About cytokeratin 19 and the drivers of liver regeneration

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The postnatal liver is a resting organ with little parenchymal cell turnover. It is estimated that each hepatocyte is replaced 3 to 4 times during the lifespan of a mammalian organism. Stem cells around the portal tract have been proposed to drive the slow physiological cell turnover replacing senescent hepatocytes, which undergo apoptosis close to central veins[1]. A more recent study located physiological hepatocyte regeneration to the central vein, where Wnt signals from central vein endothelial cells provide a proliferation advantage to adjacent hepatocytes[2].

After acute toxic injuries the lost hepatocytes are rapidly replaced by neighboring hepatocytes. Surgical removal of liver tissue also induces proliferation of mature hepatocytes and results in hyperplasia of remaining liver lobes. The paradigm of liver regeneration that only hepatocytes replace hepatocytes and cholangiocytes replace cholangiocytes may, however, not be extrapolated to chronic inflammatory states. In the early search for protocols and compounds, which can induce chronic liver damage and carcinogenesis, a distinct histological feature of ductular reactions was observed, initially in the rat liver[3,4]. As a prominent feature of these ductular reactions small oval cytokeratin (Krt) 19^+ cells were visible in large numbers. These small cells morphologically resembled progenitor cells, which obviously descended in close vicinity to cholangiocellular structures (canals of Hering). In vitro assays unlocked the bi-potential nature of these oval cells with the appearance of hepatocyte, cholangiocyte and, in some studies, also fetal hepatoblast markers. There has been a long debate concerning the contribution of Krt 19^+ cells for the *de novo* generation of hepatocytes after recovery from chronic liver injury. Several recent studies addressed this question, to which extent Krt 19^+ cells with stem/progenitor phenotype contribute to parenchymal liver regeneration. To answer this question cell tracing mouse models were analyzed for physiological regeneration or repair response after various acute and chronic injuries. In none of the models a significant contribution of Krt 19^+ stem/progenitor cells to hepatocyte regeneration was observed.

In a recently published letter to the journal “Nature” Stuart Forbes and his group demonstrated that cholangiocytes contribute to hepatocyte formation in chronic mouse liver injury models with a genetically induced block of hepatocyte proliferation[5]. For this elegant study, the authors utilized a hepatic Itgb1 (β1-integrin$^{fl/fl}$) „knock out“ phenotype to block hepatocyte proliferation in either adenoviral associated AAV8.TBG.Cre recombined R26RLS^{Ltd}Tomato (fluorescently labelled hepatocytes) or Krt19Cre^{ERT}tdTomato^{LSL} (with fluorescently labelled Krt 19^+ cholangiocytes or putative stem/progenitor cells) cell tracking mice (fluorescently labelled hepatocytes) and induced chronic liver injury by repeated application of the established toxic regimens 3,5,-diethoxcarbyonyl- 1,4dihydrocollidine (DDC), methionine – and choline deficient diet (MCD) or, thioacetamide (TAA). In the recovery phase from chronic injury they found across the various injuries 20-30 %

hepatocytes, which were either negative for green fluorescence in the first mouse model (non-hepatocyte derived) or were labelled with red fluorescence in the second model thus tracing the origin of these hepatocytes to Krt19^{+} cells. The number of Krt 19^{+} cell derived hepatocytes was significantly higher in mice with the β1-integrin "knock out" compared to the respective wild type phenotype. In an additional series of experiments they induced a block of hepatocyte proliferation by AAV-8 mediated over-expression of the p21 protein in hepatocytes with similar results. The authors concluded from their results that newly generated hepatocytes in chronic liver injury models derived from cholangiocytes.

How can these seemingly paradoxical results be explained and, if possible, reconciled with the previous reports in the field, which suggested only a minor contribution of the Krt 19^{+} hepatic cell lineage in mouse liver repair and in the generation of lost hepatocyte mass after chronic liver injury[6-8]?

It is well established that adult hepatocytes and cholangiocytes derive from bi-potential progenitors, named hepatoblasts, during embryonic development thus indicating a close developmental relationship of the two cell types[9]. In adult mammalian liver the hepatoblasts segregate functionally and positionally into Krt 8 and 18 expressing hepatocytes and Krt 9 and 19 expressing cholangiocytes. In adult rodents as well as in humans it was clear from the very first days of hepatic regeneration research that surgical loss or acute toxic injuries of the hepatocyte mass were repaired by the proliferation of mature hepatocytes. The emergence of oval cells or putative Krt 19^{+} cell lineage derived progenitor cells thus needed prolonged injury and a strong proliferation block for resident hepatocytes. Whether or not oval cells differentiated into hepatocytes in the recovery phase after chronic liver injury could only be suggested from histological analysis in the pre-genomic era, since genetic cell tracking methods were lacking. Later, with the emergence of molecular tools for mouse genome modification, the focus of hepatic regeneration research more and more switched to mice as the primary research model for human disease. Distinct cell populations could now be fluorescently labelled and, the fate of these cells could be followed.

Forbes and his group implemented a genetic block of hepatocyte proliferation and then challenged the cell-tracking mice with the various toxic compounds known to induce a ductular reaction and chronic liver injury. This experimental step was obviously critical for substantial hepatocyte generation from Krt 19^{+} cells in their study and distinct from the previous reports in mice. The data thus reveal that application of the toxic compounds, which induce ductular reactions in the liver, are not sufficient to inhibit repair by resident hepatocytes and also suggest a facultative role of the Krt 19^{+} cell lineage in hepatocyte regeneration. In line with this observation a previous study from the same group reported isolation of cells with progenitor phenotype from mice with an inducible E3 ubiquitin ligase

Mdm2 “knock out” phenotype causing apoptosis, necrosis and senescence of mature hepatocytes[10]. These cells were isolated, expanded in vitro and finally transplanted into the same mouse model followed by ablation of hepatocytes through repeated induction of the “knock out” phenotype. The transplanted progenitors cells, which obviously derived from Krt 19^+ cell lineage in this model, partially repopulated the recipient mice liver with hepatocytes und cholangiocytes thus confirming the bi-potential nature of this cell type. The potential of Krt19^+ cells to switch into Krt 8^+ and 18^+ hepatocytes reflects the common embryonic ancestry of the two cell lines and indicates a higher flexibility in cellular phenotypes in the liver than previously thought. This view is also supported by recent data indicating phenotype switches from hepatocytes to cells with cholangiocyte phenotype in mouse models of cholangiocarcinoma[11].

Another important question remains: do the small Krt 19^+ cells, as they emerge in ductular plates, reside already in small numbers in the normal hepatobiliary system and thus qualify as true stem/progenitor cells ?

The question cannot be answered with a definitive yes or no. In Wnt targeted Lgr5LacZ “knock in” mice no beta-galactosidase stained cells could be observed in normal adult liver, but emerged after a single challenge with the hepatotoxin “carbontetrachloride”. In this report the authors came to the conclusion that cells with Lgr5/Sox9/CK19 progenitor phenotype are more likely induced by toxic challenges and are not resident in a normal liver[12,13]. These cells likely derive from a subset of cholangiocytes, which express the protease ST14 on the cell surface[14]. Moreover, the Hippo pathway activity in hepatocytes was shown to guide the appearance of cells with bi-potential progenitor phenotype and morphology with characteristics typically found in ductular reactions[15]. This study and another report showed that the bi-potential progenitor cells in ductular reactions can also derive from hepatocytes and switch back to hepatocytes after cessation of the chronic liver injury[16]. Taken together, these data strongly trace the origin of cells with progenitor phenotype to mature hepatocytes and cholangiocytes. However, they do not exclude the existence of true resident stem/progenitor cells in the liver, but, at least, make it dispensable.

Although mature hepatocytes can derive from the Krt 19^+ cell lineage in the liver directly or through the intermediate stage of a stem/progenitor like cell, the efficacy for hepatocellular repopulation in an injured liver seems to be low. This is for instance reflected in experiments, where organoids derived from Lgr5/Sox9/Krt 19 progenitors were transplanted into Fah “knock out” mice, which provide a strong selection advantage for cells expressing fumaryl acetoacetate hydrolase protein. Engraftment, but incomplete repopulation of the recipient liver was observed indicating a lower capacity compared to hepatocytes in liver repopulation.

What does this mean for our understanding of liver regeneration? Certainly, the study shows that a switch of cellular phenotypes from cholangiocytic to hepatocytic can happen in mice under special experimental conditions. One important consideration might be that the mentioned experiments in mice do not necessarily extrapolate to other species including humans. We do not exactly know the complete mode of action of the various toxic compounds frequently utilized to induce chronic liver injury and ductular proliferation. Could it be that DDC, MCD or TAA and other compounds induce a stronger block of hepatocyte proliferation in rats than in mice; and do they reflect the various chronic liver disease entities in humans? The compound retrorsine gives a good example of species specific activities of certain compounds in the liver. Many researchers have successfully applied the chemical compound retrorsine to rats in hepatocyte transplantation experiments and showed a complete block of endogenous hepatocyte proliferation and expansion of the transplanted cells. No such experiments have been reported in mice and, in our hands, retrorsine was always insufficient for inhibition of mouse hepatocyte proliferation. And the question remains, whether the experimental model developed for rats and mice reflect to some extent the situation in various chronic human liver diseases? Generation of cell tracking models in several different species could at least help to study diversity in fundamental mechanisms of liver regeneration.

The take home message of the study from Forbes group is that endogenous biliary cells can give rise to hepatocytes when hepatocyte proliferation is blocked. Future studies should focus on elucidating the mechanism and implications of these findings for human liver disease.

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Figure legend: Cholangiocytes and hepatocytes are generated via replication of pre-existing cholangiocytes and hepatocytes, respectively during normal liver homeostasis. In case of chronic liver with profound of block hepatocytes proliferation, cholangiocytes are capable of differentiating into hepatocytes.