Article type : Review

Hepatobiliary organoids and their applications for studies of liver health and disease: are we there yet?

Junya Shiota[1]

Linda C Samuelson[1,2]

Nataliya Razumilava[1]

[1]Department of Internal Medicine, [2]Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI, USA

Author e-mails:

Dr. Junya Shiota jshiota@nagasaki-u.ac.jp

Dr. Linda C Samuelson lcsam@med.umich.edu

Address for Correspondence: Nataliya Razumilava, MD

109 Zina Pitcher Pl., BSRB, 2062

Ann Arbor, Michigan, USA, 48109

Tel: 734-764-5865

Fax: 734-763-4686

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/HEP.31772

E-mail: razumila@med.umich.edu

Number of: Pages: 25
Title characters: 90
Abstract words: 209
Words with references: 6547
References: 76
Figures: 2
Tables: 2

Keywords: stem and progenitor cells, pluripotent cells, regeneration, drug discovery, gallbladder, bile duct.

List of Abbreviations: 3D, 3-dimensional; ECM, extracellular matrix; IHBD, intrahepatic bile ducts; EHBD, extrahepatic bile duct; A1AT, Alpha-1 antitrypsin; CFTR, CF transmembrane conductance regulator; *COMMD1*, copper metabolism domain containing 1; FFA, free fatty acids; HCC, hepatocellular carcinoma; CCA, cholangiocarcinoma; IHC, immunohistochemistry, GGT, gamma-glutamyl transferase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase

Disclosure: JS, LCS, and NR have nothing to disclose.

This work was supported by NIDDK K08 (NR); Gilead Sciences Research Scholar Award (NR).**ABSTRACT**

Organoid culture systems have emerged as a frontier technology in liver and biliary research. These 3-dimensional (3D) cell cultures derived from pluripotent and adult hepatobiliary cells model organ structure and function. Building on gastrointestinal organoid establishment, the first hepatobiliary organoid cultures were generated from mouse LGR5+ liver progenitor cells. Subsequently, 3D hepatobiliary organoid cultures were developed from hepatocytes and cholangiocytes to model human and animal hepatobiliary health and disease. Hepatocyte organoids have been used to study Alagille syndrome, fatty liver disease, Wilson's disease, hepatitis B viral infection, and cystic fibrosis. Cholangiocyte organoids have been established to study normal cholangiocyte biology and primary sclerosing cholangitis, and to test organoid

potential to form bile ducts and gallbladder *in vitro*. Hepatobiliary cancer organoids, termed tumoroids, have been established from frozen and fresh human tissues and used as a drug-testing platform and for biobanking of cancer samples. CRISPR-based gene modifications and organoid exposure to infectious agents have permitted the generation of organoid models of carcinogenesis. This review summarizes currently available adult cell-derived hepatobiliary organoid models and their applications. Challenges faced by this young technology will be discussed, including cellular immaturity of organoid-derived hepatocytes, co-culture development to better model complex tissue structure, imperfection of extracellular matrices, and absence of standardized protocols and model validation.

INTRODUCTION

Liver cellular structure, organization and regeneration as a rationale for culturing hepatobiliary organoids

Organoids are defined as 3-dimensional (3D) structures grown *in vitro* from stem/progenitor cells that consist of organ-specific cell types that self-organize (1). These "organ-in-a-dish" systems model tissue-specific composition, architecture and function, and allow more physiological spatial cell organization and polarization compared to two-dimensional cell lines, many of which are cancer-derived. The organoid technology has evolved in parallel with definition of mechanisms of tissue development. To mimic the progenitor cell niche, organoids are cued with cytokines and growth factors and cultured in extracellular matrix (ECM) (2).

The liver is a vital organ with numerous functions, including detoxication, digestion, and metabolism (3), provided by parenchymal and non-parenchymal liver cells organized into functional units, termed lobules. Parenchymal cells are derived from endodermal hepatic diverticulum and represented by hepatocytes and biliary epithelial cells. Biliary cells line intrahepatic and extrahepatic bile ducts (IHBD and EHBD, respectively), cystic duct and gallbladder (Figure 1).

Hepatobiliary tissues have varying developmental processes. Embryonic hepatoblasts are the primary progenitor cells for the developing liver, yielding both hepatocytes and IHBD cholangiocytes. In contrast, EHBD and large IHBD cells originate from the ventral pancreatic

region (3, 4). The gallbladder is derived from a hepatic diverticulum close to the ventral pancreatic bud and the developing EHBDs. Hepatobiliary organs also include non-parenchymal cells, such as resident macrophages, stromal fibroblasts (e.g. stellate cells and portal fibroblasts in the liver), and endothelial cells.

Regenerative capacity in the adult liver is astonishing. At homeostasis, less than 2% of hepatocytes and biliary cells are proliferating (3, 5-7). However, after injury, a robust proliferative response is induced to regenerate both hepatocytes and cholangiocytes. After partial hepatectomy in mice and rats, the liver regains its original size by 3-7 days (8). In humans, after 60% liver resection, the organ achieves 75% of its original size in 10 days, which is sufficient to normalize liver biochemistries (9). This response is especially intriguing because no resident progenitor cells have been identified in the adult liver. Recent studies using unbiased lineage-tracing approaches in genetic mouse models have shown that hepatocytes in all liver zones participate in slow cell replacement during homeostasis and rapid replacement after injury (5-7). This contrasts with earlier *Axin2*-Cre lineage-tracing studies that suggested only pericentral vein region hepatocytes support liver regeneration after injury (10).

Biliary tree progenitor cells, termed “oval cells” in rodents (11), located in the canal of Hering are thought to be an additional pool of multipotent progenitors capable of giving rise to both hepatocytes and cholangiocytes to maintain adult tissues (12). Oval cells are WNT-responsive and express the R-spondin receptor LGR5, which amplifies WNT signaling (13, 14). Regeneration of large IHBDs and EHBDs during normal tissue turnover and after injury is mainly attributed to progenitor-like cells residing in peribiliary glands (15). Importantly, it has been shown that mature adult hepatocytes and cholangiocytes have plasticity to transdifferentiate upon injury to regenerate liver tissue (16-18). Identification of cellular mechanisms driving liver regeneration has relied on genetically engineered mouse models with some variable findings depending on the extent of injury. Defining mechanisms of human hepatobiliary regeneration will likely require complex human organoid models. It is possible that specific regenerative mechanisms vary depending on the type and extent of injury.

When liver function cannot be restored, the single option for patient survival is transplantation. There is a vigorous search for cell-based technologies that will provide alternatives for liver transplantation. Furthermore, there is a need for better cell culture models of

human hepatobiliary tissues to study normal tissue function and mechanisms of disease. The organoid technology, which is based on principles of organ development and repair, has become an exciting *in vitro* model system to answer questions about human liver disease and repair (Figure 1).

Classification of hepatobiliary organoids

Organoids can be derived from pluripotent stem cells (1) or from adult organ-specific stem/progenitor cells (19) (Figure 1). Pluripotent stem cells include human embryonic stem cells and induced pluripotent stem cells (1). While no professional stem cells have been identified in the liver, hepatobiliary organoids have been successfully generated from adult liver (Table 1), bile ducts and gallbladder (Table 2) using biopsies, surgical samples, bile and biliary brushings. These organoids have also been generated from numerous mammalian species, including humans, mice, rats, cats, and dogs. Their source are likely facultative progenitor cells.

Differentiation of pluripotent stem cells into liver cells is a step-wise process recapitulating stages of embryonic liver development, involving commitment into the endodermal lineage, differentiation into hepatoblasts, and eventual hepatocyte-like cell development (20). It permits generation of multi-lineage human organoid models simultaneously containing hepatocyte, stellate, and Kupffer-like cells (21), or hepatic cells with mesenchymal stem cells and umbilical vein endothelial cells (22). There are multiple sources for human pluripotent stem cells (23), including embryonic cells and skin fibroblasts, as well as commercial sources, making them more accessible than adult hepatobiliary tissues. Despite several advantages, pluripotent stem cell-derived liver organoids are generally immature and difficult to culture long-term, which can make drug and functional studies problematic (24).

This review focuses on adult tissue-derived hepatobiliary organoid models and their applications (Figure 2). It also discusses challenges of this young and rapidly evolving technology and its future directions.

HEPATOBILIARY ORGANOID MODELS AND THEIR APPLICATION

Hepatocyte organoids

The discovery that WNT is a crucial signal for stem cell maintenance in epithelial tissues became a scientific rationale for contemporary "organ-in-a dish" technology (25). Hans Clevers' group reported in 2009 the long-term culture of mouse intestinal LGR5+ stem cells in ECM that assemble into 3D-structures of progenitor and differentiated intestinal cells, organized into crypt- and villus-like compartments (26). The key was defining the niche factors required to maintain stem cell function. For intestine, this included WNT3a, R-spondin-1, EGF, and Noggin. Subsequently, human organoids have been generated from normal and diseased tissues, including hepatobiliary, gallbladder (Tables 1, 2), stomach, esophagus, pancreas, brain, lung, heart, urinary bladder, mammary gland, and fallopian tube (27).

Taking a step from mouse to human hepatocyte organoids. Hepatobiliary organoids were first established in 2013 (28) from mouse LGR5+ progenitor-like oval cells that appear in the portal triad area after liver injury and cultured in the presence of WNT3a, R-spondin-1, EGF, HGF, FGF10, and Noggin (28, 29). The resulting organoids were bipotential, forming both hepatocyte and cholangiocyte lineages. Hepatocyte differentiation of these mouse bipotential organoid cells could be induced by removal of R-spondin-1 and HGF, treatment with Notch and TGF-β pathway inhibitors to lessen stemness, and addition of FGF, BMP7, EGF, and dexamethasone to promote differentiation (28, 29). Hepatocytes formed in this mouse organoid model were binucleated and expressed mature hepatocyte markers and function, evidenced by low density lipoprotein uptake, albumin secretion, glycogen accumulation, and induction of the cytochrome P450 system (28-30). However, these cultures still included cells expressing the ductal cell marker KRT19. Interestingly, transplantation of these organoids into the injured livers of *Fah-/-* mice promoted further hepatocyte maturation, evidenced by induction of the hepatocyte marker *Hnf4a* and loss of the cholangiocyte marker *Krt19* (29).

Human liver organoids were first reported in 2015 (29), similar to mouse (28), they were derived from bipotential EpCAM+ cells sorted from dissociated human liver and contained both hepatocyte- and cholangiocyte-like cells (29). Inhibition of TGF-β, and stimulation of cAMP and WNT signaling were requisite to maintain the organoid cultures long-term. They could be differentiated towards hepatocyte cell-containing organoids using culture conditions defined for mouse organoids (28, 29). Engraftment of human hepatocyte-like organoids into carbon tetrachloride-injured mouse livers was confirmed by detection of cells marked by human KRT19

and albumin, and human albumin and alpha-1 antitrypsin (A1AT) in mouse blood, signifying the presence of functional transplanted human hepatocytes (29).

More recently, organoids have been established from purified AXIN2+ mouse hepatocytes, which produced more mature, longer-lasting hepatocyte organoids (24, 31). These liver organoids, reported independently by the Nusse (31) and Clevers groups (24) in 2018, were cultured in media containing CHIR99021 (24, 31) and TNF-α (31), in addition to R-spondin-1, HGF, EGF, gastrin, and FGF10 (24). CHIR99021 is an aminopyrimidine derivative, which potently inhibits glycogen synthase kinase, thus, functioning as a WNT activator (32). The inclusion of TNF-α was based on the observation that liver repair involves recruitment of immune cells secreting TNF-α (31). Human fetal and adult primary hepatocyte-derived organoids can also be cultured long-term in this medium, producing grape-like clusters composed of large cells with low nuclear-cytoplasm ratio and hepatocyte appearance and function (24). However, these human and mouse hepatocyte-like cells differ from mature hepatocytes because they are cycling (31), express the fetal hepatocyte marker AFP and can be transdifferentiated into cholangiocytes upon WNT pathway withdrawal (24), suggesting cell immaturity and plasticity. Exposure to the cytokine oncostatin M (24) and dexamethasone (24, 31) promoted maturation of human (24) and mouse (31) hepatocyte-like cells, which could be successfully transplanted into injured livers of mice (24, 31).

Cell production on a large-scale is a challenging prerequisite for regenerative medicine. Bipotential liver organoids produced from biobanked human fresh-frozen hepatocytes (33, 34) and hepatocytes freshly isolated from patient donors (33) have been expanded in suspension in spinner flasks containing Matrigel ECM (33, 34). This increased hepatocyte cell number over a two-week period by 7-fold compared to static cultures (33), which was attributed to improved oxygenation and more efficient access to growth factors in spinner flasks. Importantly, spinner culture-derived liver organoids could be matured into hepatocytes in the presence of FGF19, BMP7, and dexamethasone (33, 34). These differentiated human hepatocytes failed to propagate upon subcutaneous transplantation into immunodeficient mice (33) supporting their non-tumorigenic properties and potential safety for liver regeneration therapy.

Collectively, these studies in both mouse and human suggest that activation of WNT signaling is crucial for hepatocyte organoid generation (24). Additionally, HGF, a known

hepatocyte mitogen, and TNF-α are factors promoting hepatocyte culture success (29, 31). When liver organoids are derived from hepatocytes and not bipotential ductal or oval cells, they contain more functionally mature hepatocytes. In turn, both human and mouse hepatocyte maturation determines cell engraftment success (28, 29, 35). Organoid-derived hepatocyte maturity has been assessed by measuring key hepatocyte features, such as bile acid production and transport, maintenance of metabolic homeostasis, and detoxication (30).

Can we bioengineer bile ducts?

The human biliary system includes EHBDs, IHBDs, cystic duct, and gallbladder, which have distinct progenitor cell populations. Biliary organoids have been generated from a variety of human tissue sources, including liver biopsy, liver and EHBD surgical samples from live and deceased donors, and cholangiogram-obtained samples (36-38) (Table 2). Cholangiocyte organoids are commonly cystic with a large lumen (39). Interestingly, less mature mouse cholangiocyte organoids have been observed to form more branching structures (40).

Human bile ducts were bioengineered in 2017 (36) from cholangiocyte cell organoids cultured long-term with R-spondin-1, EGF, and DKK-1, a WNT signaling antagonist thought to promote biliary maturation (36). The cells demonstrated typical cholangiocyte features, including cilia, an organelle crucial for cholangiocyte sensing and signaling, tight junctions, biliary cell markers such as KRT7, KRT19, GGT and SOX9, and alkaline phosphatase and GGT activities. Functionality characteristic for cholangiocytes (41) was demonstrated by rhodamine-123 accumulation, confirming the properly functioning multidrug resistance-associated protein 1, bile acid extrusion, and response to secretin with luminal fluid accumulation. These human cholangiocyte organoids formed bile-duct like tubes upon transplantation under the mouse kidney capsule (36). After seeding on polyglycolic acid scaffolds or densified collagen, they were also shown to repair EHBD and gallbladder defects in immunodeficient mice (37). This provides an important step forward demonstrating that human cholangiocyte organoids can serve as a platform for regenerative needs.

In 2018, bile duct organoids were generated from mouse LGR5+ bipotential liver cells cultured in media containing gastrin and acetylcysteine (40). These cultures contained cells possessing several features of cholangiocytes, including cilia, *Krt7*, *Krt19*, *Hnf1b*, *Aqp1* expression, and GGT activity. They exhibited functional transport of small molecules and

responded to an FXR agonist. The cholangiocyte-like organoids could be integrated with collagen-coated polyethersulfone hollow fiber membranes to bioengineer autologous bile ducts that exhibited polarized bile acid transport activity (40). Mouse studies also identified a clonogenic subpopulation of ST14+ cholangiocytes that can proliferate and produce long-term biliary organoids (42). Cholangiocyte-like cell organoids could also be generated from dissociated mouse EHBDs (43, 44), expressing markers of mature cholangiocytes (*Krt19*, *Aqpr1*) and progenitor cells (*Lgr5*), which have been used to study normal cholangiocyte biology (44) and EHBD cancer (43).

A major advance was reported in 2018 when Soroka *et al*. (38) showed that human organoid cultures could be generated from cholangiogram-obtained bile, thereby overcoming the limitation of invasive access to human biliary tissue. These bile-derived organoids were cultured long-term with R-spondin-1, EGF, HGF, gastrin, and FGF10, and exhibited cyst-like morphology. They expressed typical cholangiocytes markers, including EpCAM, SOX9, and KRT19, and demonstrated rhodamine-123 transport and GGT activity. This approach takes advantage of a common procedure for patients with cholangiopathies, and indicates the presence of cells with progenitor properties in bile.

It was recently shown that human IHBD and EHBD organoids generated from dissociated liver or EHBD tissue and cultured long-term in medium (50) containing R-spondin-1, EGF, HGF, and gastrin, had similar gene expression profile. However, only IHBD, and not EHBD, organoids showed potential to differentiate into hepatocyte-like cells when exposed to hepatocyte differentiation medium (39), as evidenced by induction of ALB, CYP3A4 and HNF4A (50). This suggests an intrinsic IHBD-specific cell plasticity to regenerate liver not contained in EHBD-derived cells.

Gallbladder organoids

Human fetal gallbladder has been shown to contain clonogenic EpCAM+/CD44+/CD133+ cells , which, when embedded in Matrigel and stimulated with R-spondin-2, and WNT3a, EGF, and Noggin, form organoids that can engraft subcutaneously in mice (45). It was also shown that cultured human fetal EpCAM+ gallbladder cells and EpCAM+ IHBD cells express distinctive transporters (45) (Table 2) suggesting that organoids from

different parts of the biliary tree cannot substitute for each other in pharmacotoxicology and biology studies.

To take advantage of tissue accessibility, gallbladder has also been studied as a source for hepatocyte generation (46). Upon removal of growth factors and nicotinamide, mouse gallbladder organoids differentiate towards hepatocyte fate and can be used for liver transplantation when injected under the liver capsule or via mesenteric vein in mice (46) (Table 2). Transdifferentiation potential underscores the exceptional cellular plasticity of hepatobiliary cells and identifies another more readily available source of cells for regenerative purposes.

CAN ORGANOIDS MODEL LIVER DISEASES?

Alpha-1 antitrypsin deficiency. The first organoid model of human liver disease was reported in 2015 using liver biopsies from patients with A1AT deficiency (29). These hepatocyte-like A1AT-deficient organoids accumulate intracellular A1AT aggregates, and their supernatants exhibited decreased ability to block neutrophil elastase activity, the functional defect in A1AT-deficient patients. A1AT-deficient organoids also demonstrated endoplasmic reticulum stress and increased apoptosis, suggesting mechanisms of patient liver pathology.

Alagille syndrome. Mutations in the genes encoding JAGGED-1 and NOTCH2 underlie the pathology in Alagille syndrome, which is characterized by the inability to form functional bile ducts leading to biliary atresia. Huch *et al*. reported in 2015 that undifferentiated bipotential organoids generated from liver biopsies of patients with Alagille syndrome failed to upregulate biliary markers when cued with cholangiocyte differentiation media (29), suggesting that these organoids are a valid model of human liver morphogenesis. Similar findings were observed with bile duct-derived organoids from a genetic mouse model of Alagille syndrome (47).

Wilson's disease. The Bedlington Terrier dog breed has a defect in the copper metabolism domain containing 1 gene (*COMMD1*), leading to hepatitis with liver fibrosis due to cooper accumulation, modeling human Wilson's disease. Hepatocyte-like organoids from these COMMD1-deficient dogs, first reported in 2015, demonstrated increased intracellular copper accumulation, which could be reversed by infection with a lentiviral construct encoding *COMMD1* (48). Autologous organoids with the corrected gene defect transplanted via portal vein into dog liver, engraft and survive up to two years (49). These studies show the potential of

gene therapy by correcting underlying genetic defects in autologous organoids to treat genetic liver diseases.

Primary sclerosing cholangitis. Studies of bile-derived organoids from patients with primary sclerosing cholangitis (38) showed suitability to model this multifactorial human disease *in vitro*. These organoids altered expression of genes associated with immune regulation, a heightened inflammatory response to IL-17, and increased expression of *HLA-DMA* and *CCL20*, genes previously reported to be associated with primary sclerosing cholangitis (50). Future co-culture studies involving primary sclerosing cholangitis organoids, stromal and immune cells would be needed to tease out the mechanisms of cellular crosstalk leading to biliary fibrosis, the underlying pathology of this devastating disease.

Steatosis. While there are currently no human organoid models of steatohepatitis, IHBD-derived cat liver organoids have been used to model nonalcoholic fatty liver disease (NAFLD)(51) as cats are prone to a severe form of steatohepatitis, feline hepatic lipidosis. Cat hepatocyte-like organoids showed upregulation of β-oxidation genes and decreased cell viability upon treatment with free fatty acids (FFAs). This phenotype could be rescued by co-treatment with L-carnitine required for transfer of FFA across the mitochondrial membrane. Conversely, inhibition of carnitine palmitoyltransferase-1, which blocks transmembrane FFA transfer, exacerbated the phenotype.

Alcoholic liver disease. Wang *et al*. used human hepatocyte organoids co-cultured with mesenchymal cells, both derived from fetal liver (52) to study non-monogenic alcoholic liver disease. Upon exposure to alcohol, hepatocyte-like cells in these organoid co-cultures developed oxidative stress with increased reactive oxygen species, steatosis with intracellular lipid droplet accumulation, and release of inflammatory mediators. The mesenchymal cells promoted hepatocyte maturation, and showed fibrogenic responses to alcohol, as evidenced by up-regulation of *LOXL2*, *COL1A1*, *COL3A1*, *ACTA2* and *TGF-β1,* recapitulating the pathophysiology of alcoholic liver disease.

Hepatitis B viral infection. Hepatocyte organoids from livers of healthy donors and patients infected with Hepatitis B virus (HBV) were recently shown to model HBV infection and tumorigenesis (53). Exposure of healthy liver organoids to recombinant virus or HBV patient serum led to organoid infection and active virus replication. HBV-infected organoids could be

used to test anti-HBV drugs and drug toxicity. Transcriptomic analysis of liver organoids from non-tumor cirrhotic liver of patients with HBV showed the presence of an early cancer gene signature and, therefore, a potential use of these organoids in cancer biomarker discovery (53).

Cystic fibrosis. EHBD organoids have been generated from a patient with a known compound CFTR gene mutation. These organoids were shown to have a defective secretory response to forskolin due to loss of CFTR-mediated chloride ion transport (54). This model system could be useful to characterize the molecular defects observed with different *CFTR* mutations.

Organoids in Liver Cancer Research

Hepatocellular carcinoma (HCC), cholangiocarcinoma (CCA), and mixed HCC-CCA are the most common primary hepatobiliary cancers, with increasing incidence and poor outcomes worldwide (55). Until recently, *in vitro* models of hepatobiliary cancers were limited to immortalized cancer cell lines established decades ago. Hepatobiliary cancer organoids can provide advanced *in vivo* and *in vitro* models to test drugs and study cancer biology in the era of personalized medicine. Several groups showed that tumoroids, organoids derived from human hepatobiliary cancer tissue, can be successfully established from all three major hepatobiliary cancer subtypes (56, 57) and gallbladder cancer (58). The rate of organoid establishment from primary tumor tissue is directly proportional to proliferative index (56, 57). Notably, organoids from healthy tissue contaminants can outgrow tumoroids in standard liver medium (29, 56). Thus, tumoroid culture requires distinct media, including withdrawal of R-spondin-1, WNT3a, and Noggin, and supplementation with dexamethasone and a Rho-kinase inhibitor to decrease apoptosis (56). Culture conditions for HCC, intrahepatic CCA, and mixed HCC-CCA (29) are quite similar, which might indicate a similar mechanism of tumorigenesis. Notably, tumoroids from extrahepatic CCA are more difficult to establish (58), suggesting differences between intra- and extrahepatic cancers.

Human tumoroid phenotypes *in vitro* and in mouse xenografts *in vivo* closely resemble the histology of primary tumors. HCC and mixed HCC-CCA tumoroids exhibit a solid structure without a lumen, and CCA tumoroids form gland-like structures invading the lumen (57-59). The cancer-related somatic genetic variants present in the primary tumors are retained in 84-88% of corresponding tumoroids and preserved in late passages (56, 57). Mutations in *CTNNB1*, *KRAS*,

ARID1A and *ARID2*, which are common in hepatobiliary cancers, are observed in tumoroids (60, 61). Some genetic aberrations can predict patient outcomes. For example, tumoroids enriched in SOX2 expression were associated with poor cancer prognosis (58).

Cultured human tumoroids and their xenografts can be used to test sensitivity to anti-cancer compounds to predict clinical outcomes. Accordingly, the EGFR inhibitor erlotinib reduced viability of a subset of intrahepatic CCA tumoroids independent of *KRAS* or other commonly observed mutations (58). Tumoroid treatment with sorafenib, an FDA-approved multikinase inhibitor for HCC (62), decreased viability of HCC tumoroid lines (57). Drug screening HCC and intrahepatic CCA tumoroids generated from different parts of the same tumor demonstrated high intratumor drug-response heterogeneity, indicating that a tumor biopsy might not represent whole tumor responses (63). Tumoroid production from several tumor sites, including metastatic foci, and co-culture with mesenchymal cells and immune cells might help to account for tumor heterogeneity and microenvironment influence.

Organoids from genetically engineered mouse models provide an additional instrument for hepatobiliary cancer research. Exposure of *Tp53* mutant mouse gallbladder organoids to *Salmonella* induced epithelial cell transformation to carcinoma, even in the absence of immune cells (64). CCA tumoroids generated from mice with epithelial KRAS activation and deletion of *Cdh1* and *Tgfbr2* (43) unveiled peribiliary glands as a source of CCA, and demonstrated a role for the alarmin IL-33 in cancerogenesis (43). Mouse gallbladder organoids with loss-of-function mutations in *Tp53* and *Pten*, and activating mutations in *Kras* or *Erbb2*, were studied to show that tumoroid histology *in vitro* is dependent on driver oncogenes, and to test anti-tumor activity of liposomal irinotecan delivery in xenografts in mice *in vivo* (65). Studies of mouse liver tumors identified tumor-initiating properties of LGR5+ cells (66).

Collectively, the data demonstrate a high translational potential of cancer-derived organoid models which recapitulate patient tumor histology, transcriptional and genetic landscapes, and could predict clinical outcomes.

CHALLENGES AND FUTURE DIRECTIONS

We have witnessed remarkable progress with hepatobiliary organoid technology over the last few years. Disease modeling shows great promise for organoid use for cancer research and

autologous transplantation therapy, especially after gene defect correction (56, 58, 67). EHBD and gallbladder repair using human cholangiocyte organoids incorporated into scaffolds suggests the potential to use patient-derived cells in regenerative structures to avoid problems with immune rejection. However, hepatocyte maturation, culture longevity, and large-scale production of pure cultures remain challenges. In addition, access to freshly-isolated human hepatocytes is very limited and maintenance of cultures in spinner flasks (33, 34) could be cost-prohibitive.

A general challenge for the organoid field is inherent to epithelial cell monocultures, which do not reflect the tissue complexity and heterogenous cell-cell interactions in the native tissue. For hepatobiliary organoid co-cultures, it would be preferable to include mesenchymal cells, including fibroblasts, derived from liver or biliary tissue sourced from the same patient. Mesenchymal cells can potentially promote hepatocyte maturation, as demonstrated in human fetal cell-dericed co-cultures (52). Further, addition of umbilical vein endothelial cells together with mesenchymal stem cells was shown to provide paracrine signals to induce hepatocyte differentiation and spatial 3D organoid organization in induced pluripotent cell-derived organoids (68). Co-culture of tumoroids with immune-cells would be an invaluable immune-oncology research tool as would co-culture with cancer-associated fibroblasts, as reported in pancreatic cancer models (69, 70).

Another general challenge for the organoid field is the need to identify appropriate ECM to support growth. Matrigel, the most commonly utilized matrix, is a mixture of ECM proteins extracted from mouse Englebreth-Holm-Swarm sarcoma cells (71). It is complex (72), not well defined, and rich in growth factors, including insulin-like growth factor 1 and EGF (73), and does not allow stiffness manipulation. Further, as mouse-derived, Matrigel can introduce zoonosis as well as xenogenic contaminants, which might induce immune responses (74). It is also prone to batch-to-batch variability.

ECM effects on hepatobiliary progenitor cells is not yet determined and may be problematic in cancer research. Per definition, cancer cells possess anchorage-independent growth, which is difficult to model with organoids requiring ECM. Ideally, matrices would mimic native ECM biological and mechanical properties and support a complex cell mixture. Further, matrix stiffness could be manipulated to recreate homeostatic and fibrotic

microenvironments to study cirrhosis and fibroproliferative cholangiopathies. If used for pre-clinical models, drug-testing, and hepatobiliary regeneration in humans, matrices need to be more clinically relevant and meet requirements of the Federal Drug Administration's Good Manufacturing Practices. Type 1 collagen (40), polyglycolic acid scaffolds (36), and decellularized extracellular matrix hydrogels (75) have being tested among other alternative synthetic matrixes (74). In spheroid organoid models the apical surface is directed towards the lumen and is challenging to access. A recently reported a "bile duct-on-a-chip" collagen device showed proper cholangiocyte polarization and permitted separate access to apical and basolateral surfaces (76).

Lastly, journal requirements for publishing organoid work have not been standardized. To interpret human organoid studies, one should consider interpersonal variability affecting disease phenotypes, and passage number, as organoid growth slows down with time (24), indicating changes in organoid biology in culture. Preservation of earlier passages and primary tissue biobanking for organoid recovery can help standardize experimental conditions and increase sample number. Finally, protocols for organoid establishment from the same tissue vary by cell isolation techniques and growth factor use, which likely results in cultures with different cell maturity. Thus, it is important to report functional and cellular fidelity. The most optimal and standardized organoid culture conditions are yet to be discovered.

CONCLUSION

The hepatobiliary organoid technology is evolving as culture conditions and validation techniques are being refined. Biobanking of primary human tissues and organoids with simultaneous collection of clinical data can be the basis for future, more advanced translational studies (Figure 2). At the current stage, hepatocyte, biliary and gallbladder organoids already provide an unparalleled tool to study homeostasis, disease processes, and alternatives to organ transplantation to one of the most complex organs of the human body, the liver.

TABLES:

Table 1. Selected hepatocyte organoid models, their validation, and applications. Immunohistochemistry (IHC), low density lipoprotein (LDL), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alpha-1 antitrypsin (A1AT).

Table 2. Selected bile duct and gallbladder organoid models, their validation, and tested applications. Extrahepatic bile duct (EHBD), immunohistochemistry (IHC), gamma-glutamyl transferase (GGT), and alkaline phosphatase (ALP).

FIGURES:

Figure 1. Human hepatobiliary organoid classification, tested applications, and challenges**.** *Permission was obtained from Elsevier for the hepatocyte organoid image (24)*.

Figure 2. Human hepatobiliary organoid translational workflow.

REFERENCES:

1. Clevers H. Modeling Development and Disease with Organoids. Cell 2016;165:1586-1597.

2. Kretzschmar K, Clevers H. Organoids: Modeling Development and the Stem Cell Niche in a Dish. Dev Cell 2016;38:590-600.

3. Stanger BZ. Cellular homeostasis and repair in the mammalian liver. Annu Rev Physiol 2015;77:179-200.

4. Strazzabosco M, Fabris L. Development of the bile ducts: essentials for the clinical hepatologist. J Hepatol 2012;56:1159-1170.

5. Chen F, Jimenez RJ, Sharma K, Luu HY, Hsu BY, Ravindranathan A, Stohr BA, et al. Broad Distribution of Hepatocyte Proliferation in Liver Homeostasis and Regeneration. Cell Stem Cell 2020;26:27-33 e24.

6. Matsumoto T, Wakefield L, Tarlow BD, Grompe M. In Vivo Lineage Tracing of Polyploid Hepatocytes Reveals Extensive Proliferation during Liver Regeneration. Cell Stem Cell 2020;26:34-47 e33.

7. Sun T, Pikiolek M, Orsini V, Bergling S, Holwerda S, Morelli L, Hoppe PS, et al. AXIN2(+) Pericentral Hepatocytes Have Limited Contributions to Liver Homeostasis and Regeneration. Cell Stem Cell 2020;26:97-107 e106.

8. Michalopoulos GK. Liver regeneration after partial hepatectomy: critical analysis of mechanistic dilemmas. Am J Pathol 2010;176:2-13.

9. Nadalin S, Testa G, Malago M, Beste M, Frilling A, Schroeder T, Jochum C, et al. Volumetric and functional recovery of the liver after right hepatectomy for living donation. Liver Transpl 2004;10:1024-1029.

10. Wang B, Zhao L, Fish M, Logan CY, Nusse R. Self-renewing diploid Axin2(+) cells fuel homeostatic renewal of the liver. Nature 2015;524:180-185.

11. Farber E. Similarities in the sequence of early histological changes induced in the liver of the rat by ethionine, 2-acetylamino-fluorene, and 3'-methyl-4-dimethylaminoazobenzene. Cancer Res 1956;16:142-148.

12. Carpino G, Cardinale V, Onori P, Franchitto A, Berloco PB, Rossi M, Wang Y, et al. Biliary tree stem/progenitor cells in glands of extrahepatic and intraheptic bile ducts: an anatomical in situ study yielding evidence of maturational lineages. J Anat 2012;220:186-199.

13. Hu M, Kurobe M, Jeong YJ, Fuerer C, Ghole S, Nusse R, Sylvester KG. Wnt/beta-catenin signaling in murine hepatic transit amplifying progenitor cells. Gastroenterology 2007;133:1579-1591.

14. Huch M, Dolle L. The plastic cellular states of liver cells: Are EpCAM and Lgr5 fit for purpose? Hepatology 2016;64:652-662.

15. Carpino G, Nevi L, Overi D, Cardinale V, Lu WY, Di Matteo S, Safarikia S, et al. Peribiliary Gland Niche Participates in Biliary Tree Regeneration in Mouse and in Human Primary Sclerosing Cholangitis. Hepatology 2019.

16. Fan B, Malato Y, Calvisi DF, Naqvi S, Razumilava N, Ribback S, Gores GJ, et al. Cholangiocarcinomas can originate from hepatocytes in mice. J Clin Invest 2012;122:2911-2915.

17. Aloia L, McKie MA, Vernaz G, Cordero-Espinoza L, Aleksieva N, van den Ameele J, Antonica F, et al. Epigenetic remodelling licences adult cholangiocytes for organoid formation and liver regeneration. Nat Cell Biol 2019;21:1321-1333.

18. Tanimizu N, Nakamura Y, Ichinohe N, Mizuguchi T, Hirata K, Mitaka T. Hepatic biliary epithelial cells acquire epithelial integrity but lose plasticity to differentiate into hepatocytes in vitro during development. J Cell Sci 2013;126:5239-5246.

19. Dutta D, Heo I, Clevers H. Disease Modeling in Stem Cell-Derived 3D Organoid Systems. Trends Mol Med 2017;23:393-410.

20. Takebe T, Sekine K, Kimura M, Yoshizawa E, Ayano S, Koido M, Funayama S, et al. Massive and Reproducible Production of Liver Buds Entirely from Human Pluripotent Stem Cells. Cell Rep 2017;21:2661-2670.

21. Ouchi R, Togo S, Kimura M, Shinozawa T, Koido M, Koike H, Thompson W, et al. Modeling Steatohepatitis in Humans with Pluripotent Stem Cell-Derived Organoids. Cell Metab 2019;30:374-384 e376.

22. Takebe T, Sekine K, Enomura M, Koike H, Kimura M, Ogaeri T, Zhang RR, et al. Vascularized and functional human liver from an iPSC-derived organ bud transplant. Nature 2013;499:481-484.

23. Sakabe K, Takebe T, Asai A. Organoid Medicine in Hepatology. Clin Liver Dis (Hoboken) 2020;15:3-8.

24. Hu H, Gehart H, Artegiani B, C LO-I, Dekkers F, Basak O, van Es J, et al. Long-Term Expansion of Functional Mouse and Human Hepatocytes as 3D Organoids. Cell 2018;175:1591-1606 e1519.

25. Clevers H, Nusse R. Wnt/beta-catenin signaling and disease. Cell 2012;149:1192-1205.

26. Sato T, Vries RG, Snippert HJ, van de Wetering M, Barker N, Stange DE, van Es JH, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature 2009;459:262-265.

27. Li M, Izpisua Belmonte JC. Organoids - Preclinical Models of Human Disease. N Engl J Med 2019;380:569-579.

28. Huch M, Dorrell C, Boj SF, van Es JH, Li VS, van de Wetering M, Sato T, et al. In vitro expansion of single Lgr5+ liver stem cells induced by Wnt-driven regeneration. Nature 2013;494:247-250.

29. Huch M, Gehart H, van Boxtel R, Hamer K, Blokzijl F, Verstegen MM, Ellis E, et al. Long-term culture of genome-stable bipotent stem cells from adult human liver. Cell 2015;160:299-312.

30. Schulze RJ, Schott MB, Casey CA, Tuma PL, McNiven MA. The cell biology of the hepatocyte: A membrane trafficking machine. J Cell Biol 2019;218:2096-2112.

31. Peng WC, Logan CY, Fish M, Anbarchian T, Aguisanda F, Alvarez-Varela A, Wu P, et al. Inflammatory Cytokine TNFalpha Promotes the Long-Term Expansion of Primary Hepatocytes in 3D Culture. Cell 2018;175:1607-1619 e1615.

32. Ye S, Tan L, Yang R, Fang B, Qu S, Schulze EN, Song H, et al. Pleiotropy of glycogen synthase kinase-3 inhibition by CHIR99021 promotes self-renewal of embryonic stem cells from refractory mouse strains. PLoS One 2012;7:e35892.

33. Schneeberger K, Sanchez-Romero N, Ye S, van Steenbeek FG, Oosterhoff LA, Pla Palacin I, Chen C, et al. Large-scale Production of LGR5-positive Bipotential Human Liver Stem Cells. Hepatology 2019.

34. Garnier D, Li R, Delbos F, Fourrier A, Collet C, Guguen-Guillouzo C, Chesne C, et al. Expansion of human primary hepatocytes in vitro through their amplification as liver progenitors in a 3D organoid system. Sci Rep 2018;8:8222.

35. Zhang K, Zhang L, Liu W, Ma X, Cen J, Sun Z, Wang C, et al. In Vitro Expansion of Primary Human Hepatocytes with Efficient Liver Repopulation Capacity. Cell Stem Cell 2018;23:806-819 e804.

36. Sampaziotis F, Justin AW, Tysoe OC, Sawiak S, Godfrey EM, Upponi SS, Gieseck RL, 3rd, et al. Reconstruction of the mouse extrahepatic biliary tree using primary human extrahepatic cholangiocyte organoids. Nat Med 2017;23:954-963.

37. Tysoe OC, Justin AW, Brevini T, Chen SE, Mahbubani KT, Frank AK, Zedira H, et al. Isolation and propagation of primary human cholangiocyte organoids for the generation of bioengineered biliary tissue. Nat Protoc 2019;14:1884-1925.

38. Soroka CJ, Assis DN, Alrabadi LS, Roberts S, Cusack L, Jaffe AB, Boyer JL. Bile-derived organoids from patients with primary sclerosing cholangitis recapitulate their inflammatory immune profile. Hepatology 2018.

39. Sampaziotis F, Tysoe O, Brevini T, Vallier L. Use of Biliary Organoids in Cholestasis Research. Methods Mol Biol 2019;1981:373-382.

40. Chen C, Jochems PGM, Salz L, Schneeberger K, Penning LC, van de Graaf SFJ, Beuers U, et al. Bioengineered bile ducts recapitulate key cholangiocyte functions. Biofabrication 2018;10:034103.

41. Banales JM, Huebert RC, Karlsen T, Strazzabosco M, LaRusso NF, Gores GJ. Cholangiocyte pathobiology. Nat Rev Gastroenterol Hepatol 2019;16:269-281.

42. Li B, Dorrell C, Canaday PS, Pelz C, Haft A, Finegold M, Grompe M. Adult Mouse Liver Contains Two Distinct Populations of Cholangiocytes. Stem Cell Reports 2017;9:478-489.

43. Nakagawa H, Suzuki N, Hirata Y, Hikiba Y, Hayakawa Y, Kinoshita H, Ihara S, et al. Biliary epithelial injury-induced regenerative response by IL-33 promotes cholangiocarcinogenesis from peribiliary glands. Proc Natl Acad Sci U S A 2017;114:E3806-E3815.

44. Shiota J, Zaki NHM, Merchant JL, Samuelson LC, Razumilava N. Generation of Organoids from Mouse Extrahepatic Bile Ducts. J Vis Exp 2019.

45. Manohar R, Li Y, Fohrer H, Guzik L, Stolz DB, Chandran UR, LaFramboise WA, et al. Identification of a candidate stem cell in human gallbladder. Stem Cell Res 2015;14:258-269.

46. Lugli N, Kamileri I, Keogh A, Malinka T, Sarris ME, Talianidis I, Schaad O, et al. R-spondin 1 and noggin facilitate expansion of resident stem cells from non-damaged gallbladders. EMBO Rep 2016;17:769-779.

47. Andersson ER, Chivukula IV, Hankeova S, Sjoqvist M, Tsoi YL, Ramskold D, Masek J, et al. Mouse Model of Alagille Syndrome and Mechanisms of Jagged1 Missense Mutations. Gastroenterology 2018;154:1080-1095.

48. Nantasanti S, Spee B, Kruitwagen HS, Chen C, Geijsen N, Oosterhoff LA, van Wolferen ME, et al. Disease Modeling and Gene Therapy of Copper Storage Disease in Canine Hepatic Organoids. Stem Cell Reports 2015;5:895-907.

49. Kruitwagen HS, Oosterhoff LA, van Wolferen ME, Chen C, Nantasanti Assawarachan S, Schneeberger K, Kummeling A, et al. Long-Term Survival of Transplanted Autologous Canine Liver Organoids in a COMMD1-Deficient Dog Model of Metabolic Liver Disease. Cells 2020;9.

50. Jiang X, Karlsen TH. Genetics of primary sclerosing cholangitis and pathophysiological implications. Nat Rev Gastroenterol Hepatol 2017;14:279-295.

51. Kruitwagen HS, Oosterhoff LA, Vernooij I, Schrall IM, van Wolferen ME, Bannink F, Roesch C, et al. Long-Term Adult Feline Liver Organoid Cultures for Disease Modeling of Hepatic Steatosis. Stem Cell Reports 2017;8:822-830.

52. Wang S, Wang X, Tan Z, Su Y, Liu J, Chang M, Yan F, et al. Human ESC-derived expandable hepatic organoids enable therapeutic liver repopulation and pathophysiological modeling of alcoholic liver injury. Cell Res 2019.

53. De Crignis E, Romal S, Carofiglio F, Moulos P, Verstegen MMA, Mir Mubashir Khalid MM, Pourfarzad F, et al. Human liver organoids; a patient-derived primary model for HBV Infection and Related Hepatocellular Carcinoma. In: bioRxiv; 2019.

54. Verstegen MMA, Roos FJM, Burka K, Gehart H, Jager M, de Wolf M, Bijvelds MJC, et al. Human extrahepatic and intrahepatic cholangiocyte organoids show region-specific differentiation potential and model cystic fibrosis-related bile duct disease. Sci Rep 2020;10:21900.

55. Massarweh NN, El-Serag HB. Epidemiology of Hepatocellular Carcinoma and Intrahepatic Cholangiocarcinoma. Cancer Control 2017;24:1073274817729245.

56. Broutier L, Mastrogiovanni G, Verstegen MM, Francies HE, Gavarro LM, Bradshaw CR, Allen GE, et al. Human primary liver cancer-derived organoid cultures for disease modeling and drug screening. Nat Med 2017;23:1424-1435.

57. Nuciforo S, Fofana I, Matter MS, Blumer T, Calabrese D, Boldanova T, Piscuoglio S, et al. Organoid Models of Human Liver Cancers Derived from Tumor Needle Biopsies. Cell Rep 2018;24:1363-1376.

58. Saito Y, Muramatsu T, Kanai Y, Ojima H, Sukeda A, Hiraoka N, Arai E, et al. Establishment of Patient-Derived Organoids and Drug Screening for Biliary Tract Carcinoma. Cell Rep 2019;27:1265-1276 e1264.

59. Bleijs M, van de Wetering M, Clevers H, Drost J. Xenograft and organoid model systems in cancer research. EMBO J 2019;38:e101654.

60. Sia D, Villanueva A, Friedman SL, Llovet JM. Liver Cancer Cell of Origin, Molecular Class, and Effects on Patient Prognosis. Gastroenterology 2017;152:745-761.

61. Wardell CP, Fujita M, Yamada T, Simbolo M, Fassan M, Karlic R, Polak P, et al. Genomic characterization of biliary tract cancers identifies driver genes and predisposing mutations. J Hepatol 2018;68:959-969.

62. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 2008;359:378-390.

63. Li L, Knutsdottir H, Hui K, Weiss MJ, He J, Philosophe B, Cameron AM, et al. Human primary liver cancer organoids reveal intratumor and interpatient drug response heterogeneity. JCI Insight 2019;4.

64. Scanu T, Spaapen RM, Bakker JM, Pratap CB, Wu LE, Hofland I, Broeks A, et al. Salmonella Manipulation of Host Signaling Pathways Provokes Cellular Transformation Associated with Gallbladder Carcinoma. Cell Host Microbe 2015;17:763-774.

65. Erlangga Z, Wolff K, Poth T, Peltzer A, Nahnsen S, Spielberg S, Timrott K, et al. Potent Antitumor Activity of Liposomal Irinotecan in an Organoid- and CRISPR-Cas9-Based Murine Model of Gallbladder Cancer. Cancers (Basel) 2019;11.

66. Cao W, Li M, Liu J, Zhang S, Noordam L, Verstegen MMA, Wang L, et al. LGR5 marks targetable tumor-initiating cells in mouse liver cancer. Nat Commun 2020;11:1961.

67. Favier RP, Spee B, Schotanus BA, van den Ingh TS, Fieten H, Brinkhof B, Viebahn CS, et al. COMMD1-deficient dogs accumulate copper in hepatocytes and provide a good model for chronic hepatitis and fibrosis. PLoS One 2012;7:e42158.

68. Asai A, Aihara E, Watson C, Mourya R, Mizuochi T, Shivakumar P, Phelan K, et al. Paracrine signals regulate human liver organoid maturation from induced pluripotent stem cells. Development 2017;144:1056-1064.

69. Tsai S, McOlash L, Palen K, Johnson B, Duris C, Yang Q, Dwinell MB, et al. Development of primary human pancreatic cancer organoids, matched stromal and immune cells and 3D tumor microenvironment models. BMC Cancer 2018;18:335.

70. Ohlund D, Handly-Santana A, Biffi G, Elyada E, Almeida AS, Ponz-Sarvise M, Corbo V, et al. Distinct populations of inflammatory fibroblasts and myofibroblasts in pancreatic cancer. J Exp Med 2017;214:579-596.

71. Kleinman HK, Martin GR. Matrigel: basement membrane matrix with biological activity. Semin Cancer Biol 2005;15:378-386.

72. Hughes CS, Postovit LM, Lajoie GA. Matrigel: a complex protein mixture required for optimal growth of cell culture. Proteomics 2010;10:1886-1890.

73. Vukicevic S, Kleinman HK, Luyten FP, Roberts AB, Roche NS, Reddi AH. Identification of multiple active growth factors in basement membrane Matrigel suggests caution in interpretation of cellular activity related to extracellular matrix components. Exp Cell Res 1992;202:1-8.

74. Aisenbrey EA, Murphy WL. Synthetic alternatives to Matrigel. Nat Rev Mater 2020;5:539-551.

75. Lewis PL, Yan M, Su J, Shah RN. Directing the growth and alignment of biliary epithelium within extracellular matrix hydrogels. Acta Biomater 2019;85:84-93.

76. Du Y, Khandekar G, Llewellyn J, Polacheck W, Chen CS, Wells RG. A Bile Duct-on-a-Chip With Organ-Level Functions. Hepatology 2019.

Cellular source	Validation	Applications	References
Human and mouse LGR5+/EpCAM+ bipotential liver cells	• IHC and mRNA abundance of progenitor, epithelial, hepatocyte, biliary, and proliferation markers • Functional studies for albumin secretion, LDL uptake, cytochrome P450 activity, glycogen storage, Rhodamine 123 transport, bile acid production, and ammonia elimination	Disease modeling (A1AT), Alagille syndrome, hepatitis B viral infection, tumorigenesis, regenerative medicine, gene therapy, large scale hepatocyte-like organoid production	28, 29, 33, 53
Primary human hepatocytes and mouse AXIN2+ hepatocytes	• IHC and mRNA abundance of progenitor, epithelial, hepatocyte, biliary, and proliferation markers • Functional studies for albumin secretion, LDL uptake, cytochrome P450 activity, glycogen storage, and Rhodamine 123 transport	Liver regeneration potential	24, 31
Commercially purchased primary mature human hepatocytes	• IHC and mRNA abundance of progenitor, epithelial, hepatocyte, biliary, proliferation markers	Large scale hepatocyte-like organoid production	34
Primary human liver cancer	• Histological and genetic correlation with primary tumors	Xenograft studies, drug screening, biobanking	56, 57, 58, 63
Cat and dog bipotential liver cells	• IHC and mRNA abundance of progenitor, epithelial, hepatocyte, biliary, and proliferation markers • Functional studies for albumin secretion, AST and ALT levels, cytochrome P450 activity, and glycogen storage	Disease modeling (hepatic steatosis, Wilson's disease), gene therapy	48, 49, 51

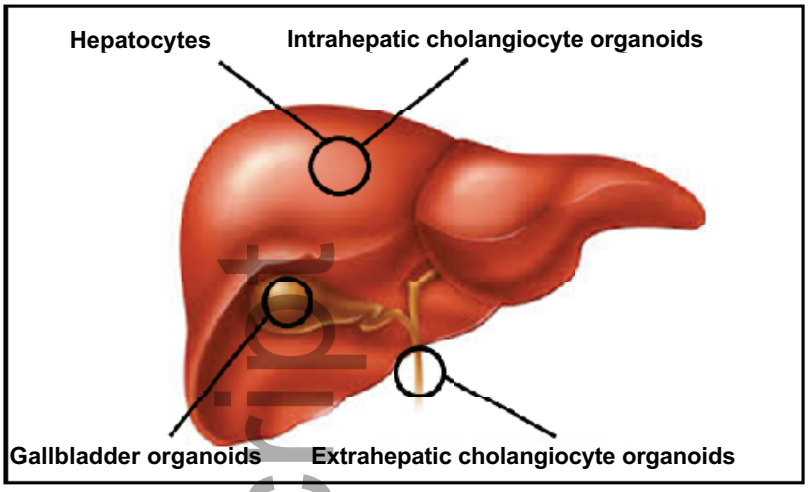
Table 1.

Cellular source	Validation	Application	References
Primary human liver cells, EHBD	• IHC and mRNA abundance of progenitor, epithelial, hepatocyte and biliary markers • Functional studies for Rhodamine 123 and Cholyl-L-lysyl-fluorescein transport and GGT and ALP activity	Bile duct bioengineering, regenerative medicine, cystic fibrosis	36, 37, 54

cholangiocytes and gallbladder	• Secretory response to Foskolin		
Human bile-derived cells	• IHC and mRNA abundance of progenitor, epithelial, hepatocyte, biliary, and immune markers • Functional studies for Rhodamine 123 transport and GGT and ALP activity	Disease modeling (primary sclerosing cholangitis)	38
Human fetal gallbladder	• IHC and mRNA abundance of progenitor, epithelial, hepatocyte, biliary, and proliferation markers	Regenerative medicine	45
Mouse bile ducts	• IHC and mRNA abundance of progenitor, epithelial, hepatocyte and biliary markers • Functional studies for Rhodamine 123 transport and response to FXR agonists	Bile duct bioengineering	40
Mouse EHBD cholangiocytes	• IHC and mRNA abundance of progenitor, epithelial, hepatocyte, biliary, and proliferation markers	Genetic models of biliary cancer and disease modeling	43, 44
Mouse gallbladder	• IHC and mRNA abundance of progenitor, epithelial, hepatocyte and biliary markers	CRISPR-Cas9-assisted mutagenesis, carcinogenesis, and regenerative potential	46, 64, 65

Table 2.

Fig. 1



Applications:

- Hepatobiliary biology
- Disease modeling: Alpha-1 antitrypsin deficiency, Wilson's disease, hepatic steatosis, primary sclerosing cholangitis, hepatobiliary cancers, viral hepatitis B, cystic fibrosis
- Regenerative medicine: cell transplantation, gene therapy (Wilson's disease)
- Personalized medicine:
  - Drug testing platforms
  - Animal cancer avatars (xenografts)
  - Biobanking

Pluripotent stem cell-derived organoids:

- Induced pluripotent stem cell-derived
- Embryonic stem cell-derived

Adult tissue-specific stem cell-derived organoids:

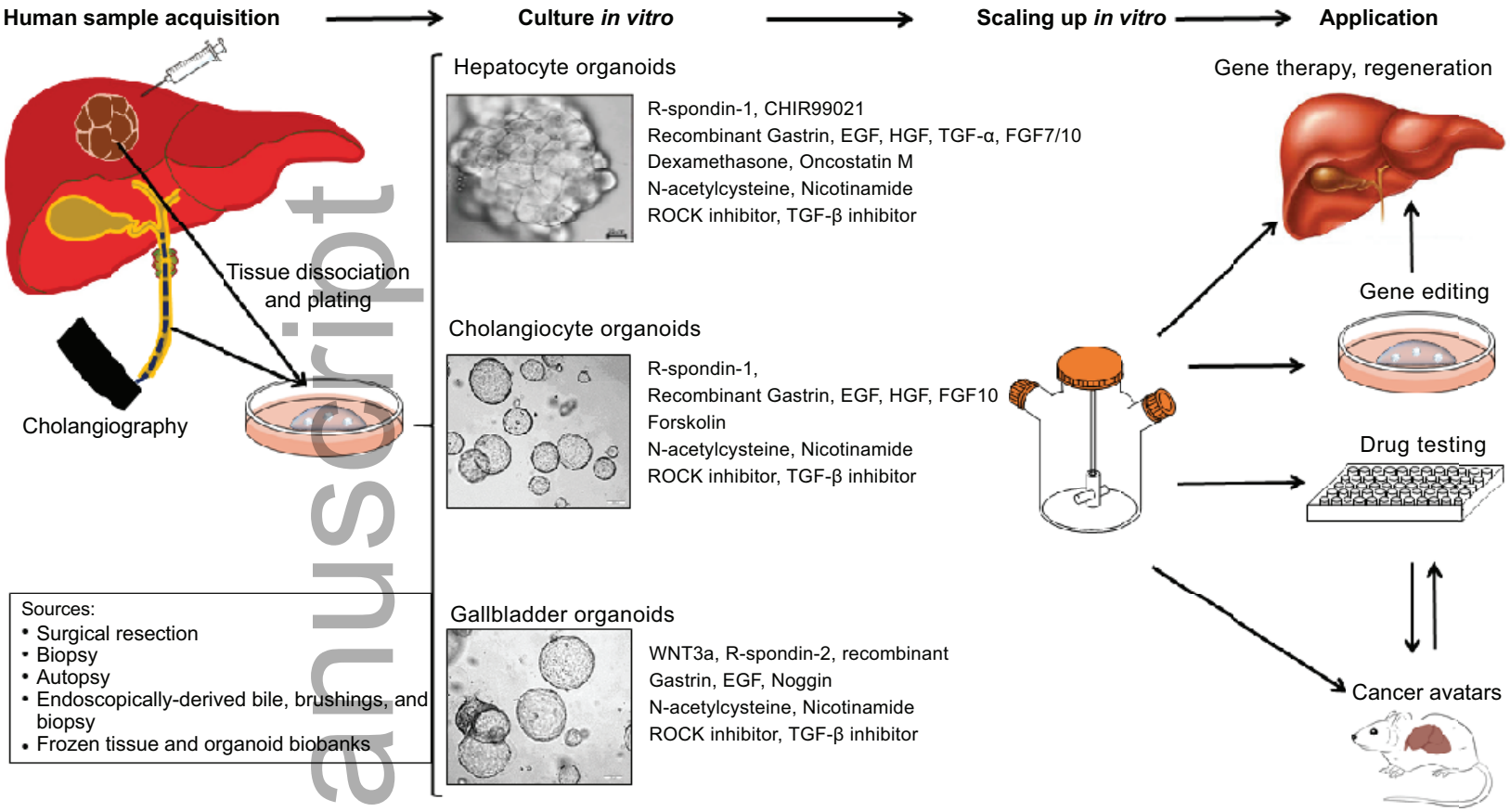
- Bipotential ductal cell-derived
- Hepatocyte-derived
- Cholangiocyte-derived
  - Intrahepatic
  - Extrahepatic
- Gallbladder-derived

Challenges:

- Hepatocyte maturation and large-scale production
- Human issue access
- Suboptimal extracellular matrices
- Co-culture with other cell types
- Standardization of generation and validation
- Undetermined safety for liver regeneration

hep_31772_f1.eps

Fig. 2



hep_31772_f2.eps