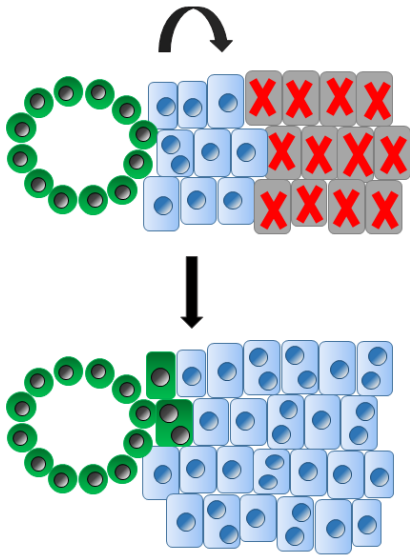
32 Figure legends

33 Fig. 1. Rodent models of chronic liver disease, in which the biliary-derived hepatocytes 34 contribute to parenchymal regeneration

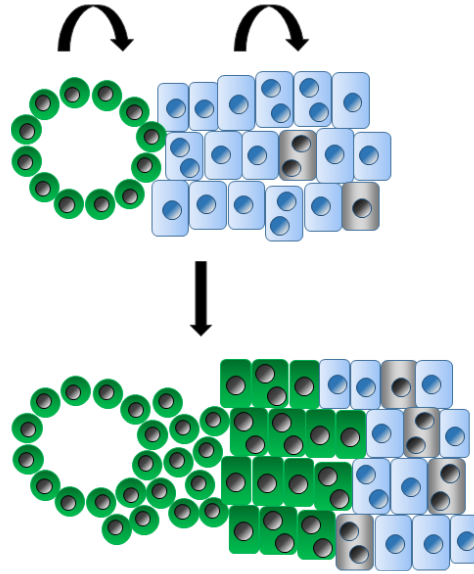
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37 In chronic injury, when the native hepatocytes are able to proliferate normally, the biliary-derived
38 hepatocytes contribute minimally to parenchymal restoration even in injury scenarios with widespread
39 hepatocyte loss (1.). In chronic injury, when the capacity of the native hepatocytes to proliferate is
40 impaired, the biliary-derived hepatocytes contribute significantly to parenchymal regeneration (2.). In
41 prolonged chronic injury and compromised native hepatocyte proliferation, the biliary-derived
42 hepatocytes contribute to parenchymal restoration. Importantly, the damaged, native hepatocytes are
43 susceptible to malignant transformation (3.).
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Figure

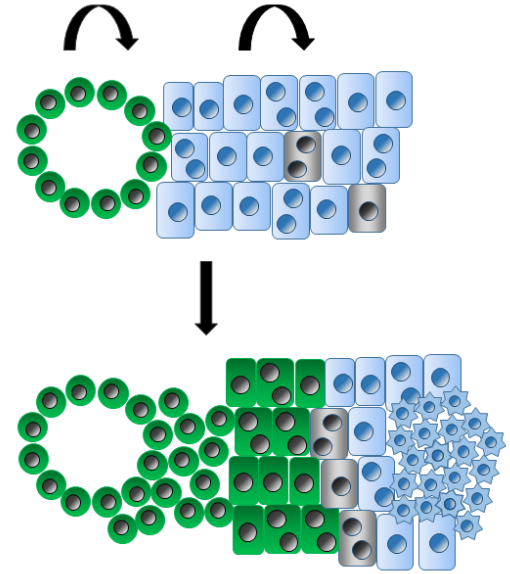
1. normal hepatocyte proliferation
chronic injury









2. impaired hepatocyte proliferation
chronic injury



3. impaired hepatocyte proliferation
chronic injury and cancerogenesis



-  biliary cell
-  healthy hepatocyte
-  biliary-derived hepatocyte

-  dead hepatocyte
-  senescent hepatocyte
-  malignant hepatocyte