

EDITORIAL

Outside influence: The extrahepatic duct as a source for bile duct regeneration

Rare genetic liver diseases collectively account for one fifth of liver transplants.^[1] Though representing a significant clinical burden, monogenic liver disorders also provide unique opportunities for fundamental and clinical insights into mechanisms of health, disease, and recovery. One such hallmark disorder is Alagille syndrome (ALGS), caused by mutations in the Notch ligand, *JAGGED1*, or in the receptor, Notch receptor 2 (*NOTCH2*), which is characterized by cholestasis attributable to a developmental absence of peripheral bile ducts in the liver and congenital defects in other organ systems.^[2,3] A fascinating particularity of ALGS is that some patients display spontaneous alleviation of cholestasis,^[4] thought to resolve thanks to *de novo* generation of bile ducts. The cellular origin of these new bile ducts has been the subject of intense investigation, and multiple hepatic cellular sources have been demonstrated in various animal models upon biliary injury and regrowth of the biliary tree.^[5] In this issue, Zhao et al. provide evidence that an extrahepatic progenitor cell pool can contribute to liver repair in an animal model of ALGS.

The extraordinary regenerative capacity of the human liver has likely been appreciated for millennia, as demonstrated by the ancient Greek myth of Prometheus, condemned to having his liver eaten daily by an eagle, followed by nightly regrowth. Today, it is well established that liver regeneration usually uses phenotypic fidelity: Hepatocytes generate hepatocytes, and cholangiocytes generate cholangiocytes.^[5] But when either cell type is compromised, transdifferentiation of one cell type into the other can occur.^[5] In ALGS, cholangiocytes are compromised by the loss of Notch signaling, and thus other cellular sources may be needed for *de novo* generation of bile ducts. Zhao et al., from the Dong lab, use zebrafish models of ALGS to investigate cellular sources and molecular mechanisms contributing to liver repair, demonstrating that extrahepatic duct (EHD) progenitors can contribute to liver regeneration, in a Notch- and fibroblast growth factor

(Fgf)-dependent manner.^[6] This suggests an alternative mechanism to that shown by previous work from the Huppert and Willenbring labs, who used a Notch-compromised mouse model of ALGS to demonstrate that Tgfb β -driven hepatocyte transdifferentiation into cholangiocytes establishes the peripheral bile ducts in the *de novo* grown biliary tree.^[7] In this model, all *de novo*-generated peripheral ducts were determined to be the result of hepatocyte transdifferentiation. In the current study, no hepatocyte transdifferentiation was observed in regenerated *jag1b/2b* morphants; but in *jag2b*^{-/-} fish, low levels of hepatocyte transdifferentiation were present.^[6] Thus, both EHD cells and hepatocytes can contribute to bile duct *de novo* generation, depending on the model and genetic perturbation (Figure 1).

Previous work from the Dong lab showed that zebrafish lacking *jag1b* and *jag2b* exhibit cardiac defects and pronephric edema and do not develop intrahepatic cholangiocytes,^[8] mimicking several hallmarks of ALGS. Here, the investigators took advantage of the resumption of Notch signaling in transient Notch knockdown fish, and show that whereas compound mutant fish die by 9 days postfertilization (dpf), with severe liver damage, transient knockdown of *jag1b/2b* with morpholinos results in a milder phenotype with initial bile duct growth failure, but a Notch-dependent rescue of bile duct growth initiating at 4 dpf.^[6] The requirement for Notch in bile duct regeneration in this model appears to contradict results from a mouse model for ALGS, in which Notch is dispensable for *de novo* biliary growth.^[7] However, these two models, in fact, leverage different genetic components to uniquely reveal two mechanisms that the liver can use. The mouse model relies on conditional albumin promoter-driven Cre recombinase (*Alb-cre*) deletion of the Notch transcription factor, recombination signal binding protein for immunoglobulin kappa J region (*Rbpjk*), which could lead to derepression of Notch target genes, and lacks hepatocyte nuclear factor 6 (*Hnf6*; also known as *Onecut1/one cut homeobox 1*).^[7] Schaub et al. reasoned that because HNF6 inhibits the expression of transforming growth factor beta receptor 2 (TGFB β 2), the absence of *Hnf6* may lead to up-regulation of TGFB β 2 and

Abbreviations: *Alb-cre*, albumin promoter-driven Cre recombinase; ALGS, Alagille syndrome; EHD, extrahepatic duct; Fgf, fibroblast growth factor; *Hnf6*, hepatocyte nuclear factor 6.

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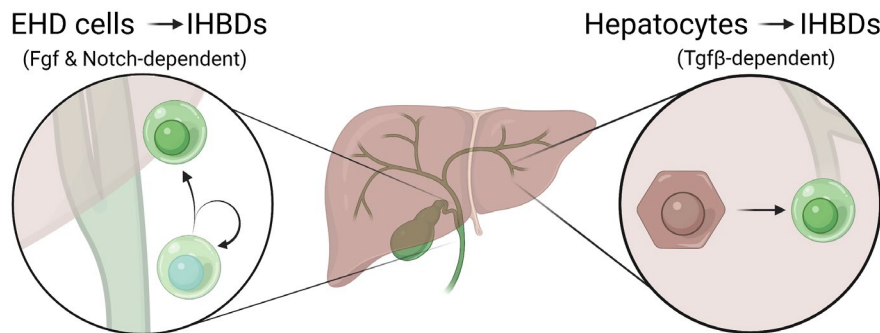


FIGURE 1 In models of ALGS, two sources of cells have now been demonstrated to contribute to *de novo* generation of the biliary tree. In this issue, at left, Zhao et al. demonstrate that EHD cells contribute to *de novo* generation of intrahepatic bile ducts (IHBDs) in a zebrafish model of ALGS, in a Notch- and Fgf-dependent manner.^[6] At right, Tgf β -driven hepatocyte transdifferentiation into cholangiocytes/IHBDs is responsible for the generation of peripheral bile ducts in a mouse model for ALGS.^[7] For the sake of simplicity, a human liver was chosen for this schematic, although the two models were derived from fish and mice, respectively. Which mechanisms are most relevant to human patients will be a future exciting avenue of research. Created with BioRender.com

hyper-responsiveness of hepatocytes to Tgf β signaling, allowing transdifferentiation in the mouse model. Therefore, in the fish model, wherein the *jag1b/2b* morphants have normal *hnf6* levels, little to no hepatocyte transdifferentiation would be expected. Conversely, Zhao et al. show that it is loss of Notch signaling itself that induces proliferation of cells in the EHD—a mechanism that would not occur in *Alb-cre^{+/-}Rbpjk^{fl/fl}Hnf6^{fl/fl}* mice, which would be expected to have a normal EHD. Thus, the use of these different genetic models unveiled multiple mechanisms contributing to repair of the biliary system.

The EHD is thus a potentially clinically relevant source of cells for intrahepatic duct repair. Importantly, previous work from Cardinale et al. also showed that human EHD peribiliary gland progenitor cells can generate cholangiocytes *in vitro*.^[9] A major question is therefore which mechanisms are most relevant in patients with ALGS. Which regenerative pathways are used in the spontaneous recovery that sometimes occurs, and which pathways would be easiest to safely modulate in patients? Interestingly, another mouse model for ALGS, the *Jag1^{Ndr/Ndr}* mouse, which also displays initial bile duct paucity and postnatal *de novo* generation of the biliary tree, suggests that both mechanisms might occur in parallel. A recently developed three-dimensional dual portal vein/biliary tree analysis method, termed double resin casting micro-CT, showed that liver regeneration in *Jag1^{Ndr/Ndr}* mice occurred with two architecturally distinct modes: hyperbranching of the ductular tree in hilar liver and tortuous ducts in the periphery, often traversing the hepatic parenchyma between portal tracts.^[10] Perhaps hilar hyperbranching regeneration occurs by EHD cell contribution, whereas tortuous peripheral bile ducts, sometimes present in parenchyma, are generated by hepatocyte transdifferentiation. Although global Notch loss of function in this model should activate EHD cell proliferation,^[6] this model does not have explicit

Hnf6 knockout and concurrent Tgf β hypersensitivity,^[7] which would predispose to hepatocyte transdifferentiation. However, based on the presence of phosphorylated SMAD (small mothers against decapentaplegic, a mediator of Tgf β signaling) in *de novo*-generated bile ducts in liver samples from patients with ALGS,^[7] Schaub et al. suggested that Tgf β -driven hepatocyte transdifferentiation also occurs in patients without requiring *Hnf6* silencing. Further lineage tracing and gain-/loss-of-function studies should be undertaken to determine whether different modes of regeneration preferentially occur in different parts of the liver. Indeed, Zhao et al. showed that when hepatocyte transdifferentiation could be detected in *jag2b^{-/-}* mutants, transdifferentiated cells were present in distal (peripheral) liver—further supporting the concept of regional differences in regeneration.

Multiple signaling pathways play key roles in maintaining the stem cell state, or driving differentiation and/or morphogenesis of biliary cells in the *de novo*-generated biliary tree. Zhao et al. show that Notch plays a dual role in this process: Notch must be silenced in order to prompt proliferation of progenitor/stem cells in the EHD, but resumption of Notch signaling (once the EHD cells have migrated into the liver) is required for their subsequent differentiation into biliary cells. Similarly, and importantly, the investigators show that Fgf signaling in the EHD has two roles: It both maintains the progenitor cell state and retains extrahepatic progenitor cells in the EHD. Using molecular inhibitors of Fgf signaling and multiple elegant genetic models, the investigators show that repression of Fgf signaling leads to ectopic expression of biliary markers by cells in the EHD, and also demonstrate the rapid displacement of Fgf-repressed cells from the EHD into the liver. Carefully modulating Notch, Fgf, or Tgf β signaling in patients could thus hypothetically balance both displacement and activation of the EHD progenitor pool to generate cholangiocytes, and induce

transdifferentiation of hepatocytes into cholangiocytes to accelerate the regenerative process in patients.

In conclusion, multiple cellular sources can contribute to bile duct *de novo* generation *in vivo*. Despite the classical model of phenotypic fidelity in liver repair,^[5] in various animal models of ALGS, new cholangiocytes have been demonstrated to originate either from EHD cells^[6] or hepatocytes^[7] to generate a fully functional biliary tree. Although these two models appear to point to mutually exclusive conclusions, in particular given that each study showed near-complete contribution to the new biliary tree by either EHD cells or hepatocytes, in fact the use of different genetic approaches has unveiled two fundamentally different mechanisms that the liver can use to repair the biliary tree. Whether phenotypic fidelity is more or less conserved in ALGS, in which hilar bile ducts are present, and could contribute to repair, remains to be determined. Which cell sources are most relevant, and which mechanisms are predominantly active in human patients? Which cells and mechanisms drive “spontaneous” recovery? Furthermore, which mechanisms can be most easily harnessed therapeutically for patients with ALGS? Answering these questions will pave the way to therapeutics for this pediatric liver disease. What is certain is that, once again, the liver’s reputation for regeneration and repair has outdone itself—now with an outside source for repair.

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I apologize to the many authors whose important and relevant publications could not be cited because of space constraints.

CONFLICT OF INTEREST

Nothing to report.

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REFERENCES

1. Karlsten TH, Sheron N, Zelber-Sagi S, Carrieri P, Dusheiko G, Bugianesi E, et al. The EASL–Lancet Liver Commission: protecting the next generation of Europeans against liver disease complications and premature mortality. *Lancet*. 2021;399:61–116.
2. Alagille D, Odièvre M, Gautier M, Dommergues JP. Hepatic ductular hypoplasia associated with characteristic facies, vertebral malformations, retarded physical, mental, and sexual development, and cardiac murmur. *J Pediatr*. 1975;86:63–71.
3. Kohut TJ, Gilbert MA, Loomes KM. Alagille syndrome: a focused review on clinical features, genetics, and treatment. *Semin Liver Dis*. 2021;41:525–37.
4. Emerick KM, Rand EB, Goldmuntz E, Krantz ID, Spinner NB, Piccoli DA. Features of Alagille syndrome in 92 patients: frequency and relation to prognosis. *Hepatology*. 1999;29:822–829.
5. Michalopoulos GK, Bhushan B. Liver regeneration: biological and pathological mechanisms and implications. *Nat Rev Gastroenterol Hepatol*. 2021;18:40–55.
6. Zhao C, Lancman JJ, Yang Y, Gates KP, Cao D, Barske L, et al. Intrahepatic cholangiocyte regeneration from an Fgf-dependent extrahepatic progenitor niche in a zebrafish model of Alagille syndrome. *Hepatology*. 2022;75:567–83.
7. Schaub JR, Huppert KA, Kurial SNT, Hsu BY, Cast AE, Donnelly B, et al. De novo formation of the biliary system by TGFβ-mediated hepatocyte transdifferentiation. *Nature*. 2018;557:247–51.
8. Zhang D, Gates KP, Barske L, Wang G, Lancman JJ, Zeng XXI, et al. Endoderm Jagged induces liver and pancreas duct lineage in zebrafish. *Nat Commun*. 2017;8:769.
9. Cardinale V, Wang Y, Carpino G, Cui CB, Gatto M, Rossi M, et al. Multipotent stem/progenitor cells in human biliary tree give rise to hepatocytes, cholangiocytes, and pancreatic islets. *Hepatology*. 2011;54:2159–72.
10. Hankeova S, Salplachta J, Zikmund T, Kavkova M, Van Hul N, Brinek A, et al. DUCT reveals architectural mechanisms contributing to bile duct recovery in a mouse model for Alagille syndrome. *eLife*. 2021;10:848481.