NEWS AND VIEWS

Cholangiocyte organoids as a cell source for biliary repair

Jeremy J. Velazquez[1,2] & Mo R. Ebrahimkhani[1,2,3,4]

1 Department of Pathology, Division of Experimental Pathology, School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA
2 Pittsburgh Liver Research Center, University of Pittsburgh, Pittsburgh, PA, USA
3 Department of Bioengineering, Swanson School of Engineering, University of Pittsburgh, Pittsburgh, PA, USA
4 McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA, USA
E-mail: mo.ebr@pitt.edu

Preoccupied with the astounding regenerative capabilities and metabolic importance of hepatocytes in the liver, the role of the resident 'plumbers' of the liver, the cholangiocytes, can be overlooked in comparison. Cholangiocytes form the ductal network which makes up the biliary tree and transports bile from the liver to the gallbladder for secretion into the intestine during digestion. Chronic accumulation of bile in the liver is cytotoxic, causing fibrosis and eventually leads to cirrhosis and liver failure. Liver transplantation is the only treatment option for survival at this progression of disease, and the demand for liver transplantations far exceeds the supply [1]. Additionally, biliary complications are significant contributors to liver transplant failure, being prone to developing strictures or leaks post-transplantation [2,3]. This paucity of treatments and understanding of biliary-related pathologies, combined with the increasing recognition of biliary-related disease and transplant burden, spurred the development of alternative therapies to liver transplantation.

Organoids are three-dimensional cellular systems developed from stem cells and their progeny via process of cell sorting, differentiation and self-organization [4]. They can capture key features of tissue function and structure. They can also provide a near-physiological platform that enables long-term expansion of cells *in vitro* while maintaining their genomic stability [5-7] that can offer an important cell source for regenerative engineering [8,9]. Cholangiocyte organoids from adult stem/progenitor cells or pluripotent stem cells were produced previously and enabled disease modelling as well as reconstruction of mouse extrahepatic biliary tree [10-12]. However, it was not clear if biliary organoids from different regions of liver can be expanded and employed to repair biliary epithelium of the human liver. In a recent study [13], Sampaziotis et al. provide compelling data that pave a path towards translation of organoid technology to regenerative engineering in human liver. In their study, the team analysed region-specific cholangiocytes [intrahepatic bile duct (IHD), common bile duct (CBD) and gallbladder (GB)] from human donors using single cell RNA sequencing (scRNA-seq) to characterize cholangiocyte diversity and compared their transcriptomic signatures to their regionally derived organoids. First, Sampaziotis et al. were able to show using differential gene expression and imaging that, once expanded with their *in vitro* organoid culture protocol, the cells lose most of their regional-specific gene expression while maintaining core cholangiocyte gene expression (KRT7, KRT19, SOX9, HNF1B, CFTR) and function (ALP and GGT activity). Interestingly, they showed that regional-specific gene expression of the gallbladder could be recovered by addition of bile suggesting the importance of microenvironmental factors in the loss of the regional gene expression and function, and that it might be recovered if reintroduced to its proper niche. Of critical note, is that regardless of the region the primary cells were sourced (IHD, CBD or GB) for cholangiocyte organoid expansion, the cultures all showed ability to express gallbladder-specific genes with the addition of bile. Their findings show high degree of plasticity in cholangiocytes and importance of the microenvironmental cues in shaping the cell state. In fact, the regional control of cell state or even positioning in liver was shown in hepatocytes [14] and immune cells [15]. Hence, the reintroduction of cells into the host environment can re-specify the appropriate characteristics according to the region.

To validate their findings, they asked whether cholangiocyte organoids from human gallbladder can rescue mice with cholangiopathy. They used 4,4'-methylenedianiline (MDA) to induce biliary injury and performed intraductal

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delivery of cells derived from the organoids. The therapeutic effect of the transplanted cells was robust, yielding a 100% survival (terminated after 3 months) of the cholangiocyte injected mice compared the control injected mice that died within 3 weeks. Immunofluorescence, histology and magnetic resonance cholangiopancreatography (MRCP) confirmed developing cholestasis and cholangiopathy of MDA administered control mice and showed resolution of cholangiopathy in transplanted mice. Furthermore, transplanted cells accounted for a considerable amount of the regenerated bile ducts (25-55%) and importantly downregulated gallbladder markers such as SOX17 and upregulated expression of intrahepatic bile duct markers SOX4, DCDC2 and BICC1. The authors also probed the interchangeability of the organoid origin by expanding common bile duct-derived human cholangiocytes as organoids and transplanting to the gallbladder of immunocompromised mice. All-in-all, results highlight the therapeutic capability of the cholangiocyte organoids and the interchangeability of biliary tree region used for derivation of these organoids.

In their final experiments, Sampaziotis et al. address potential concerns of the known differences between mouse and human liver microenvironment and further address the ability of cell therapy to combat ischaemic reperfusion injury in transplanted livers. Utilizing normothermic machine perfusion (NMP), nutrients and warm oxygenated blood were circulated through primary donor human livers with measured low bile pH, an indicator of ischaemia and increased risk for duct damage. These livers were transplanted with gallbladder organoid-derived cholangiocytes dissociated cell suspension (or small cell clumps) into the terminal branches of the intrahepatic ducts and perfused for 100 hours *ex vivo*. As predicted, the transplanted cells again lost gallbladder marker expression and gained intrahepatic gene expression. Appearance of the treated ducts was more open and did not have the tendency to collapsed like portions of the untreated ducts where ischaemia damage was likely to have occurred. This finding was corroborated with higher volume of bile aspirated from treated ducts supporting functional recovery.

During the mouse *in vivo* or human *ex vivo* studies, no cancerous growths were detected, although long-term studies with appropriate controls would be necessary to determine safety of this approach from tumorigenesis. Timescale of the experiments and maintenance of in vivo human liver function over time present as next barriers to overcome in proving safety and efficacy of this approach. Because of the *in vitro* expansion of the cells, this strategy also allows for genetic modification or correction, which could be of great value for assisting with genetic disease diseases such as cystic fibrosis. Further study of the impact of cholangiocyte organoid therapies on polygenetic or lesser understood acute and chronic cholangiopathies such as biliary atresia and Alagille syndrome could be extremely beneficial. Additionally, the induced pluripotent stem cell (iPSC)-derived iterations of these technologies are attractive because of vast expansion capability and donor flexibility. All-in-all, the implications from the work of Sampaziotis et al. indicate significant improvements for promising cholangiopathy-related therapeutics on the horizon that can increase cholangiopathy treatment options, increase the viability of donor transplants, and decrease organ transplant burden. It was shown that cholangiocytes can act as facultative stem cells to produce hepatocytes [16] when native hepatocyte regeneration is impaired. However, the transplanted biliary cells in this study did not produce hepatocyte cells and were locked in biliary fate after delivery. It is possible that upon right environmental cues such as inhibition of hepatocyte proliferation, the delivered cells gain additional plasticity to produce hepatocyte as well, which warrants future studies.

Authorship

JJV and MRE wrote and edited the paper.

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Conflict of Interest

The authors declare no conflict of interest.

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REFERENCES

1. Squires RH, Ng V, Romero R, *et al*. Evaluation of the pediatric patient for liver transplantation: 2014 practice guideline by the American Association for the Study of Liver Diseases, American Society of Transplantation and the North American Society for Pediatric Gastroenterology. *Hepatol Nutrition Hepatology* 2014; **60**: 362.
2. Coelho JCU, Leite LO, Molena A, Freitas ACT, Matias JEF. Biliary complications after liver transplantation. *Arq Bras Cir Dig* 2017; **30**: 127.
3. Kochhar G, Parungao JM, Hanouneh IA, Parsi MA. Biliary complications following liver transplantation. *World J Gastroenterol* 2013; **19**: 2841.
4. Lancaster MA, Knoblich JA. Organogenesis in a dish: modeling development and disease using organoid technologies. *Science* 2014; **345**: 1247125.
5. Huch M, Gehart H, van Boxtel R, *et al*. Long-term culture of genome-stable bipotent stem cells from adult human liver. *Cell* 2015; **160**: 299.
6. Karthaus WR, Iaquinta PJ, Drost J, *et al*. Identification of multipotent luminal progenitor cells in human prostate organoid cultures. *Cell* 2014; **159**: 163.
7. Sato T, Stange DE, Ferrante M, *et al*. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. *Gastroenterology* 2011; **141**: 1762.
8. Yui S, Nakamura T, Sato T, *et al*. Functional engraftment of colon epithelium expanded in vitro from a single adult Lgr5(+) stem cell. *Nat Med* 2012; **18**: 618.
9. Shaker A, Rubin DC. Stem cells: One step closer to gut repair. *Nature* 2012; **485**: 181.
10. Ogawa M, Ogawa S, Bear CE, *et al*. Directed differentiation of cholangiocytes from human pluripotent stem cells. *Nat Biotechnol* 2015; **33**: 853.
11. Sampaziotis F, de Brito MC, Madrigal P, *et al*. Cholangiocytes derived from human induced pluripotent stem cells for disease modeling and drug validation. *Nat Biotechnol* 2015; **33**: 845.
12. Sampaziotis F, Justin AW, Tysoe OC, *et al*. Reconstruction of the mouse extrahepatic biliary tree using primary human extrahepatic cholangiocyte organoids. *Nat Med* 2017; **23**: 954.
13. Sampaziotis F, Muraro D, Tysoe OC, *et al*. Cholangiocyte organoids can repair bile ducts after transplantation in the human liver. *Science* 2021; **371**: 839.
14. Russell JO, Monga SP. Wnt/beta-catenin signaling in liver development, homeostasis, and pathobiology. *Annu Rev Pathol* 2018; **13**: 351.
15. Gola A, Dorrington MG, Speranza E, *et al*. Commensal-driven immune zonation of the liver promotes host defence. *Nature* 2021; **589**: 131.
16. Raven A, Lu WY, Man TY, *et al*. Cholangiocytes act as facultative liver stem cells during impaired hepatocyte regeneration. *Nature* 2017; **547**: 350.