Bile Duct Repair in Human Liver Grafts: Effective Cholangiocyte Organoid Engraftment and Plasticity

Organoid technology holds great promise for regenerative medicine but has not yet been applied to humans. With the groundbreaking paper in *Science*,[1] by Sampaziotis and colleagues from the University of Cambridge, UK, clinical applications of cholangiocyte organoids come into view. The authors describe how cholangiocyte organoids can be used in the context of cholangiopathies and show bile duct repair by successfully transplanting these organoids in the human liver. Using single-cell RNA sequencing, they furthermore demonstrate transcriptional diversity in primary human cholangiocytes and that this is mostly lost in organoid culture. Despite this, cholangiocyte organoids remain plastic and resume their *in vivo* signatures when transplanted in the biliary tree. The authors use a cell engraftment model in human livers undergoing *ex vivo* normothermic machine perfusion (NMP) to demonstrate the regenerative properties of extrahepatic cholangiocyte organoids to repair intrahepatic ducts after ductal infusion. Supported by mouse experiments, this publication provides important proof-of-principle that cholangiocyte organoids have regenerative capacities and potential as treatment for cholangiopathies. Biliary diseases often occur due to destruction of the local biliary epithelial cells (cholangiocytes), which leads to biliary scar formation, cholestasis and eventually liver damage. Overall, cholangiopathy indications account for 70% of pediatric and up to a third of adult liver transplantations.[2] In addition to this and despite the high demand for donor organs, approximately 20% of potential liver grafts are declined for transplantation due to ischemia-related damage of bile ducts.[3] Although oxygenated (hypothermic) machine perfusion could potentially prevent this ischemic bile duct damage, currently no therapy is available that restores already affected bile ducts. Cholangiocyte organoids are considered to be promising cell sources for this. Previous studies by this group revealed that primary cholangiocytes can be expanded in the long term as organoids. These were used to tissue-engineer functional extrahepatic bile ducts that could replace the common bile duct in mice.[4] They and others showed that these cholangiocyte organoids can be initiated from bile duct tissue collected at different regions alongside the biliary tree,[5] from brushes, or from bile.[6] Although cholangiocyte organoids grown from these different sources are very similar, subtle differences were observed in phenotype and differentiation potential.[5] This obviously raises the question of how these differences will affect their use in regenerative applications. Related to this, it is important to know whether cholangiocyte organoids possess a certain degree of phenotypic plasticity and are able to adapt to the local environment after transplantation. The answer is now provided by Sampaziotis et al.[1]

Cholangiocytes were isolated from intrahepatic and extrahepatic bile ducts as well as from gallbladders (Fig. 1A). Analyses of the single-cell transcriptomes by small conditional RNA sequencing revealed that cholangiocytes from all regions shared a similar core profile but also have regional differences. These region-specific genes included SRY (sex-determining region Y)-box 4 (SOX4) expression in the intrahepatic cholangiocytes, trefoil factor 2 in the extrahepatic cholangiocytes, and SOX17 in the gallbladder cholangiocytes. Overall, the cholangiocytes displayed a gradual shift in their transcriptional profile alongside the biliary tree. One hypothesis is that different factors expressed by the local environment, such as bile composition, drive these changes in cholangiocyte gene expression. It is indeed known that bile composition changes as it moves down the biliary tree (by water and bicarbonate secretion, bile salt and nutrient extraction). This hypothesis was further investigated by assessing the phenotypic plasticity of cholangiocyte organoids derived from these three regions. The organoids maintained expression of the core cholangiocyte transcriptional signature but lost the expression of regional markers in culture. Interestingly, when gallbladder bile was added to the culture, the organoids from all three regions up-regulated gallbladder cholangiocyte marker SOX17, indicating that differences in bile composition

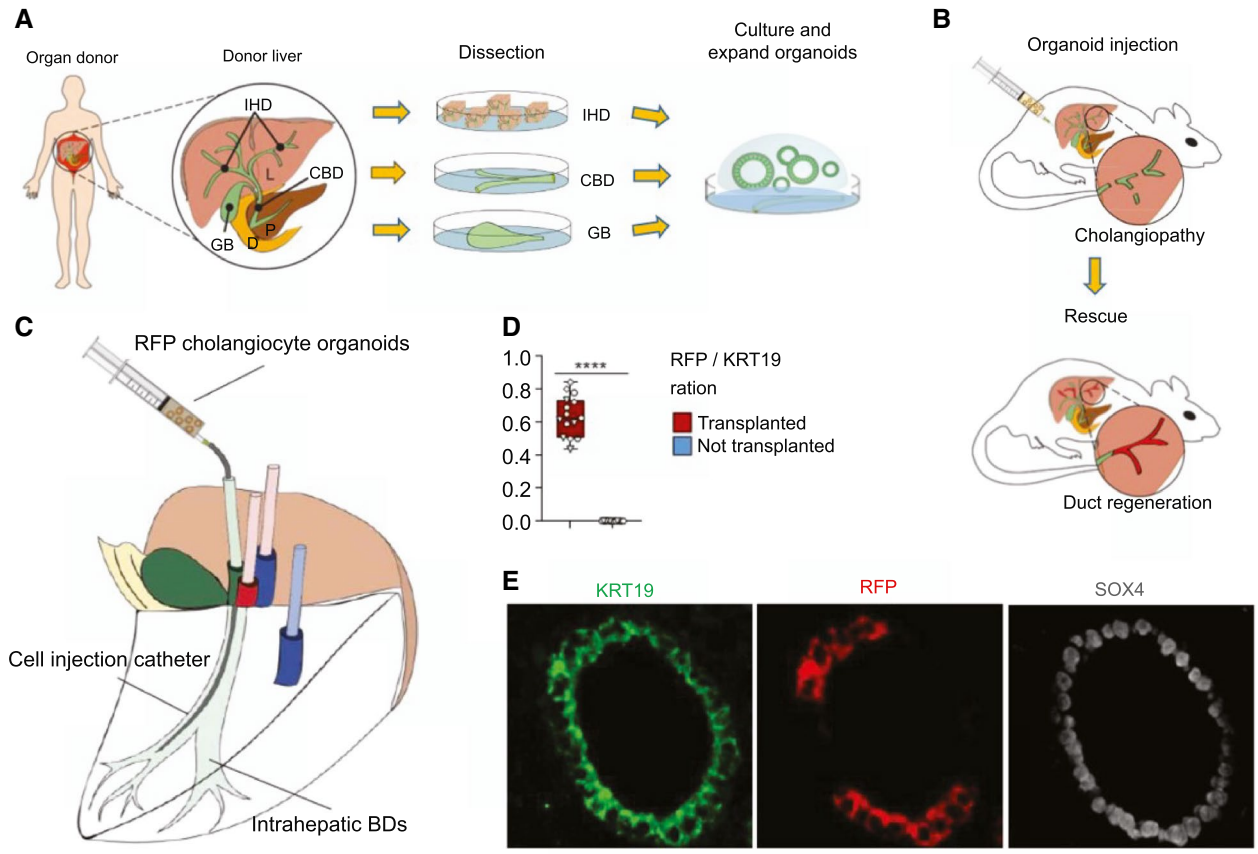

FIG. 1. Cholagiocyte organoids can repair bile ducts. (A) Cholangiocyte populations in different regions of the biliary tree were isolated and cultured as organoids. (B) Cholangiocyte organoids promote ductal regeneration and rescue experimentally induced lethal cholangiopathy after engraftment in mice. (C) Cholangiocyte organoids engraft in a human liver graft during NMP. RFP-labeled gallbladder organoids were infused in intrahepatic bile duct branches. (D) Up to 100 hours after organoid infusion, between 50% and 70% of all cholangiocytes of injected ducts were RFP-positive. (E) After engraftment, the RFP-positive gallbladder cholangiocyte organoids assumed an intrahepatic identity, illustrated by gain of SOX4 expression and loss of SOX17 (not shown). From Sampaziotis et al.,[1] reprinted with permission from the American Association for the Advancement of Science. Abbreviations: BDs, bile ducts; CBD, common bile duct; D, duodenum; GB, gallbladder; IHD, intrahepatic duct; KRT19, keratin 19; L, liver; P, pancreas.

contribute to the regional identity. This bile-induced effect on SOX17 gene expression was lost with the inhibition of bile acid farnesoid X receptor.

Further evidence for cholangiocyte and organoid plasticity was extracted from *in vivo* experiments using an intrahepatic cholangiopathy mouse model. Fluorescently labeled gallbladder-derived organoids were administered intraductally to rescue the mouse phenotype (Fig. 1B). Mice receiving the organoid infusion survived for 3 months and resolved their cholangiopathy, whereas mice receiving a carrier with no organoids died within 3 weeks. Engraftment of organoid cells was very effective as the labeled cells represented 25%-55% of the regenerated cholangiocytes. The phenotype of gallbladder organoid cells that engrafted upstream in the mouse intrahepatic bile ducts again showed clear evidence of cholangiocyte plasticity and adaptation to the intrahepatic environment. Cells lost expression of gallbladder-specific genes (i.e., SOX17) and gained expression of intrahepatic cholangiocyte-related genes (SOX4). Whether this plasticity is also observed when intrahepatic cholangiocyte organoids engraft downstream was not shown; however, a recent *in vitro* study demonstrated that repopulation of the extrahepatic bile duct matrix by intrahepatic cholangiocyte organoids was less profound than their extrahepatic counterparts.[7] Interestingly, when freshly isolated human cholangiocytes were infused, engraftment was limited and survival benefits were observed. One possible explanation for this is that the organoid culture environment, which drives cell proliferation and maintains a stem

cell–like phenotype, potentiates their capacity to survive after engraftment and promotes bile duct regeneration. Remarkably, even mesenchymal stromal cells, which are commonly used to stimulate organ repair through paracrine signaling, were unable to rescue the mice when infused to the bile duct.

To demonstrate proof-of-principle that organoid therapy could be effective in restoring human bile duct injury, the authors performed a series of experiments using NMP of damaged human liver grafts (Fig. 1C). It is well known that the biliary tree is particularly vulnerable to ischemia and that extended warm ischemia periods of the donor often cause bile duct damage resulting from cell necrosis and shedding/sloughing of cholangiocytes.[3] This bile duct injury often leads to biliary complications after transplantation. Similar to the mouse experiments, fluorescently labeled, gallbladder-derived cholangiocyte organoids were infused through an intraductal catheter while the graft was perfused for up to 100 hours with normothermic oxygenated blood. As a control, a bile duct branch of another liver segment was infused with the carrier alone. Organoids effectively engrafted most of the ducts, with between 50% and 70% of all cholangiocytes being red fluorescent protein (RFP)–positive (Fig. 1D). Like in the mouse experiments, the engrafted gallbladder organoid cells adapted to their intrahepatic environment, as demonstrated by SOX4 positivity (Fig. 1E). Of note, organoid engraftment did not cause any bile duct obstruction or other complications. As a matter of fact, bile duct regeneration by organoids significantly improved bile secretion and bile pH compared to bile from the carrier control duct. This suggests that cells became functional through the contact with bile and adjusted the bile pH, which is an important marker for biliary damage during NMP procedures. Both the high level of regeneration and the bile adjustments were unique to the specific parts of the biliary tree that were repaired by the organoids.

Although this study shows the feasibility of organoid-based regeneration in human livers, many challenges remain in the translation to broad clinical application. For this, high numbers of organoid cells expanded under clinical-grade conditions are required. Now, the first steps are being taken in upscaling cholangiocyte organoid cultures for large-scale production, for instance, using a spinner flask setup.[8] The first clinical application with cholangiocyte organoids may be in the setting of liver transplantation by full graft repair of bile duct injury during NMP. However, this remarkable study by the Cambridge team also paves the way for exploring direct applications in patients with biliary diseases.

Luc J.W. van der Laan, Ph.D.
Floris J.M. Roos, M.Sc.
Monique M.A. Verstegen, Ph.D.
Department of Surgery
Erasmus MC–University Medical Center and
Erasmus MC Transplant Institute
Rotterdam, the Netherlands

REFERENCES

1) Sampaziotis F, Muraro D, Tysoe OC, Sawiak S, Beach TE, Godfrey EM, et al. Cholangiocyte organoids can repair bile ducts after transplantation in the human liver. Science 2021;371:839-846.
2) Squires RH, Ng V, Romero R, Ekong U, Hardikar W, Emre S, 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, Hepatology and Nutrition. Hepatology 2014;60:362-398.
3) op den Dries S, Westerkamp AC, Karimian N, Gouw ASH, Bruinsma BG, Markmann JF, et al. Injury to peribiliary glands and vascular plexus before liver transplantation predicts formation of non-anastomotic biliary strictures. J Hepatol 2014;60:1172-1179.
4) Sampaziotis F, Justin AW, Tysoe OC, Sawiak S, Godfrey EM, Upponi SS, et al. Reconstruction of the mouse extrahepatic biliary tree using primary human extrahepatic cholangiocyte organoids. Nat Med 2017;23:954-963.
5) Rimland CA, Tilson SG, Morell CM, Tomaz RA, Lu WY, Adams SE, et al. Regional differences in human biliary tissues and corresponding in vitro–derived organoids. Hepatology 2021;73:247-267.
6) Roos FJM, Verstegen MMA, Muñoz Albarinos L, Roest HP, Poley J-W, Tetteroo GWM, et al. Human bile contains cholangiocyte organoid-initiating cells which expand as functional cholangiocytes in non-canonical Wnt stimulating conditions. Front Cell Dev Biol 2021;8:630492.
7) Willemse J, Roos FJM, Voogt IJ, Schurink IJ, Bijvelds M, Jonge HR, et al. Scaffolds obtained from decellularized human extrahepatic bile ducts support organoids to establish functional biliary tissue in a dish. Biotechnol Bioeng 2021;118:836-851.
8) Schneeberger K, Sánchez-Romero N, Ye S, Steenbeek FG, Oosterhoff LA, Pla Palacin I, et al. Large-scale production of LGR5-positive bipotential human liver stem cells. Hepatology 2020;72:257-270.

View this article online at wileyonlinelibrary.com.

DOI 10.1002/hep.31877

Potential conflict of interest: Nothing to report.