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Cellular plasticity in liver regeneration - spotlight on cholangiocytes

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HEPATOLOGY

Cellular plasticity in liver regeneration - cholangiocytes take centre stage.

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Manuscripts

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3 **Cellular plasticity in liver regeneration - cholangiocytes take centre stage.**
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3 1 The liver's remarkable capacity to self-repair and regenerate following tissue injury
4 2 has been recognised since the ancient Greek myth of Prometheus. However the
5 3 diverse potential sources of this regenerative capacity have been an area of hot debate
6 4 and only recently have studies started to unravel the actual degree of hepatic cell
7 5 plasticity. The article by **Deng X, Zhang X, Li W *et al.* Chronic liver injury
8 6 induces conversion of biliary epithelial cells into hepatocytes. *Cell Stem Cell*
9 7 **2018; 23:114-122** established through lineage tracing experiments using a double-
10 8 fluorescent reporter system that biliary epithelial cells significantly contributed to
11 9 hepatocyte regeneration in two murine chronic liver injury models. Furthermore,
12 10 during the cholangiocyte-to-hepatocyte conversion, bi-phenotypic cells were
13 11 identified in both mouse models as well as in human cirrhotic livers. Following
14 12 analysis of liver progenitor cell markers and mature cholangiocytes, the authors
15 13 concluded that cholangiocytes directly lineage-converted to hepatocytes without a
16 14 progenitor cell intermediate and suggested these bi-phenotypic cells as potential
17 15 cellular sources for future therapeutic transplantation strategies.**

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17 17 The landscape of published evidence supporting the regenerative capacity and
18 18 plasticity of hepatocytes and cholangiocytes has changed rapidly over the last few
19 19 years and a novel working model is gradually emerging, describing several potential
20 20 routes to liver regeneration. It involves (a) lineage-restricted regeneration, where
21 21 mature hepatocytes or biliary epithelial cells proliferate and generate new hepatocytes
22 22 or cholangiocytes, respectively, or (b) non-lineage-restricted regeneration, mediated
23 23 by immature, bipotential liver progenitor cells (LPCs), giving rise to either of the two
24 24 main hepatic epithelial lineages, or - only recently fully recognised -
25 25 'transdifferentiation-based regeneration', where mature hepatocytes or cholangiocytes
26 26 convert to the opposite lineage to replace lost tissue.

27 27 Several recent papers have indicated the heterogeneity of hepatocytes in both the
28 28 physiological maintenance of liver mass and following injury.⁽¹⁻³⁾ Of note, a periportal
29 29 source of regenerative hepatocytes was described. These hybrid hepatocytes
30 30 (HybHP), located at the limiting plate, were positive for both hepatocyte nuclear
31 31 factor 4 α (HNF4 α) and the ductal transcription factor Sox9 but were negative for the
32 32 cholangiocyte and liver progenitor cell marker cytokeratin 19 (CK19). HybHPs
33 33 regenerated hepatocytes following chronic and carcinogenic injury.⁽¹⁾ Hepatocyte-to-

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3 34 cholangiocyte transdifferentiation has been shown by Schaub *et al.* in a mouse model
4 35 of Alagille syndrome that results in cholestatic injury at birth and leads to postnatal *de*
5 36 *novo* cholangiocyte formation. This cellular plasticity was mediated via transforming
6 37 growth factor β signalling.⁽⁴⁾ In this model, there is likely a strong selective pressure
7 38 on hepatocytes due to the lack of pre-existing peripheral bile ducts. Similarly,
8 39 selective pressures facilitated the significant transdifferentiation of biliary epithelial
9 40 cells to hepatocytes following substantial hepatocyte depletion using zebrafish as the
10 41 model organism.⁽⁵⁾ For some time it had been controversial whether cholangiocyte-to-
11 42 hepatocyte conversion was an effective mechanism of hepatocyte regeneration in
12 43 zebrafish alone or whether it also occurred in mouse and humans. Experiments in
13 44 mice, combining significant liver injury with the inhibition of hepatocyte proliferation
14 45 by either knockdown of the transmembrane heterodimeric protein β 1-integrin or
15 46 overexpression of the cyclin-dependent kinase inhibitor p21 in hepatocytes, led to
16 47 induction of cholangiocyte-derived ductular reactions and the formation of functional,
17 48 biliary epithelial cell-derived hepatocytes.⁽⁶⁾ Together these data have demonstrated
18 49 functionally relevant cellular plasticity in the epithelial compartment of the injured
19 50 liver, adding cholangiocytes to the list of potential cell sources for hepatocyte
20 51 regeneration and *visa versa*.

21 52 In a recent issue of *Cell Stem Cell*, Deng *et al.* corroborated and extended these
22 53 findings by confirming cholangiocyte-to-hepatocyte transdifferentiation in murine
23 54 lineage tracing models in the absence of genetic interventions.⁽⁷⁾ The authors used
24 55 thioacetamide (TAA) administration as a model of progressive fibrosis and cirrhosis
25 56 and 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) as a model of cholangitis and
26 57 biliary fibrosis. The study took advantage of double-fluorescent Cre reporter mice,
27 58 which displayed global expression of membrane-targeted tandem dimer Tomato,
28 59 unless excision had taken place through the expression of Cre recombinase. The
29 60 authors achieved this in hepatocytes using an adeno-associated virus expressing Cre
30 61 recombinase under the control of the hepatocyte-specific thyroxine-binding globulin
31 62 promoter. Hence, cells ubiquitously fluoresced red except for adult hepatocytes,
32 63 which exhibited green fluorescent protein (GFP) expression. In both injury models,
33 64 following extended injury, patches of HNF4 α ⁺ hepatocytes developed that were red
34 65 (i.e. were not Cre-deleted), suggesting a non-hepatocyte origin. However, in this
35 66 system the authors cannot formally exclude the possibility that small numbers of

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3 67 hepatocytes that escaped the Cre-deletion expanded throughout the parenchyma.
4 68 However, this scenario is not considered likely as any putative Cre-escaped
5 69 hepatocytes would not have any known selective advantage over the Cre-deleted
6 70 hepatocytes. Importantly, following TAA-injury, putative transdifferentiated
7 71 hepatocytes exhibited markers of spatial zonation, including expression of carbamoyl
8 72 phosphate synthetase I in periportal and glutamine synthetase in pericentral areas, and
9 73 were therefore demonstrated to have functionally integrated into the liver
10 74 parenchyma. In addition, none of the liver cancers that formed after 52-week TAA
11 75 treatment were derived from transdifferentiated hepatocytes. To formally prove the
12 76 non-parenchymal origin of these new hepatocytes, Deng *et al.* used positive lineage
13 77 tracing. Co-staining of HNF4 α and CK19 revealed double-positive cells in periportal
14 78 liver areas. Lineage tracing for CK19⁺ biliary epithelial cells was performed, which
15 79 revealed that these cells migrated from ductal to parenchymal areas, adopted
16 80 hepatocyte shape and consequently expressed HNF4 α , cytochrome P450 3A4 and
17 81 multidrug resistance protein 4. Furthermore, these cholangiocyte-derived cells lacked
18 82 expression of CK19 and Sox9. It was estimated that approximately 9-10% of
19 83 hepatocytes were derived through hepatocyte transdifferentiation of biliary epithelial
20 84 cells in the TAA- and DDC-induced liver injuries. Bi-phenotypic cells that co-
21 85 expressed HNF4 α and CK19 and exhibited columnar and stratified epithelial
22 86 morphology were further analysed. The majority of bi-phenotypic cells lacked
23 87 primary cilia as well as expression of the polarity marker protein kinase C zeta,
24 88 suggesting that cells had lost their typical apical-basal polarity during the conversion
25 89 process. A few mature cholangiocytes displayed co-expression of HNF4 α and
26 90 primary cilia, which prompted the authors to propose that cholangiocyte-to-
27 91 hepatocyte transdifferentiation was the result of a previously unrecognised direct
28 92 lineage conversion. The fact that bi-phenotypic cells did not express the liver
29 93 progenitor cell markers Lgr5 and alpha-fetoprotein led Deng *et al.* to conclude that
30 94 this conversion had taken place without a liver progenitor cell intermediate. It should
31 95 be noted that (a) the putative liver progenitor cell pool consists of a very
32 96 heterogeneous cell population, which necessitates the simultaneous use of multiple
33 97 markers to identify all subpopulations and (b) alpha-fetoprotein is only suitable as a
34 98 liver progenitor cell marker in rats and not in mice.⁽⁸⁾ Therefore, a more detailed
35 99 analysis of these bi-phenotypic cells would need to be undertaken before the

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3 100 conclusion of a direct cholangiocyte-to-hepatocyte lineage conversion without a
4 101 progenitor cell intermediate can be formally proven.
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6 102 While underlying mechanisms may vary between species and are certainly context-
7 103 specific, these data emphasise that the liver has an abundance of effective
8 104 regenerative sources, inducing the most appropriate cell in a given injury scenario.
9 105 Collectively, these recent papers^(5, 8, 9) have highlighted the plasticity of the liver's
10 106 epithelial cell population, and clearly shown that this response is dependent upon the
11 107 type of injury and regenerative failure of the 'native epithelial cell'. This has also
12 108 helped to uphold the liver's reputation as a regeneration super star among solid
13 109 organs.
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For Peer Review

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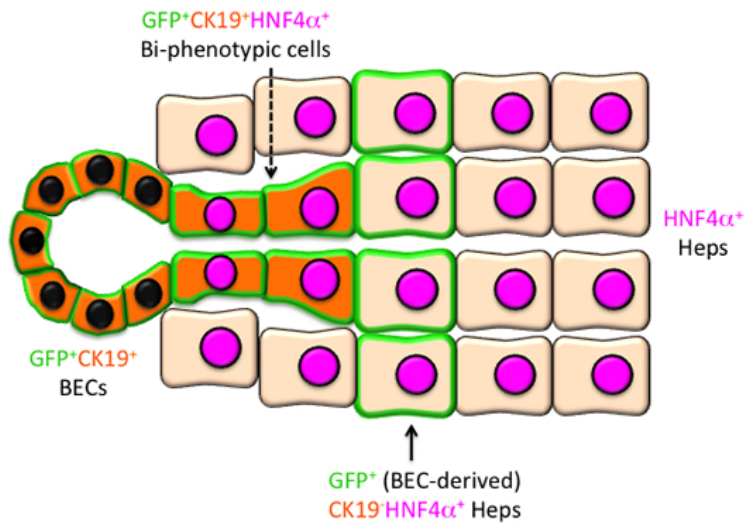


Figure 1: Biliary epithelial cell-to-hepatocyte conversion.

60x44mm (300 x 300 DPI)

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3 **Figure 1:** Biliary epithelial cell-to-hepatocyte conversion. Deng *et al.* used tamoxifen
4 treatment in CK19^{CreERT}:mTmG mice to label cytokeratin 19 (CK19)-expressing
5 biliary epithelial cells (BECs) with green fluorescent protein (GFP). Chronic liver
6 injury through treatment with thioacetamide or 3,5-diethoxycarbonyl-1,4-
7 dihydrocollidine led to the generation of bi-phenotypic cells that co-expressed CK19
8 and hepatocyte nuclear factor α (HNF4 α). In addition, single-cell lineage tracing was
9 performed in thioacetamide-treated CK19^{CreERT}:mTmG mice through one round of
10 low-dose tamoxifen administration. Single GFP⁺ BECs gave rise to small hepatic
11 nodules of CK19⁻HNF4 α ⁺ cells, providing evidence for a BEC-to-hepatocyte
12 conversion.
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