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LIVER

Cholangiocytes regenerate hepatocytes during severe liver injury

> …these results confirm the contribution of cholangiocytes to hepatic regeneration during liver injury…

New mouse models of hepatocyte regeneration have identified cholangiocytes as the cellular source of regeneration in the injured adult liver, according to research published in *Nature*. These findings could help reveal regenerative signalling pathways in the liver and ultimately aid the development of new therapies for hepatic regeneration.

The liver is famous for its ability to regenerate but the identity of the liver stem or progenitor cell remains elusive. Partial hepatectomy models of liver injury show that hepatocytes can efficiently regenerate themselves. "However, I rarely see this scenario in clinical practice," reports corresponding author Stuart Forbes. "Far more often, I see patients with severely or chronically injured livers. Analysis of liver tissue from such patients reveals that hepatocytes are not proliferating efficiently and are often senescent."

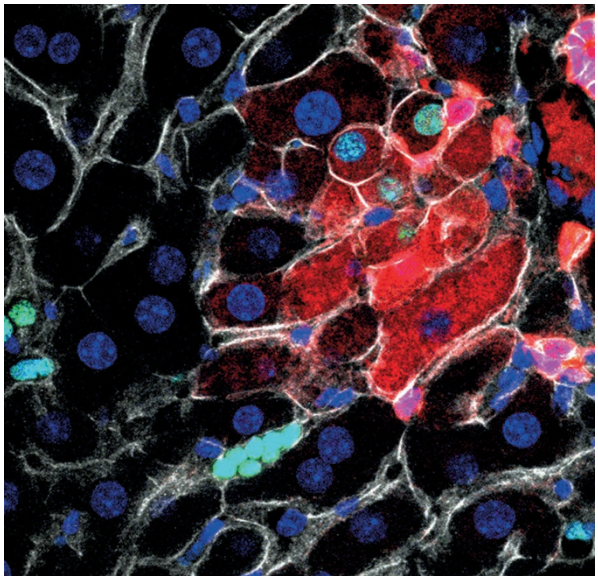

Image of red (tdTomato$^+$) hepatocytes derived from tdTomato-labelled cholangiocytes. Courtesy of W.-Y. Lu.

In this situation, cells are thought to emerge from bile ducts within the liver and contribute to regeneration. This process has been shown in zebrafish after hepatocyte loss, but not in mammalian models. In lineage tracing experiments with mice, ductular cells show an inconsistent and limited ability to form new hepatocytes. However, these mouse models are believed to not fully recapitulate human liver disease as they show a lack of hepatocyte senescence that leads to uninhibited liver regeneration. "We therefore set out to build a better mouse model of liver injury and regeneration that had impaired hepatocyte regeneration in response to injury, and then perform lineage tracing experiments in these mice. The question was whether the lack of regeneration from bile ducts was absolute, or due to the animal models used," explains Forbes.

The investigators developed two transgenic mouse systems to diminish hepatocyte regeneration during injury and evaluate the contribution to regeneration from non-hepatocyte sources. In the first system, hepatocyte β1-integrin was ablated, inhibiting hepatocyte growth factor signalling and impairing regeneration, before severe liver injury was induced using three different diet regimens. To determine whether non-hepatocytes regenerated hepatocytes, adeno-associated virus–Cre labelling of hepatocytes was used along with a tdTomato reporter.

Following liver injury, ~25% of hepatocytes positive for mature hepatocyte markers were tdTomato$^-$, indicating a non-hepatocyte origin. Furthermore, these cells were typically found adjacent to ductal cells positive for CKY19 and SOX9, suggesting a ductular origin. In the second system, cholangiocytes were lineage traced using similar site-specific recombinase technology and a tdTomato reporter. Using this system, the investigators showed that when hepatocyte proliferation was impaired by overexpression of p21 (a cell senescence marker), patches of hepatocytes derived from cholangiocytes (tdTomato$^+$) were formed after diet-induced liver injuries.

These cholangiocyte-derived hepatocytes were shown to store glycogen. Also, when the global transcriptional states of these cells, as well as the tdTomato$^-$ hepatocytes from the β1-integrin model, were compared with hepatocytes and cholangiocytes from injured wild-type mice, the profiles were highly similar to hepatocytes and distinct from ductular cells.

Taken together, these results confirm the contribution of cholangiocytes to hepatic regeneration during liver injury, but only when hepatocyte proliferation is inhibited. The investigators are now keen to understand how this backup system of liver regeneration is controlled. "If we know what the signals are controlling the proliferation of bile ducts, their migration into the liver and their differentiation into hepatocytes, then we can seek to control these processes using drugs or other agents," concludes Forbes.

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ORIGINAL ARTICLE Raven, A. *et al.* Cholangiocytes act as facultative liver stem cells during impaired hepatocyte regeneration. *Nature* http://dx.doi.org/10.1038/nature23015 (2017)