Research article

JHEP|Reports

Tumor organoids for primary liver cancers: A systematic review of current applications in diagnostics, disease modeling, and drug screening

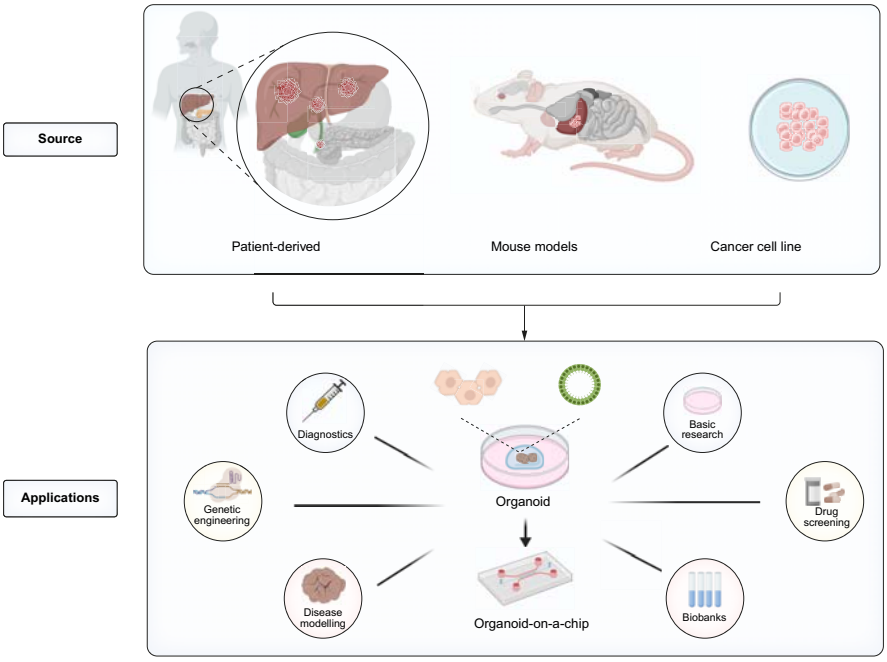
Authors

Ayesha A. Qureshi, Chase J. Wehrle, Sofia Ferreira-Gonzalez, …, Jos Melenhorst, Federico Aucejo, Andrea Schlegel

Correspondence

schlega4@ccf.org (A. Schlegel).

Graphical abstract



Highlights:

- This study underscores the utility of primary liver cancer organoids in diagnostic precision, disease modeling, and drug screening.
- Patient-derived organoids maintain the genetic traits and mutational profiles of primary tumors.
- Innovative approaches, such as co-culturing systems, significantly enhance the physiological relevance of organoid models.
- Despite these advances, standardizing *in vitro* protocols remains critical for translating organoid research into clinical practice.

Impact and implications:

This study provides an overview of the current understanding of tumor-derived organoids in primary liver cancers, emphasizing their potential in diagnostics, disease modeling, and drug screening. The scientific foundation rests on the organoids' ability to replicate the tumor microenvironment and genetic landscape, opening new avenues for personalized therapies. These insights are crucial for both researchers and clinicians, as patient-derived organoids can help identify biomarkers and therapeutic targets. Physicians and policymakers can harness these advances to drive progress in precision medicine, while recognizing the challenges involved in standardizing organoid models for clinical implementation.

https://doi.org/10.1016/j.jhepr.2024.101164

 JHEP Reports, 2024, 6,

Research article

JHEP|Reports

Tumor organoids for primary liver cancers: A systematic review of current applications in diagnostics, disease modeling, and drug screening

Ayesha A. Qureshi[1,†], **Chase J. Wehrle**[2,†], Sofia Ferreira-Gonzalez[3], Chunbao Jiao[4], Hanna Hong[2], Neda Dadgar[7,8], Jorge Arpi-Palacios[5], Yee Phoon Phong[5], Jaekeun Kim[2], Keyue Sun[4], Koji Hashimoto[2], David CH. Kwon[2], Charles Miller[2], Nic Leipzig[6], Wen Wee Ma[7], Jos Melenhorst[5], Federico Aucejo[2], Andrea Schlegel[2,4,*]

JHEP Reports **2024**. vol. 6 | 1–19

Background & Aims: Liver cancer-related deaths are projected to exceed one million annually by 2030. Existing therapies have significant limitations, including severe side effects and inconsistent efficacy. Innovative therapeutic approaches to address primary liver cancer (PLC) have led to the ongoing development of tumor-derived organoids. These are sophisticated three-dimensional structures capable of mimicking native tissue architecture and function *in vitro*, improving our ability to model *in vivo* homeostasis and disease.

Methods: This systematic review consolidates known literature on human and mouse liver organoids across all PLC subtypes, emphasizing diagnostic precision, disease modeling, and drug screening capabilities.

Results: Across all 39 included studies, organoids were most frequently patient-derived, closely followed by cancer cell line-derived. The literature concentrated on hepatocellular carcinoma and intrahepatic cholangiocarcinoma, while exploration of other subtypes was limited. These studies demonstrate a valuable role for PLC organoid cultures in biomarker discovery, disease modeling, and therapeutic exploration.

Conclusions: Encouraging advances such as organoid-on-a-chip and co-culturing systems hold promise for advancing treatment regimens for PLC. Standardizing *in vitro* protocols is crucial to integrate research breakthroughs into practical treatment strategies for PLC.

Introduction

Liver cancer is the third leading cause of cancer-related death worldwide and is estimated to account for one million deaths annually by 2030.[1,2] Hepatocellular carcinoma (HCC) constitutes approximately 80% of all primary liver cancers (PLCs), followed by intrahepatic cholangiocarcinoma (iCCA) and other rarer cancer types.[2,3] Owing to the liver's extensive functional reserve and robust compensatory capacity, most patients are diagnosed at advanced stages of PLC, rendering conventional therapies like radical resection and ablation ineffective.[2,4] Thus, treatment of advance-staged PLC often relies on systemic interventions including chemotherapy, radiation, targeted molecular therapy, and immunotherapy. However, these options are limited by their severe side effects and treatment efficacy.[2,3] As such, there is an immediate need for innovative therapeutic approaches to address PLC treatment.[5] Unfortunately, the low rate of *in vivo* success following *in vitro* discovery underscores the need for effective translation from bench to bedside, pivotal for improving therapeutic discovery in clinical practice.[6]

There is an ongoing transformative shift in cancer research with the advent of organoids, or complex three-dimensional structures with self-differentiation and self-organizing capacities, which simulate elements of the native tissue architecture and function *in vitro.* Organoids can be developed from a variety of sources including cell lines, stem cells, and primary cells.[7] Due to the intratumor heterogeneity and intricate tumor microenvironment (TME) that comprise PLCs, liver organoids are ideal pre-clinical models that recapitulate the molecular and structural features of patient tumors.[2] It is also possible to create multi-stage PLC organoids and study the initiation and progression of liver cancer through the assessment of novel biomarkers and disease-driving mutations that occur during tumorigenesis, greatly enhancing diagnostic precision and our basic understanding of the molecular events driving cancer progression. Liver organoids additionally facilitate high-throughput drug screening, allowing for cost-effective, rapid, and realistic evaluations of patient responsiveness to targeted medications, the ability to assess therapeutic resistance, and finally to develop personalized cancer therapeutics.[4,8]

* Corresponding author. Address: 9500 Euclid Avenue, Cleveland, OH 44195, USA; Tel. (216) 339-0741.
E-mail address: schlega4@ccf.org (A. Schlegel).
@ChaseWehrle *(C.J. Wehrle),* @KojiHashimotoMD *(K. Hashimoto)*
† Shared first authorship
https://doi.org/10.1016/j.jhepr.2024.101164

In recent years, a significant upsurge in published literature has highlighted the effectiveness of PLC organoids across diverse *in vitro* applications, incorporating novel developments such as co-culture models and organoids-on-a-chip. Despite this progress, limitations with clinical implementation and sample scarcity hinder a comprehensive realization of liver cancer organoid potential. This review seeks to consolidate the prevailing knowledge concerning utilization of liver organoids across all PLCs for diagnostic precision, disease paradigm, and drug screening, ultimately paving the way for further advances in hepatology.

Materials and methods

This systematic review is written in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (Fig. 1). The PICO (participants, interventions, comparators, and outcomes) process was also used to help detail the aims of this review. The International Prospective Register of Systematic Reviews (PROSPERO) was checked for similar reviews. Registration number is as follows: CRD42024513847.

Search strategy

Studies were identified by conducting a literature search on PubMed, Embase, and Web of Science databases. The following key words used for the search strategy are as follows: “organoid” OR “3D cell culture” OR “tissue spheroids” OR “mini organs”; “hepatocellular carcinoma” OR “hepatoma” OR “liver cancer” OR “liver transplant” OR “liver graft” OR “biliary tract carcinoma” OR “bile duct cancer” OR “intrahepatic cholangiocarcinoma”; “diagnosis” OR “drug” OR “therapy” OR “therapeutic” OR “gene expression” OR “biomarker” OR “organoid transplant” OR “inject”. An additional search of references from previous reviews and expert recommendations was undertaken to identify relevant studies. The full search strategy is available in Table S1.

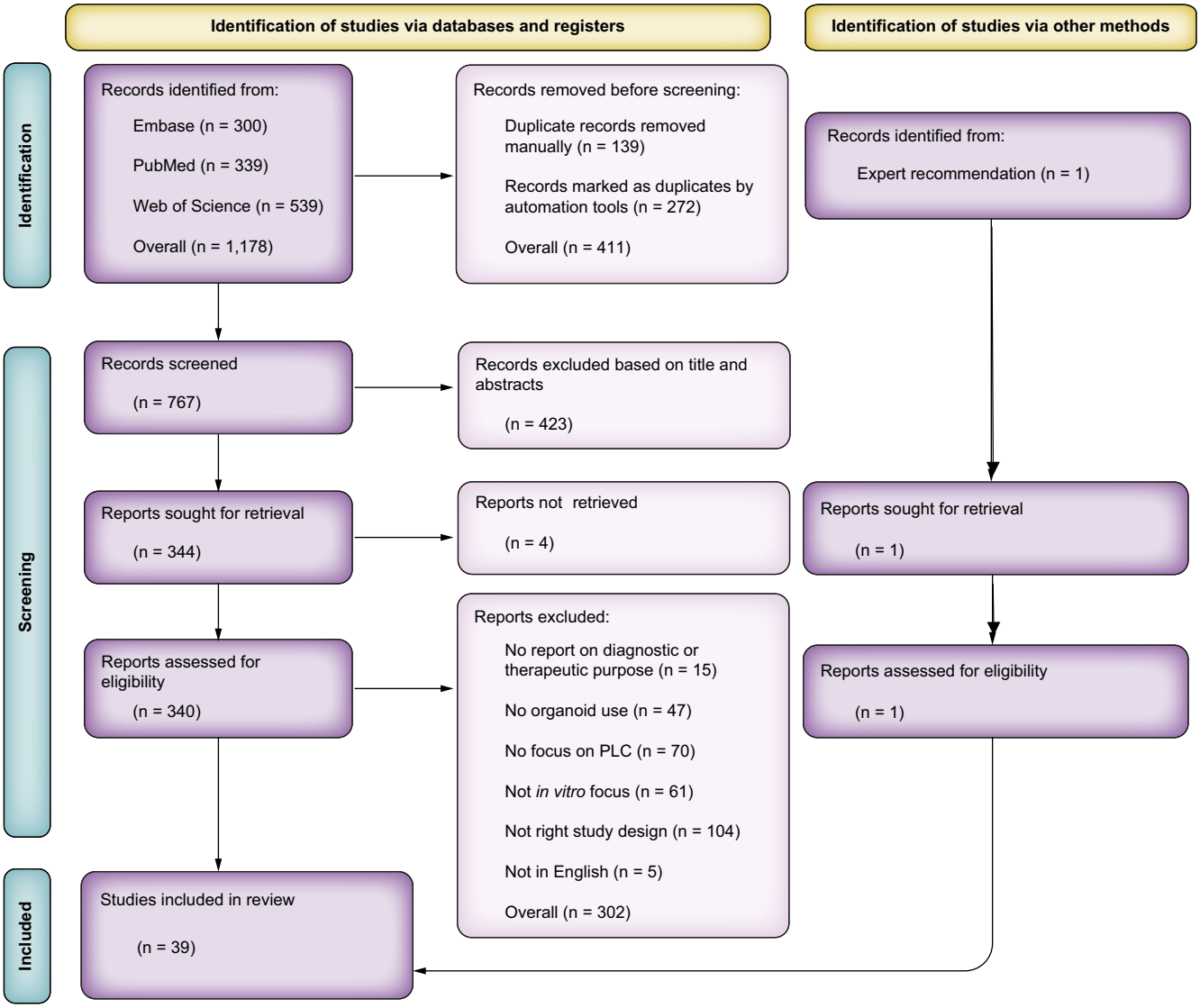


Fig. 1. Study selection framework: PRISMA flow diagram and inclusion and exclusion criteria. PLC, primary liver cancer.

Selection process

Eligible studies were screened against a pre-defined inclusion and exclusion criteria (Table 1) during both title/abstract review and full text review. A second reviewer (CJW) independently analyzed results against the inclusion and exclusion criteria. Duplicate results were removed using EndNote 20, followed by a manual check to identify remaining duplicates.

Data acquisition

The final list of articles was recorded as follows: authors, year of publication, organoid model type, organoids' role in diagnostics, disease modeling, and therapeutics. Data was categorized according to PLC type (Tables 2-4). Additionally, a critical analysis of the limitations present in the selected studies was conducted to provide a comprehensive understanding of the research landscape.

Results

Literature search

The database search yielded 1,178 results. An additional search of references from previous reviews and expert recommendations produced one result. A total of 411 duplicates were identified and removed. The remaining 767 results were screened on their title and abstract content, excluding a further 423 articles. After the exclusion of four articles that were not retrievable, the remaining 344 publications were evaluated based on the pre-defined inclusion and exclusion criteria (as outlined in Table 1). Thirty-nine studies met the inclusion criteria. Fig. 1 illustrates the application of the inclusion (*e.g.*, English language) and exclusion (*e.g.*, only conference abstract available) criteria for this systematic review.

Identification of organoid model type across all studies

The selected articles illustrated a diverse array of organoid models utilized in liver cancer research (Fig. 2). Patient-derived organoids (PDOs) obtained from whole liver preparations were the most frequently employed and were the organoids of choice in 25 out of 39 articles (64%).[9–33] Seven articles described cancer cell line-derived organoids.[34–40] Five articles used mouse models, with two authors using mouse iCCA cells[41] and mouse biliary cells,[42] and three authors using mouse liver tumor tissues.[43–45] Sun *et al.*[46] directly reprogrammed human hepatocytes (hiHeps) to establish organoids possessing liver architecture and function. Similarly, Ruland *et al.*[47] CRISPR-engineered human hepatocyte organoids to recreate liver cancer background.

Primary liver cancer classification across all studies

Articles covered the entire spectrum of primary PLC types (Fig. 3). HCC was the most prevalent cancer type and was described in 22 out of 39 articles (56%). While 14 of these articles solely investigated HCC,[12,15,19,26,29,31,33,34,36,37,39,40,43,44] others also included cancers such as cholangiocarcinoma (CCA),[9,11,13,17,21,25,32,46] gallbladder cancer,[25,32] combined hepatocellular-cholangiocarcinoma (CHC),[9,13] and hepatoblastoma.[13] Of note, biliary tract cancers such as gallbladder cancer[22,25,27,32] and neuroendocrine carcinoma of the ampulla of Vater,[22] were included in four studies; however, all four studies also assessed PLC, as part of the inclusion criteria of this review.

Cholangiocarcinoma was described in 21 out of 39 articles.[9–11,13,14,16–18,21,22,24,25,27,28,30,32,38,41,42,45,46] Of these, 14 were classified as solely iCCA[10,13,14,16,17,22,25,28,30,32,41,42,45,46] and five were not specified to either the intrahepatic or extrahepatic subtype.[9,11,21,24,38] Lieshout *et al.*[18] used both iCCA and extrahepatic CCA, and Wang *et al.*[27] used solely extrahepatic CCA. Two articles included hepatoblastoma,[13,23] two articles assessed CHC,[9,13] and three evaluated the rare fibrolamellar carcinoma (FLC).[20,35,47] Ji *et al.* was the only study to study four different types of PLC: HCC, iCCA, CHC, and hepatoblastoma.[13]

Utility in diagnostics

Of the 39 articles reviewed, 29 (74%) indicate potential diagnostic tools for PLC. Among these, 17 studies focused on the identification and validation of biomarkers linked to the initiation, progression, and prognosis of liver cancer.[9,10,13,16,18–22,29,30,32,34,36,38,40,43] The investigations included confirmation of the presence of well-established tumor markers in organoid models, such as Roos *et al.* showcasing the widespread expression of the CCA tumor marker KRT7.[38] Yet other studies focused on discovery of clinically linked biomarkers. Zhang *et al.*, for instance, reported that the heightened expression of tRNA-Lys-CUU in tumors correlated with overall worse clinical outcomes.[30] Saito *et al.* further highlighted increased levels of KLK6 and CPB2 significantly correlated with an unfavorable prognosis in CCA.[22] Notably, Broutier *et al.* identified previously unrecognized genes closely linked with an adverse prognosis in primary liver cancer.[9] Specifically, they reported the presence of *C19ORF48, UBE2S, DTYMK* (for HCC), and *C1QBP and STMN1* (for CCA) as novel prognostic markers within an organoid culture system.

Twenty-two articles explored gene expression in PLC organoids,[10,12–18,22,23,25,26,28–30,32,34,35,38,40,42,43,46] primarily utilizing PCR-based methods. Ji *et al.*, however, integrated transcriptomic data with other omics datasets including genomic, epigenomic, and proteomic data, to provide a

Table 1. Inclusion and exclusion criteria.

Category	Inclusion	Exclusion
Language	English	
Study design		Reviews, conference abstracts, editorials, opinion and commentary, protocols and techniques
Intervention	Articles specific to primary liver cancer (PLC) organoids *in vitro*	
Outcome	Articles reporting results on diagnostic potential Articles reporting results on disease modeling Articles reporting results on drug screening	

Table 2. HCC organoid data extraction table: HCC organoids in diagnostics, disease modeling, and therapeutics.

Author, year	Organoid	Species	Diagnostics	Disease modeling	Therapeutics	Limitations
Zou *et al.*, 2023	PDO +MSC-PBMC +CAF-PBMC	Human	N/A	MSC boosts PDO culture success by 27-54%, akin to CAFs' impact on HCC PDO growth. MSC-PDO-PBMC organoids mirror primary HCC tissues. Microfluidic platform accelerates PDO growth, enhances organoid uniformity.	Multi-layer microfluidic chip for drug screening. MSC-PDO-PBMC and CAF-PDO-PBMC models exhibit similar responses to various drugs, with superior predictive accuracy for anti-PD-L1 drug responses in assessing patients.	MSC passages 5-8 used, but concerns remain for long-term translation stability.
Peng *et al.*, 2023	CCO	Human	N/A	N/A	Niclosamide downregulated the Sorafenib-induced gene expression associated with glycolysis, stemness and drug resistance and enhanced the ability of Sorafenib to reduce the mitochondrial membrane potential in vitro. Niclosamide increases Sorafenib sensitivity in resistant HCC organoid.	Evaluation of HCC heterogeneity and diverse patient subgroups would provide clinical relevance.
Zhang *et al.*, 2023	PDOs	Human	N/A	N/A	Inhibition of ROS levels and reduced redox status in Lenvatinib-resistant HCC. LINC01607 regulated the p62-Nrf2 axis to enhance drug resistance by affecting mitophagy and antioxidant pathway. Silencing LINC01607 combined with Lenvatinib reversed resistance.	Potential off-target effects and consequences associated with targeting LINC01607.
Zhu *et al.*, 2022	CCO	Human	Implication of BNIP3 in heightened liver cancer cell tumorigenicity. CD24 elevation observed in liver cancer organoids alongside BNIP3. BNIP3 is mostly expressed by epithelial cells, but not immune cells in the TME. BNIP3 upregulated cancer cells might be armed with immune evasion arsenals.	N/A	N/A	Validate as a therapeutic target and explore immune evasion implications.
Xu *et al.*, 2022	PDO	Human	Overexpression of DUT found in 42% of HCC tumors, correlates with advanced stage HCC.	N/A	Stably expressed DUT in liver progenitor organoids confers drug resistance to TKI Sorafenib. TAS-114 targeting dUTPase potentiates suppression of HCC growth, synergizes with Sorafenib for better treatment sensitivity.	Need downstream effector pathways and mechanistic connections between DUT and signaling.
Konopa *et al.*, 2022	PDO	Human	G protein-coupled LPAR1 is a novel interaction partner of MRTF-A and FLNA. LPAR1 promotes FLNA phosphorylation at S2152 which enhances the complex formation of FLNA and MRTF-A, actin polymerization, and MRTF transcriptional activity.	N/A	Pharmacological blockade or depletion of LPAR1 prevents FLNA phosphorylation and complex formation with MRTF-A, resulting in reduced MRTF/SRF target gene expression and oncogene-induced senescence. Inhibition of the LPAR1–FLNA–MRTF-A interaction represents a promising strategy.	Validation of the identified mechanisms and interactions, with role of LPAR1.

(*continued on next page*)

Table 2. (*continued*)

Author, year	Organoid	Species	Diagnostics	Disease modeling	Therapeutics	Limitations
Wang *et al.*, 2021	PDO	Human	DHFR is therapeutically targetable.	N/A	Metformin treatment increases sensitivity to methotrexate by suppressing DHFR expression. Combination inhibits nucleotide metabolism, cell cycle progression, and tumorigenesis. Metformin represses DHFR transcriptionally via E2F4 and promotes DHFR degradation in lysosomes.	Mechanistic details of metformin induced DHFR degradation and its therapy.
Oz *et al.*, 2021	CCO	Human	Cell lines exhibit distinct expression patterns of Ki-67, CK18, CK7, and vimentin. Mesenchymal-like lines strongly express vimentin, with varying CK18. SNU449 differs in CK7 and CD44 from SNU398. Heterogeneous expression of progenitor markers and EMT markers.	Hep3B forms diverse colonies, Huh7 is highly proliferative, and HepG2 shows intense staining with small cells. Mesenchymal-like lines (SNU398, SNU449) differ in 3D structures and biomarker expressions. Study reveals heterogeneous biomarker expression in 3D-cultured HCC cell lines. All five HCC-derived 3D organoids exhibit compact structures, resembling primary HCC organoids. Hepatoblast-like organoids are more compact than mesenchymal ones.	N/A	Highlights cellular heterogeneity, warranting further exploration of genetic stability and the role of stem/progenitor subpopulations.
Liu *et al.*, 2021	PDO	Human	Top mutant genes in HCC: TP53, CTNNB1, ARID1A, AXIN1. HANs value correlates significantly with better OS, a potential prognostic biomarker. Positive correlation between HAN value and frequency of CD39+CD8+ TILs. Higher CD39+CD8+ TIL frequency linked with better OS.	CD39+CD8+ TILs from HAN-high groups exhibit enhanced antitumor activity when cultured with autologous tumor organoids. Organoids offer a valuable platform for evaluating immune cell antitumor potential, particularly concerning HAN status.	CD39+CD8+ TILs from HAN-high group demonstrate superior tumor-killing activity. Specific peptides induce peptide-specific T-cell responses in CD39+CD8+ TILs, suggesting potential therapeutic targets.	Mechanistic basis of HAN-induced activation of CD39+CD8+ T cells to discern molecular pathways, potential targets for therapeutic interventions.
Fan *et al.*, 2021	PDO	Human	Knockdown of CD47 reduced the migration and EMT triggered by sublethal heat treatment. The enzyme METTL3, involved in m6A modification, was induced by the 46 °C treatment, leading to increased CD47 expression in HCC cells. CD47 mRNA degradation was found to be stabilized in an IGF2BP1-dependent manner.	PDOs confirmed the stimulation of CD47 expression and EMT transition by sublethal heat treatment. Sublethal heat treatment (46 °C) increased the expression of CD47 in HCC cells compared to those treated at 37 °C.	Potential of the METTL3/IGF2BP1/CD47 axis as a therapeutic target for incomplete ablation-induced metastasis in HCC cells.	Organoid models acknowledged for evaluating phenotypic changes, additional in vivo investigations are warranted to validate the proposed mechanism.

(*continued on next page*)

Table 2. (*continued*)

Author, year	Organoid	Species	Diagnostics	Disease modeling	Therapeutics	Limitations
Cho *et al.*, 2021	CCO	Human	Elevated YAP/TAZ signaling in tumors associates with increased expression of stromal activation markers (α-SMA, fibronectin, vimentin) in TH tumors compared to S7HM tumors or normal liver tissues. Upregulation of master regulators of hepatic fibrosis (TGF-β, CTGF) in TH tumors.	Multicellular HCC organoid (MCHO) models established, containing hepatic stellate cells, fibroblasts, endothelial cells, and HCC cells.	High YAP/TAZ activity in HCC cells hinders verteporfin penetration. MCHOs with activated YAP/TAZ signaling exhibit stromal activation, impeding verteporfin penetration. Inhibiting YAP/TAZ activity increases drug penetration into MCHOs. YAP/TAZ signaling impairs drug delivery to liver cancer. Targeting activated tumor stroma may enhance drug delivery in HCC with elevated YAP/TAZ activity.	Mechanistic studies for molecular pathways and interactions: YAP/TAZ activity on drug delivery.
Cao *et al.*, 2020	MDCO	Mouse	Observation of higher levels of LGR5-expressing cells, a recognized stem cell marker, in both mouse liver tumors and human hepatocellular carcinoma. This upregulation suggests a potential role of LGR5 in liver cancer initiation and progression.	Single-cell suspension was directly mixed with Matrigel. Cells were cultured in organoid culture medium, which was based on advanced DMEM/F12. For the first 8–12 days, organoids were supplemented with Y-27632, Noggin, and Wnt3a-conditioned medium.	Displayed resistance to conventional treatments like Sorafenib and 5-FU. Ablation of LGR5 lineage significantly inhibits both the initiation of organoids and the growth of tumors. Combination of LGR5 ablation with 5-FU, but not Sorafenib, enhances therapeutic efficacy.	Use of LGR5 as an independent prognostic biomarker remains inconclusive.
Chen *et al.*, 2019	MDCO	Mouse	N/A	Cells were mixed with Matrigel. After Matrigel formed, a solid gel, medium was added softly. supplemented with B27, N2, N-acetylcysteine, gastrin, nicotinamide, EGF, FGF10, HGF, and R-spondin1. During the first 3 days, Noggin and Wnt3a (produced by 293T-HA-Noggin and L-Wnt3a cell lines, respectively) were added.	MPA effectively inhibited the growth of formed organoids shown by morphological appearance. MPA robustly inhibited the initiation of organoids from the dissociated single organoid cells.	Validate the observed inhibitory effects of MPA through prospective clinical trials.
Wang *et al.*, 2017	CCO +HUVEC-HPFFL	Human	N/A	Co-seeding of HCC cells and non-parenchymal cells formed tumor organoid-like structures and maintained viability. Models expressed more neo-angiogenesis-related markers, tumor-related inflammatory factors and molecules related to induced epithelial-mesenchymal transition compared with organoids containing only HCC cells.	N/A	Valuable organoid model for HCC, but validation needed to understand long-term functionality.

CAFs, cancer-associated fibroblasts; CCOs, cancer cell line-derived organoids; EMT, epithelial-mesenchymal transition; HANs, high-affinity neoantigens; HCC, hepatocellular carcinoma; HUVECs, human umbilical vein endothelial cells; ICIs, immune checkpoint inhibitors; LPAR1, lysophosphatidic acid receptor 1; MCCs, mouse cancer cells; MDCO, mouse-derived cancer organoids; N/A, not available or not applicable; OS, overall survival; PDOs, patient-derived organoids; TKIs, tyrosine kinase inhibitors; VECs, vascular endothelial cells.

comprehensive profile of patient-derived liver cancer organoids.[13] Two studies demonstrated the upregulation of proteins such as BNIP3 and DUT in HCC.[29,40] Of the 22 studies, 14 reported specific genes as potential therapeutic targets. Identified gene targets included DHFR, G6PD, and β-catenin-TCF4-CEGRs/ALCDs pathway.[13,26,35] Ten articles explored direct genetic alterations.[10,13,14,16–19,25,42,46] A CCA organoid model was identified to have a spectrum of mutant genes including those related to kinase signaling (*ARID1A, DDR2, ERBB2, FGFR1, IGF1R, KRAS, MTOR, NRAS, PIK3R1, ROS1); KMT2C* and *PTCHD3; FMN2* and *USP2*; *ARID1B, RTKs,* and *HDAC5; BAP1, IDH1; PBRM1, SMAD4*, and *TP53*.[10,17,18]

Seven studies assessed molecular and cellular processes, reporting signaling pathways and protein interactions to decode the dynamics of PLC gene expression. Notably, Konopa *et al.*[15] described the role of *LPAR1* in amplifying FLNA phosphorylation at S2152, subsequently augmenting the assembly of FLNA and MRTF-A complexes. This process facilitated actin polymerization and heightened MRTF transcriptional activity.

Disease modeling

Most of the reviewed articles, 30 out of 39 (77%) reported the efficacy of organoid models in mirroring PLC pathogenesis.[9,11–14,16–25,27,32–36,38,39,41–46] HCC organoids were established across 16 studies,[9,11–13,17,19,21,25,32–34,36,39,43,44,46] of which several underscored organoid precision in retaining genetic alterations observed in HCC. Wang *et al.*'s results reported that tumor organoids replicated neoantigen-related gene variations and maintained patient-specific heterogeneous profiles. 66.73% of neoantigen-associated mutations (range of 28.57–88.89%) were shared by primary tissues and organoids on average.[25] Broutier *et al.* found a 92% retention of genetic variants in early tumoroid cultures compared to each patient's tissue, a highly faithful preservation of the mutational landscape.[9] Despite a 26% organoid generation rate (10 out of 38 HCC biopsies) by Nuciforo *et al.*, HCC organoids exhibited comparable somatic mutation numbers (median 165, range 117–180) to corresponding tumor biopsies (median 146, range 127–207; $p = 0.78$, Mann-Whitney *U* test).[21] Cao *et al.* had a 70.8% organoid generation rate (63 out of 89 tumor tissues). These organoids maintained a population of LGRF5-positive cells, which was consistent with the upregulation seen in HCC tissues compared to tumor-free liver tissues ($p = 0.0066$).[43]

Zou *et al.* tested the influence of co-culturing HCC PDOs with mesenchymal stem cells (MSCs), overall improving the rate of successfully establishing biopsy-derived PDO culture from 27% (3 out of 11) to 54% (6 out of 11). MSCs did not alter the 82% (9 out of 11) success rate of surgical resection-derived PDOs.[33] Cho *et al.* co-cultured PDOs with hepatic stellate cells, fibroblasts, and endothelial cells. Incorporating stromal cells resulted in a denser organoid structure compared to organoids consisting only of HCC cells.[34] Wang *et al.* also discussed the role of non-parenchymal cells, reporting a statistically significant increased expression of neo-angiogenesis-related and inflammatory markers in co-seeded organoids ($p < 0.05$).[39] Eight articles specifically noted the ability of their developed organoids to capture the intratumor multiclonal diversity seen in liver cancer.[11,13,17,18,25,32,34,36]

CCA organoids were developed in 17 studies.[9,11,13,14,16–18,21,22,24,25,27,32,38,41,42,45,46] Lee *et al.* assessed genetic similarities between iCCA organoids and original tumor specimens. Of the 28 organoids evaluated, 96.4% displayed somatic mutations, primarily involving *TP53* (71%). Concordance evaluation with matching primary tumors consistently exceeded 70% for every organoid.[16] Saito *et al.* failed to establish more than three iCCA organoids, with a 50% success rate (3 out of 6 tissue specimens). The three iCCA organoids showed similar CK7, MUC1, and PAS staining patterns to the original primary tissue.[22] Histological features were evaluated to ascertain the preservation of parental tumor characteristics. CCA organoids were also demonstrated to have widespread glandular domains, with carcinoma cells invading the lumen and forming cribriform structures, mirroring observations in the patient's tissue.[9] Another study utilized RNA sequencing analysis and identified a common *KRAS* mutation (G12D) in organoids, consistent with the known prevalence of this mutation in iCCA.[14] Li *et al.* found that matched iCCA PDOs and primary tumors display similar staining for all markers tested, including EPCAM, CK19 and CK7, LGR5, and SOX9.[17]

Two studies each focused on hepatoblastoma[13,23] and CHC,[9,13] while three studied FLC.[20,35,47] Saltsman *et al.* initially established six human liver organoid lines from three patients with hepatoblastoma. After multiple passages, two of the organoids derived from tumor tissue failed to exhibit the mutations present in their associated tumor tissue samples. The profiling of transcriptomes identified 3,413 genes differentially expressed (false discovery rate <0.05, $|\text{Log}_2\text{fold change}| > 1$) between normal and tumor tissues. Tumor organoids exhibited distinct clustering, while normal organoids showed separation from both tumor and normal tissues.[23] The expression pattern of CHC organoid markers was maintained in a patient-specific manner. Notably, MUC5B expression was exclusive to CHC-1 organoids and absent in CHC-2, consistent with the tissue from the respective patients and with intrasubtype heterogeneity. Narayan *et al.* identified a transcriptome of 509 genes altered in FLC. Clustering analysis showed distinct patterns among FLC tumors, patient-derived FLC organoids, normal tissue, and patient-derived normal organoids. Differential expression analysis revealed 270 upregulated and 43 downregulated genes between FLC tumors and organoids, with a Pearson correlation coefficient of 0.82 for the fibrolamellar signature genes, such as *AKAP12, VCAN, OAT, NTS,* and *COL1A1*.[20] Rüland *et al.* CRISPR-engineered human hepatocyte organoids to mimic different FLC backgrounds, including the DNAJB1-PRKACA fusion and mutations in BAP1 and PRKAR2A. The mutant organoids exhibited similarities to primary FLC tumor samples, with combined loss of BAP1 and PRKAR2A leading to hepatocyte transdifferentiation into ductal/progenitor-like cells. While all FLC mutations caused hepatocyte dedifferentiation, DNAJB1-PRKACA fusion organoids display milder phenotypes.[47]

Four articles showcased innovative methodologies in establishing tumor organoid systems.[11,24,33,38] Zou *et al.*[33] used MSC and peripheral blood mononuclear cell (PBMC) co-culture to construct HCC organoid-on-a-chip. This effectively mimicked the original TME, shortened the growth time of PDOs, and enhanced dimensional uniformity. Van Tienderen *et al.*[24] introduced the potential of organoid technology and

Table 3. CCA organoid data extraction table: CCA organoids in diagnostics, disease modeling, and therapeutics.

Author, year	Organoid	PLC	Species	Diagnostics	Disease modeling	Therapeutics	Limitations
Cho *et al.*, 2023	PDO	iCCA	Human	Three clinically supported subtypes (stem-like, poorly immunogenic, and metabolism) were identified in iCCA and revealed intratumor heterogeneity in iCC. Specific mutated genes: *ARID1A, BAP1, IDH1, KRAS, PBRM1, SMAD4,* and *TP53.*	N/A	NCT-501 exhibited synergism with nanoparticle albumin-bound–paclitaxel in the organoid model for the stem-like subtype. Proposed combination treatment for stem-like subtype: NCT-501 with nab-paclitaxel. ALDH1A1 identified as a marker and therapeutic target for the stem-like subtype. Poorly immunogenic subtype linked to KRAS alterations, suggesting resistance to immunotherapy. Potential targeted therapies for KRAS mutations: Sotorasib and other developing KRAS-targeting drugs.	Low frequency of FGFR2 fusions (4%), but promising targets in iCCA.
Lee *et al.*, 2023	PDO	iCCA	Human	SD-type gene expressions: *APOE, SPARC,* and *BMP10* Higher *BAP1* (37.5%) and *IDH1/2* (12.5%) mutations in SD type. LD type: 'cholangiocarcinoma class 2,' 'KRAS dependency,' 'TGFβ-up gene,' 'ERBB-up gene'. Key LD-type transcription factors: *ATF2*, *ELK1*, *CTNNB1*, *FLI1*, *ZNF217*. LD-type gene expression: *GPRC5A, MUC5AC, TFF1.*	27 of 28 samples (96.4%) share somatic mutations between original tumors and organoids. iCCA organoids mirror primary tumor characteristics, retaining PD-L1 expression and abnormal chromosomal numbers. No statistical significance in organoid establishment time to progression comparisons. iCCA organoids enable CAA tumor sub-classification into LD type (S100P+) and SD type (N cadherin CD56).	IC_{50} values for the gemcitabine and cisplatin combination were higher for the LD type than for the SD type ($P = 0.002$). SD-type patients had a larger median tumor size (6.9 cm) compared with LD-type patients (4.2 cm) and more advanced cancer stage regardless of their subtype.	Enrolled patients may not represent iCCA spectrum due to tumor heterogeneity and small sample size.
Xin *et al.*, 2023	PDO	iCCA	Human	Identifying and classifying BRAF variants may be able to help guide precise treatment for patients with iCCA.	N/A	Results showed broad differences among organoids with different BRAF variant subtypes in sensitivity to BRAF or MEK inhibitors.	Exclusive focus on surgically resectable patients: inherent selection bias.
Van Tienderen *et al.*, 2022	PDO	CCA*	Human	N/A	ICO can self-assemble in microcapsules. Encapsulated CCAO exhibit a relatively similar gene and protein expression profile compared to conventional BME culture.	Gemcitabine and Cisplatin showed clear variation in the drug response to singular therapies. Probing similarities between patient and organoid drug response in the future is necessary to validate the ability of standardized production	Validating standardized tumor organoid production: correlate patient and organoid drug responses in broad or patient-specific therapeutics.
Roos *et al.*, 2022	BRCO BRCCAO	CCA*	Human	BRCCAOs showed widespread expression of the CCA tumor marker KRT7. BRCCAOs show higher expression of genes related to complex cellular pathways including tumor-associated hypoxia.	A branching morphology can be induced in adult intrahepatic cholangiocyte organoids. Branching cholangiocyte organoids resemble functional tubular structures in vitro. The branching characteristics are comparable to in vivo branching organs.	As CCAs *in vivo*, BRCCAOs are chemoresistant. Gemcitabine and cisplatin combinational therapy provides patients with only a modest benefit in overall survival and BRCCAOs closely reproduce this response.	To enhance BRCOs' functionality, must co-culture with hepatocyte-like cells.

(*continued on next page*)

Table 3. (*continued*)

Author, year	Organoid	PLC	Species	Diagnostics	Disease modeling	Therapeutics	Limitations
Pang *et al.*, 2022	MDO	iCCA	Mouse	N/A	Generated using established protocol via hydrodynamics induced mouse primary intrahepatic cholangiocarcinoma.	Three compounds 9, 12, and 26 significantly repressed tumor colony and sphere formation in both cell lines. The three analogues possessed an inhibitory role of organoid formation established from hydrodynamic induced mouse primary intrahepatic cholangiocarcinoma. 26 could significantly repress cancer stem markers.	Findings are specific to steroidal glycosides isolated from T. tschonoskii rhizomes.
Lieshout *et al.*, 2022	PDO	CCA**	Human	Mutant genes related to kinase signaling were *ARID1A, DDR2 ERBB2, FGFR1, IGF1R, KRAS, MTOR, NRAS, PIK3R1, ROS1*.Target kinases signify potential predictors of response. Potential of kinase activity profiles as biomarkers.	Each CCAO line displayed distinct kinomic pathways. Utility of organoids in modeling disease-specific cellular activities and responses.	Kinome profiling is a feasible method to identify druggable targets for CAA. Resistance to most drugs at lower concentrations. At higher concentrations, a few drugs showed promising results. Eight drugs were identified as pan-effective in reducing viability across all three CCAO lines. Among these, multi-tyrosine kinase inhibitors and specific inhibitors (e.g., Cobimetinib, Trametinib) were effective. EGFR, PDGFRβ, and MAPK are potential druggable targets for CCA,	Efficient killing of healthy adjacent organoids by targeted therapeutics may not accurately represent patient risk.
Koch *et al.*, 2022	PDO	iCCA	Human	*KRAS* in iCCA organoids exhibited the G12D mutation associated with cancer progression.	Established a robust analysis pipeline combining bleftfield microscopy and a straightforward image processing approach for the label-free growth monitoring of patient-derived organoids.	iCCA organoid growth was inhibited by sorafenib in a time- and dose-dependent fashion, while iCCA free organoids were unaffected. Quantification of the proliferation marker Ki67 confirmed inhibition of iCCAO growth by roughly 50% after 48 h of treatment with 4 μM sorafenib.	Broader multi-omics and clinical integration needed to understand mutations, morphology, and treatment links.
Bai *et al.*, 2022	MCC	iCCA	Mouse	N/A	iCCA mouse model was constructed by hydrodynamic transfection method. Mouse primary iCCA cells were purified from the induced mouse iCCA tumor tissues and then cultured the mouse iCCA organoids in three-dimensional (3D) medium.	Combination of Hinokitiol and Palbociclib showed a significant inhibitory effect on human iCCA cells and mouse iCCA organoids. Hinokitiol may have the potential to be developed as a clinical therapeutic drug for iCCA treatment.	Need for further investigation into the underlying mechanisms of Hinokitiol.
Zhang *et al.*, 2021	PDO	iCCA	Human	*KARS1* was upregulated in patient CC tumor tissues. High expression of tRNA-Lys-CUU in tumor is potentially associated with poor clinical outcomes. Biological or pharmacological targeting of the interface of charging lysine to tRNA-Lys-CUU inhibits cancer cell growth and migration.	N/A	N/A	Small cohort size (69 pairs of tumors and matched TFL tissues).

(*continued on next page*)

Table 3. (*continued*)

Author, year	Organoid	PLC	Species	Diagnostics	Disease modeling	Therapeutics	Limitations
Fujiwara *et al.*, 2019	MCC	iCCA	Mouse	Mutant IDH1 increased the formation of IBOs as well as accelerated glucose metabolism. Upregulation of PFKP.	Knockdown of the PFKP gene alleviated the mutant IDH1-induced increase in IBO formation. High expression of PFKP was observed more frequently in patients with IDH-mutant iCCA compared to in those with wild-type IDH ($p < 0.01$, 80.9% vs. 42.5%, respectively). IBOs expressing mutant IDH1 survived the suppression of ATP production caused by growth factor depletion and matrix detachment. Findings provide systematic understanding as to how mutant IDH induces tumorigenic preconditioning by metabolic rewiring in intrahepatic cholangiocytes.	N/A	Metabolic traits triggered by IDH mutation can be different among cell lineages. Findings may not be widely applicable.

BRCOs, human branching cholangiocyte organoids; CAFs, cancer-associated fibroblasts; CCAOs, encapsulated cholangiocarcinoma organoids; CCOs, cancer cell line-derived organoids; eCCA, extrahepatic cholangiocarcinoma; IBOs, intrahepatic biliary organoids; iCCA, intrahepatic cholangiocarcinoma; ICI, immune checkpoint inhibitors; ICO, healthy intrahepatic cholangiocyte organoids; LPAR1, lysophosphatidic acid receptor 1; MCCs, mouse cancer cells; MDCO, mouse-derived cancer organoids; N/A, not available or not applicable; OS, overall survival; PDO, patient-derived organoids; TKIs, tyrosine kinase inhibitors; VECs, vascular endothelial cells; CCA*, cholangiocarcinoma not specified as either intrahepatic or extrahepatic; CCA**, used both intrahepatic and extrahepatic subtype.

microfluidics convergence by demonstrating a one-step fabrication of hybrid microcapsules. Microcapsules enabled self-assembly and 3D culture of human cholangiocyte and cholangiocarcinoma organoids. This easily scalable method also produced size-standardized microcapsules (average diameter was within 157 μm, SD ± 14 μm), reducing the size variability in organoid culture and providing uniform scaffolding. Dong *et al.*[11] demonstrated the efficacy of alginate-gelatin hydrogel capsules, and successfully cultured 18 out of 28 patient-derived multicellular clusters as PDOs. The resulting PDOs preserved stromal cells, maintained a stable expression of molecular markers, and a similar tumor heterogeneity to the primary tissues. Roos *et al.*[38] proved that human adult intrahepatic cholangiocyte organoids can be induced to form a branching tubular architecture resembling bile ducts. Branching biliary organoids exhibited a stronger correlation with CCA tumors (correlation coefficient 0.80 ± SD 0.05) than non-branching organoids and CCA tumors (CC 0.55 ± SD 0.08).

Primary liver cancer organoids in therapeutic applications

Thirty-three studies[9–31,33–35,37,38,41,43–46] identified PLC organoid applications in therapeutic development, with 28 of them[10,11,13,14,16–27,29,32–35,37,38,41,43–46] specifically conducting drug screenings on their models. Narayan *et al.*[20] conducted the largest preliminary drug screening using patient-derived FLC organoids, testing approximately 650 drugs. Eight compounds exhibited over 50% survival inhibition across multiple test days. Similarly, Lit *et al.*[17] performed high-throughput drug screening on 27 PDOs derived from five primary liver cancers, treating them with 129 drugs and generating 3,483 data points.

Several studies assessed the efficacy of multi-tyrosine kinase inhibitors (TKIs). Li *et al.*'s[17] analysis revealed that ineffective drugs showed little variability, while targeted drugs such as TKIs showed higher variability in effectiveness, primarily due to inter-tumoral differences. Sorafenib and crizotinib effectively reduced viability across all three CCA organoid lines.[18] Koch *et al.*[14] further observed a time- and dose-dependent inhibition of iCCA organoid growth by sorafenib. Ji *et al.*[13] evaluated drug responses in various liver cancer organoids (iCCA, HCC, and CHC), demonstrating a strong correlation in predicting responses to already-approved liver cancer therapeutics such as regorafenib, lenvatinib, and sorafenib.

Two studies showed significant progress in understanding the interaction between neoantigen-specific peptides and the immune system's ability to target and destroy liver tumor organoids.[19,25] Wang *et al.*[25] explored the neoantigen landscape. Peptide-reactive T cells exhibited effectiveness in reducing live tumor organoid cells. The study also highlighted that immune checkpoint inhibitors heightened the sensitivity of tumor cells to neoantigen peptide-reactive T cells. Liu *et al.*[19] delved into immunological tumoricidal potential, noting that CD39+CD8+ tumor-infiltrating lymphocytes (TILs) from the high-affinity neoantigens (HAN)-high group displayed superior tumor-killing activity compared to those from the HAN-low group. Additionally, specific peptides inducing peptide-specific T-cell responses in CD39+CD8+ TILs were identified, suggesting potential therapeutic targets.

Nine studies investigated drug resistance within primary liver cancer organoids.[10,13,18,29,32,37,38,43] Zhao *et al.*[32] reported that organoids with metabolic advantages and enriched hypoxia

Table 4. Rare and mixed PLC organoid data extraction table: Rare and mixed PLC organoids in diagnostics, disease modeling, and therapeutics.

Author, year	Organoid	PLC	Species	Diagnostics	Disease modeling	Therapeutics	Limitations
Rüland *et al.*, 2023	CRISPR-edited human hepatocyte	FLC	Human	N/A	Organoids reflect different FLC mutations. BAP1KO;PRKAR2AKO mutants showed significant changes. Transcriptomic analysis mirrored FLC tumors. Loss of BAP1 and PRKAR2A shifted hepatocytes to a ductal/progenitor-like phenotype. BAP1KO, PRKAR2AKO organoids had drastic changes in cell identity and grew selectively in a ductal environment. BAP1 mutation primed hepatocytes for cell cycle progression, but PRKAR2A loss overrode mitotic arrest. This highlights the importance of these mutations in driving trans-differentiation and cancer stemness in FLC.	N/A	Does not state if multiple clonal lines represent a diverse range of genetic backgrounds or if they are derived from a limited pool of starting material.
Ji *et al.*, 2023	PDO	HCC iCCA CHC HB	Human	Mutated genes in both HCC and iCCA including *TP53*, *KMT2C*, *RB1*, and *PBRM1*, HCC-specific mutated *CTNNB1*, and iCCA-specific mutated *KRAS* and *BAP1*. L-PL exhibited the worst prognosis, L-LM showed the best, and L-DM, focused on drug metabolism, had an intermediate survival. L-LM had increased glycolysis and lipid metabolic pathways, whereas L-DM showed pentose phosphate metabolism and glutathione pathways. *G6PD* was significantly upregulated in the L-DM subtype compared to other subtypes, and its higher expression correlated with worse survival outcomes in HCC	Mutation clusters are highly consistent between organoids and original tissues. LICOB organoids better represent liver cancers than cell lines. LICOB recapitulated the histological and molecular features of the original cancer tissues and may serve as reliable models. LICOB models preserved intrinsic molecular traits and diversity of different liver cancer types. Four subtypes were characterized—LICOB (L)–iCCA, L-PL (proliferative subtype), L-LM (enriched in lipid metabolism), and L-DM (focused on drug metabolism).	Different subtypes within LICOB exhibited distinct drug response patterns. Some subtypes within L-iCCA were more resistant to tyrosine kinase inhibitors. The models showed good correlation in predicting responses for approved liver cancer therapeutics like Regorafenib, Lenvatinib, and Sorafenib. The study established an interactive website for comprehensive exploration of proteogenomic and pharmacological data from the LICOB cohort, aiming to facilitate broader biomedical applications.	Low success rate in establishing cancer organoids: small sample sizes in study.
Wang *et al.*, 2022	PDO	HCC iCCA GBC	Human	Higher neoantigen load correlated with early tumor stage. Discovered the prevalence of 11mer peptides as possible neoantigens, which had efficient MHC binding and transporter-associated antigen processing. Correlation between mutational patterns and neoantigen potential.	Tumor organoids recapitulated neoantigen-related gene variations of the primary tissues and maintained patient-specific heterogeneous neoantigen profiles.	Activation of peptide-reactive T-cell response under immune checkpoint inhibitors could be induced or boosted in a minority population. Peptide-reactive T cells effectively reduced live tumor organoid cells. ICIs increased the sensitivity of tumor cells to neoantigen peptide-reactive T cells.	Variability in tumor composition: certain tumor tissues unable to form organoids, need for refinement in the culture system.

(*continued on next page*)

Table 4. (*continued*)

Author, year	Organoid	PLC	Species	Diagnostics	Disease modeling	Therapeutics	Limitations
Narayan *et al.*, 2022	PDO	FLC	Human	Most of the patient-derived FLC organoids were positive for both CD68 and CK7, as were the patient tumors from which they were derived	DNAJB1-PRKACA fusion transcript was detected in FLC tumor tissue and PDO FLC. At both low and high magnification, the PDO FLC the tumors from which they were derived. Driver mutation in FLC, DNAJB1-PRKACA, was detected in all the PDO FLC and not in the patient-derived normal organoids.	A preliminary drug screening using organoids tested around 650 drugs. Eight showed over 50% survival inhibition on multiple test days. Of these, two compounds—finasteride and methotrexate—previously deemed safe in other cell lines, didn't display toxicity. Initial screening highlights the potential of FLC organoids in identifying new therapies.	Challenges in reproducibility during FLC drug screening, requiring altered coatings and treatment durations.
Gulati *et al.*, 2022	CCO	FLC	Human	The DNAJB1-PKAc- β-catenin-TCF4-CEGR/ALCD pathway is the main activator of fibrosis in patients with FLC. Targets of the β-catenin-TCF4-CEGRs/ALCDs pathway are mostly collagens. Elevation of SPARC in patients with FLC. SPARC is activated by β-catenin in patients with FLC and in FLC organoids.	Floating aggregates of FLC cells, referred to as FLC organoids were prepared and cultured from the xenografts established using the patient-derived FLC tumor line transplanted mice.	Inhibition of β-catenin by PRI-724 dramatically (175-fold) downregulated SPARC and other fibrotic genes. Rationale for considering β-catenin inhibitors as a therapy for FLC as well as a potential inhibitor of metastases in patients with FLC.	Complexity of CEGRs/ALCDs and their numerous oncogenic targets raises challenges in understanding all roles in cancer.
Dong *et al.*, 2022	PDO	HCC CCA*	Human	N/A	Simulation of the liver TME with suspended alginate-gelatin hydrogel capsules encapsulating patient-derived liver tumor multicellular clusters, and the culture of patient-derived tumor organoids. PDTOs, along with hepatocyte growth factor (HGF) of non-cellular components, preserve stromal cells, including cancer-associated fibroblasts (CAFs) and vascular endothelial cells (VECs). They also maintain stable expression of molecular markers and tumor heterogeneity similar to those of the original liver tumors. .	Drugs, including cabazitaxel, oxaliplatin, and sorafenib, were tested in PDTOs. The sensitivity of PDTOs to these drugs differs between individuals. The sensitivity of one PDTO to oxaliplatin was validated using magnetic resonance imaging (MRI) and biochemical tests after oxaliplatin clinical treatment of the corresponding patient.	Should further compare the therapeutic effects with clinical combination drugs.

(*continued on next page*)

Table 4. (*continued*)

Author, year	Organoid	PLC	Species	Diagnostics	Disease modeling	Therapeutics	Limitations
Zhao *et al.*, 2021	PDOs	HCC iCCA GBC	Human	*CTNNB1*, *GAPDH*, and *NEAT1* are commonly shared among hepatobiliary organoids. Metabolism-associated clusters feature similar genes: *GAPDH*, *NDRG1*, *ALDOA*, and *CA9*. Combination of *GAPDH* and *NDRG1* serves as an independent risk factor and predictive marker for patient survival.	Hepatobiliary tumor organoids are generated to explore heterogeneity and evolution. Intratumoral heterogenic subpopulations renders malignant phenotypes and drug resistance.	High epithelial-mesenchymal transition in HCC272 associated with broad-spectrum drug resistance. CD44 positive population may render drug resistance in HCC272. Enrichment of hypoxia signal upregulate NEAT1 expression in CD44 subgroup and mediate drug resistance that relies on Jak-STAT pathway.	Limited by a small number of clinical samples, affecting interpretation of tumor heterogeneity.
Wang *et al.*, 2021	PDO	GBC eCCA	Human	N/A	Successfully established five GBC and one eCCA PDOs. Different PDOs exhibited diverse growth rates during in vitro culture. Marker expression in cancer PDOs was similar to that of the original specimens	Gemcitabine was most efficient drug for eBTC treatment. Results from drug screening were confirmed to a certain extent by three clinical cases.	Insufficient representation of eCCA in the study's cancer PDOs due to low incidence.
Saltsman *et al.*, 2020	PDO	HB	Human	JQ1 is an inhibitor of the bromodomain and extra-terminal domain (BET) family of proteins	Hepatoblastoma tumor organoids recapitulate the key elements of patient tumors, including tumor architecture, mutational profile, gene expression patterns, and features of Wnt/β-catenin signaling that are hallmarks of hepatoblastoma pathophysiology.	Tumor organoids were successfully used alongside non-tumor liver organoids to perform a drug screen using 12 candidate compounds. JQ1, demonstrated increased destruction of liver organoids from hepatoblastoma tumor tissue relative to organoids from the adjacent non-tumor liver.	Low yield of tumor organoids, potential for optimizing culturing techniques.
Sun *et al.*, 2019	hiHep	HCC iCCA	Human	Excessive mitochondrion–endoplasmic reticulum coupling induced by c-Myc facilitated HCC. RAS- induced human iCCA-enriched mutations relied on Notch and JAK-STAT.	Directly reprogrammed human hepatocytes (hiHeps) and inactivation of p53 and RB: possessed liver architecture and function. HiHep organoids were genetically engineered to model the initial alterations in human liver cancers. RAS^{G12V} possess the capacity to drive the conversion from hepatocytes to iCCAs.	Combination Crenigacestat and Nifuroxazide led to a profound decrease in cell numbers and the expression of iCCA-related genes. Inhibition of Notch and JAK-STAT would provide a possible preventive strategy for RAS-induced iCCA formation.	HiHep organoids exhibit low expression levels of hepatocyte genes compared to primary human hepatocytes.
Saito *et al.*, 2019	PDO	iCCA GBC NE-CAV	Human	SOX2, KLK6, and CPB2 are prognostic biomarkers for BTC and CCA. High expression of KLK6 and CPB2 led to significantly poorer prognosis in CCA. GSTT1 is a candidate gene specific to CCA. BTC utlin-3a could be a potential therapeutic drug for refractory cancers harboring wild-type TP53.	Establishment of organoids derived from patients with BTC. Biological similarity between the primary BTC tissues and established organoids.	Drug screening identified antifungal drugs as potential therapeutic agents for BTC. High expression of CPB2 may indicate resistance to Erlotinib in BTC cancers	Non-cancer cells contaminating surgically resected tumor tissues may dominate the culture.

(*continued on next page*)

Table 4. (*continued*)

Author, year	Organoid	PLC	Species	Diagnostics	Disease modeling	Therapeutics	Limitations
Li *et al.*, 2019	PDO	HCC iCCA	Human	CCA PDOs displayed a frame- shift mutation in fibroblast growth factor receptor 1 (FGFR1). All PDOs have KMT2C and PTCHD3 mutations, CCA8-10 has FMN2 and USP2 mutations, PDOs CCA8-6 and CCA8-10 have ARID1B mutations, CCA8-10 and CCA8-11 have RTK mutations, and CCA8-5, CCA8-9, CCA8-10, and CCA8-11 have HDAC5 mutations.	Confirmed that PDO cultures displayed marker profiles similar to the original primary human tumors.	Cisplatin had no effect on PDOs, while gemcitabine had a moderate effect. Bortezomib exhibited significant inhibitory effects. Combination therapies didn't enhance the impact on PDOs. Nine were pan-effective (seven were novel) across all lines, belonging to five classes of antineoplastic agents. Variability in drug response (73%) stemmed from differences between tumors. Targeted drugs like TKIs showed higher variability	Classification of cancer drugs may oversimplify the complexity of drug responses in a clinical context.
Nuciforo *et al.*, 2018	PDO	HCC CCA*	Human	Consistent distribution and expression intensity of AFP, Glypican 3, glutamine synthetase, and heat shock protein 70 between organoids and their original tumor biopsy tissue. Some of the HCCs along with organoids stained positive for the biliary cell markers Keratin 7 (KRT7) and Keratin 19 (KRT19).	All HCC organoids are derived from poorly differentiated tumors HCC organoids maintained the growth pattern and differentiation grade of the originating primary tumors HCC organoids derived from tumor biopsies largely maintain the genetic alterations and mutational signatures observed in their originating HCCs.	Sorafenib reduced HCC organoid growth in a dose-dependent manner A CCA organoid derived from a rare subtype of CCA responded to sorafenib treatment in vitro Organoids derived from biopsies of PLC can be used to test tumor-specific sensitivities to growth-inhibitory substances.	Success rates for establishing organoids derived from BTCs are relatively low: modify culture conditions.
Broutier *et al.*, 2017	PDO	HCC CCA* CHC	Human	Tumor-derived organoid cultures could represent a valuable resource for biomarker discovery, especially for prognostic markers.C19ORF48, UBE2S, DTYMK (for HCC) and C1QBP and STMN1 (for CC) as previously unidentified genes associated with poor prognosis for primary liver cancer.	PLC-derived organoid cultures preserve the histological architecture, gene expression and genomic landscape of the original tumor. PLC tissue grown as organoid cultures faithfully models the genetic complexity of human PLC in vitro. Established cultures from tumors derived from eight individuals with HCC, CC and CHC.	Correlation between some drug sensitivities and the mutational profile in the tumoroid lines. De novo identification of the ERK inhibitor SCH772984 as a potential novel therapeutic agent for PLC. Future studies aimed at validating the efficacy of ERK inhibition in a bigger collection of tumoroid lines will be required.	Inability to model tumor microenvironment interaction without an immune system and stromal components in the culture system.

BRCOs, human branching cholangiocyte organoids; BTC, biliary tract carcinoma; CAFs, cancer-associated fibroblasts; CCAO, encapsulated cholangiocarcinoma organoids; CCOs, cancer cell line-derived organoids, CHC, combined hepatocellular-cholangiocarcinoma; eCCA, extrahepatic cholangiocarcinoma; HANs, high-affinity neoantigens; HB, hepatoblastoma; HCC, hepatocellular carcinoma; HUVECs, human umbilical vein endothelial cells; IBOs, intrahepatic biliary organoids; iCCA, intrahepatic cholangiocarcinoma; ICIs, immune checkpoint inhibitors; ICOs, healthy intrahepatic cholangiocyte organoids; LPAR1, lysophosphatidic acid receptor 1; MCCs, mouse cancer cells; MDCOs, mouse-derived cancer organoids; N/A, not available or not applicable; NECAV, neuroendocrine carcinoma of the Ampulla of vater; OS, overall survival; PDOs, patient-derived organoids; TKIs, tyrosine kinase inhibitors; VECs, vascular endothelial cells. CCA* = cholangiocarcinoma not specified as either intrahepatic or extrahepatic.

signals upregulate NEAT1 expression in the CD44 subgroup, inducing drug resistance through the Jak-STAT pathway. Xu *et al.*[29] discovered that the stable expression of DUT in liver progenitor organoids confers resistance to the TKI sorafenib. Cao *et al.*'s.[43] mouse liver tumor-based HCC organoid models displayed resistance to conventional liver cancer therapies like sorafenib and 5-FU. Cho *et al.*[10] identified a poorly immunogenic subtype associated with KRAS alterations, hinting at potential resistance to immunotherapy. Roos *et al.*'s[38] exploration unveiled that *in vivo*, branching cholangiocyte organoids demonstrated chemoresistance, underlying the modest benefits of gemcitabine/cisplatin combinational therapy on overall patient survival. Peng *et al.*[37] showed that niclosamide effectively downregulated sorafenib-induced gene expression related to glycolysis (*GLUT1, HK2, LDHA,* and *PEPCK*), stemness (*OCT4*), and drug resistance (*ABCG2*). Moreover, it boosted sorafenib's ability to reduce the mitochondrial membrane potential *in vitro*.

Three studies introduced innovative approaches for high-throughput drug screening using organoid models. Zou *et al.*[33] devised a multi-layer microfluidic chip specifically tailored for high-throughput co-culture (*e.g.* with MSCs and cancer-associated fibroblasts) in drug screening. Their models, MSC-PDO-PBMCs and cancer-associated fibroblast-PDO-PBMCs, exhibited comparable responses to chemotherapeutic or targeted antitumor drugs. Notably, they displayed enhanced precision in predicting patient responses to anti-PD-L1 drugs. Ji *et al.*[13] established a patient-derived liver cancer organoid biobank (LICOB), enabling high-throughput drug screening that unveiled distinct response patterns associated with specific multiomics signatures for each subtype. By integrating LICOB pharmacoproteogenomic data, they identified molecular features linked to drug responses, predicting potential personalized treatment combinations. Van Tienderen *et al.*[24] assembled encapsulated CCA organoids and demonstrated their suitability for drug screening. Their screening of gemcitabine and cisplatin revealed clear variations in drug responses to individual therapies.

Discussion

This systematic review covered 39 articles describing the utility of tumor organoids in primary liver cancer research. Most articles described utility of organoids for therapeutic discovery, closely followed by studies highlighting diagnostic potential and their role in disease modeling. Organoid systems are well-suited for conducting extensive studies in drug discovery, as previously cited by Vandana *et al.*[48] However, there was still a significant portion of studies (51%, 20/39), which evaluated organoids across all parameters: diagnostic precision, disease modeling, and therapeutic applications, underlining the expanding and versatile applications of organoids in primary liver cancer research.

Most articles described PDOs. PDOs represent advanced 3D cell culture models faithfully replicating the intricate structure and functionality of tumor tissue. They vividly demonstrate complex cell-to-cell and cell-to-matrix interactions while exhibiting pathophysiological traits akin to differentiated tumor tissue in laboratory settings. As a model, primary liver tumor organoids can retain the histological architecture, gene expression patterns, and genomic landscape of the original tumor. This fidelity renders them invaluable tools for identifying biomarkers and conducting drug screening, offering a platform that closely mirrors real tumor behavior.[49] They can also provide exciting tools for precision medicine, allowing for the *in vitro* testing of drugs on a patient's tumor in real time. Proposed utility in precision medicine was described in multiple articles covered in this review.[13,28,31,33,44] Xin *et al.* emphasized significant variations in the response to BRAF or MEK inhibitors across organoids with diverse BRAF variant subtypes.[28] Identifying and classifying these variants can guide precise treatment for patients with PLC. Ji *et al.* identified subtype-specific drug response patterns and multiomics signatures, enabling the prediction of personalized treatment combinations through LICOB data integration.[13] In addition to the development of novel therapeutics, as models improve, there might be a scalable methodology allowing for selection of ideal therapeutic regimens for patients after testing of their own tumor biology using PDOs.

Included articles demonstrate proof of concept that PLC organoid cultures serve as a valuable resource for biomarker discovery. Notably, much of the research focused on biomarker reporting in CCA, revealing that heightened expression of tRNA-Lys-CUU, KLK6, and CPB2 in tumors correlated with unfavorable clinical outcomes.[22,30] However, identification of prognostic biomarkers in HCC seems more challenging. Oz *et al.*'s study highlighted diverse biomarker expression among HCC cell lines in 3D culture, hinting at varied cellular characteristics and potential phenotypic flexibility.[36] This aligns with prior studies that found the tumor mutational burden lacked correlation with specific neoantigens in the HCC microenvironment, rendering it unsuitable as a predictive biomarker. Interestingly, higher tumor mutational burden and/or neoantigens displayed significant correlations with improved survival in other cancers like non-small-cell lung cancer and melanoma.[19,50] However, recognizing the unique ability of PLC-derived organoids to maintain the original tumor's mutational landscape and expression profile even after prolonged culture expansion, Broutier *et al.* hypothesized the possibility of identifying prognostic biomarkers specific to HCC. This study[9] reported the first ever specific prognostic biomarkers from an HCC organoid culture system, with a set of previously unidentified genes - *C19ORF48, UBE2S, DTYMK* (for HCC), and *C1QBP* and *STMN1* (for CCA) – being tied to adverse oncologic outcomes.

Traditionally, organoids are cultured in tumor-derived basement membrane extracts (BMEs), a complex mixture of extracellular matrix components. BME promotes self-organization, allowing organoids to form as three-dimensional structures, closely mimicking organs. The choice of BME is frequently Matrigel, an extract of the EHS mouse tumor,[51] which comprises the key constituents found in the structural matrix of various tissues (Fig. 4). High batch-to-batch variability and many undefined factors in Matrigel pose similar challenges encountered with other serum-based cell culture methods such as FBS. This uncontrolled process leads to a disparity in sizes among organoids, affecting reproducibility and scalability. Dong *et al.*[11] proposed a methodology involving suspended alginate-gelatin hydrogel capsules to simulate the liver TME. These capsules surround patient-derived liver tumor multicellular clusters, allowing for the cultivation of PDOs. The 3D matrix environment mimics the mechanical and biological properties of the *in vivo* liver and facilitated the successful

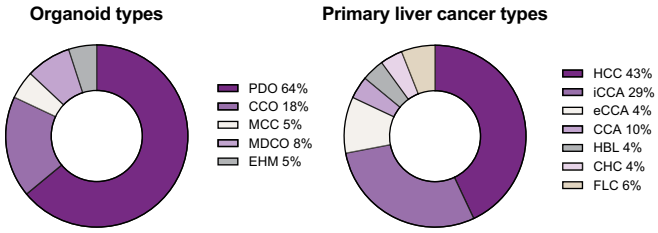


Fig. 2. Identification of organoid model type and primary liver cancer classification across all studies. CCA, cholangiocarcinoma (not specified as either intrahepatic or extrahepatic subtype); CCOs, cancer cell line-derived organoids; CHC, combined hepatocellular-cholangiocarcinoma; eCCA, extrahepatic cholangiocarcinoma; EHM: extrahepatic metastases; FLC, fibrolamellar carcinoma; HBL, hepatoblastoma; HCC, hepatocellular carcinoma; hiHeps, reprogrammed human hepatocytes; iCCA, intrahepatic cholangiocarcinoma; MCCs, mouse cancer cell-derived organoids; MDCOs, mouse-derived cancer organoids; PDOs, patient-derived organoids. Numbers are provided in percent based on the systematic literature review.

culturing of 18 out of 28 patient-derived multicellular clusters as PDOs. The resulting organoids exhibited stable expression of molecular markers and retained tumor heterogeneity comparable to the original liver tumors, highlighting the high fidelity of this approach. However, it is also possible these hydrogels would still fail to resolve issues related to organoid size heterogeneity and they do require a time-intensive culture process. In response to these challenges, Van Tienderen *et al.*[24] introduced a microfluidic method utilizing hybrid microcapsules containing liver-derived extracellular matrix. These microcapsules demonstrated a gene and protein expression profile relatively akin to conventional culture methods utilizing BMEs. This approach offers a more standardized and scalable environment, potentially addressing the constraints associated with organoid size heterogeneity and the time-consuming culture process observed with the use of hydrogel capsules.

Ensuring the fidelity of organoids to their parental tumors is paramount for their utility in PLC research. While the reviewed studies highlight the potential of organoids in recapitulating the complexities of PLC, systematic validation to affirm their resemblance to original tumors remains inconsistent. Rigorous validation procedures encompassing comprehensive analyses of gene expression, histological characteristics, and functional assays are imperative. Studies like those by Broutier *et al.*[9] and Wang *et al.*[25] have assessed the retention of genetic alterations in HCC organoid models, demonstrating high fidelity preservation of the mutational landscape with over 90% retention of genetic variants in early tumoroid cultures. Wang *et al.*[25] further revealed a significant overlap

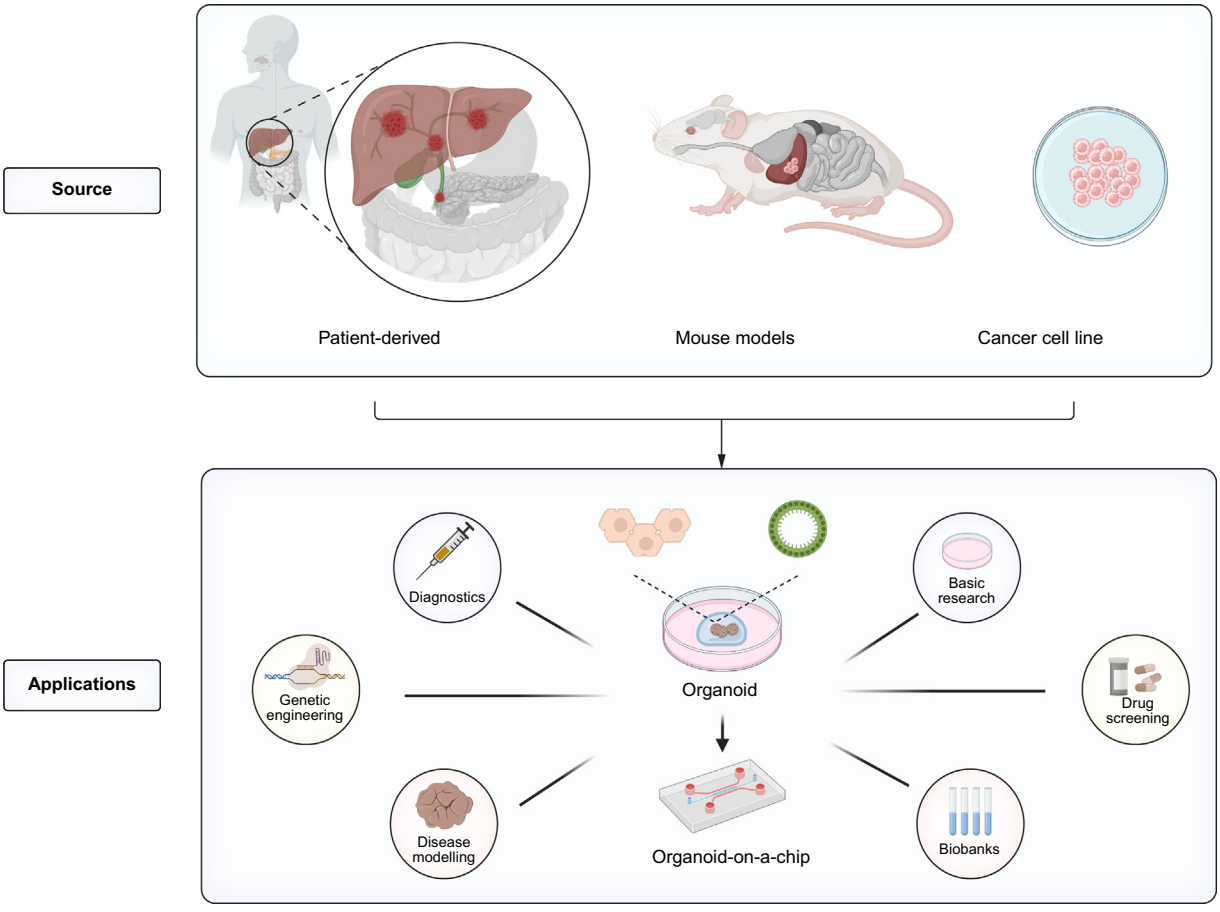


Fig. 3. Sources and applications of primary liver cancer organoids. (A) Primary liver cancer organoids are mainly built from patients' tissue, mouse models and cell lines. (B) Based on different research needs, primary liver cancer organoids are widely explored in disease modeling, therapeutic exploration, drug screening. With the encouraging advances of organoid-on-a-chip, more promising treatments and breakthrough basic science research are emerging.

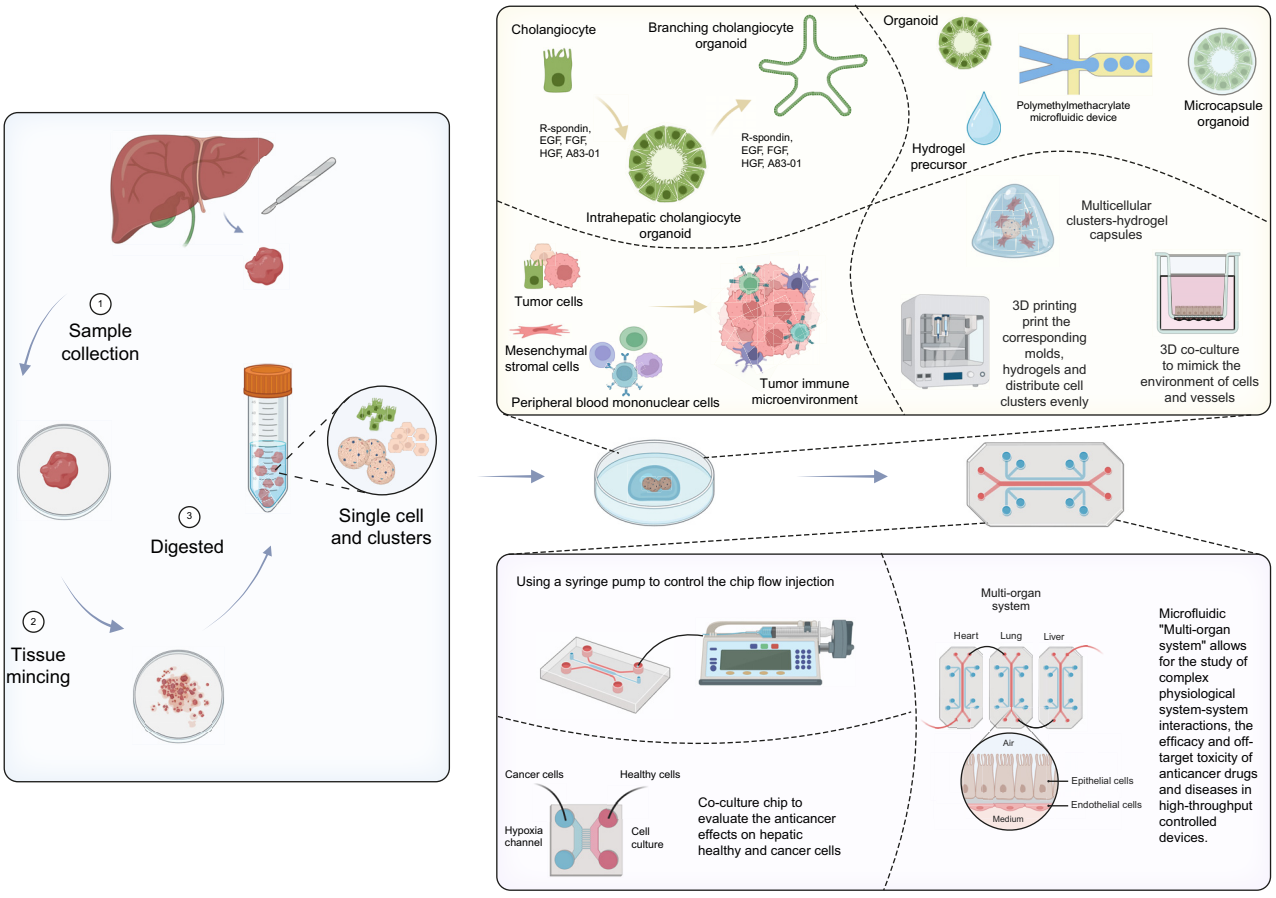


Fig. 4. Methodologies in establishing patient-derived organoid systems. (A) Isolation: Patient-derived organoid protocols typically begin with obtaining single cells or tissue clusters from liver biopsies or surgically resected tissues. Such tissues undergo mechanical dissociation (mincing) and enzymatic digestion to generate a cell suspension. (B) Organoid culture (HCC and CCA): To closely replicate the *in vivo* environment, mesenchymal stem cells and peripheral blood mononuclear cells can be co-cultured, creating a tumor microenvironment; CCA can be further differentiated into branching tubular structures that better mimic the *in vivo* architecture of the bile ducts. Techniques like 3D bioprinting or hybrid microcapsules can aid in better distribution of cells and clusters. (C) Organ-on-a-chip platforms: Microfluidic platforms, also referred as "organ-on-a-chip" systems, provide a dynamic environment for studying cell/organ interactions. These platforms allow for co-culture of healthy and/or cancer cells under controlled conditions, including hypoxia. Syringe pumps or micro-perfusion systems can be used to mimic physiological flow rates within the chip. By connecting multiple chips, researchers can build more complex “multi-organ systems” facilitating the investigation of inter-organ communication and disease processes. CCA, cholangiocarcinoma (not specified as either intrahepatic or extrahepatic subtype); HCC, hepatocellular carcinoma.

between neoantigen-associated mutations in primary tissues and organoids through extensive transcriptome profiling. Gene expression profiles obtained from comprehensive transcriptome analysis and single-cell RNA sequencing also play a critical role in this validation process. Ji *et al.*[13] exemplified this by integrating transcriptomic data with genomic, epigenomic, and proteomic datasets to offer a detailed profile of PDOs. However, validation approaches varied significantly between articles. while some researchers, like Xu *et al.*,[24] conduct exhaustive genetic analyses, others opt for more rudimentary assessments or even forego RNA validation entirely. Equally vital are histological evaluations encompassing tissue architecture and cellular morphology, often assessed through careful histopathological examination and immunohistochemistry. Saito *et al.*[22] confirmed morphological fidelity in iCCA organoids, reflecting original tissue staining patterns. Functional assays emerged as another metric in assessing organoid fidelity. In their study, Roos *et al.*[38] used viability assessments, metabolic profiling, and phenotypic evaluations, integrated within the protocol for initiating and maintaining branching organoids. Manual selection procedures and cryopreservation protocols were also implemented to ensure the functionality and consistency of the cultures for further experimentation.

Limitations in the culture system have been frequently reported secondary to lack of both immune and stromal components, which hinder model fidelity to true *in vivo* TME.[9] However, Liu *et al.*[19] showed that CD39+CD8+ TILs derived from HAN-high groups had enhanced antitumor activity when cultured with autologous tumor organoids. These immune cells induced more apoptosis in the organoids from the HAN-high group compared to those from the HAN-low group. This suggests that their HCC PDOs provide a useful platform for evaluating the antitumor potential of immune cells, particularly in relation to the HAN status. Many organoid models reviewed lacked an immune component. For example, Zhu *et al.*[40] mentioned BNIP3-upregulated cancer cells’ potential immune evasion but did not explore this further. Broutier *et al.*[9]

offered insights into prognostic markers and tumor histology but noted that the absence of immune and stromal components limits the model's ability to depict TME interactions accurately. Patient-derived xenografts, or human cancer organoids transplanted into animal models, could also have a role in addressing this limitation, as they retain tumor histopathology including TILs and stromal components. Further studies could focus on the utility of organoid auto- & allotransplantation in animal models. Importantly, the introduction of co-culture has shifted the paradigm and allowed for the introduction and maintenance of an enhanced stromal system. Within the past 2 years, studies have explored avenues to enhance success rates of organoid cultures, by co-culturing with stromal cells such as MSCs, endothelial cells, hepatic stellate cells and cancer-associated fibroblasts.[33,34,39]

Organoids have shown promise in replicating key physiological and pharmacological aspects of full organs, yet they still fall short in capturing the intricate interactions between multiple organs, and their metabolic significance as seen in the body. Additionally, the time needed to grow an organoid can hinder clinical utility, and these approaches are generally very resource intensive. However, a promising avenue lies in merging organoid technology with organ-on-a-chip technology. This innovation combines three-dimensional human/mouse organoid systems (single or multicellular) with a plastic surface, utilizing microfluidic techniques to precisely control fluid flow and O_2 environment. Zou *et al.*[33] have developed a sophisticated multi-layer microfluidic chip specifically engineered to enhance the consistency of high-throughput cultured PDOs. These microfluidic chips feature microarray units tailored for 3D cell culture and targeted drug delivery. Each microwell has a volume approximately one-thousandth that of a standard 96-well plate, accelerating experimentation and saving time in PDO culture and drug screening. The top-layer microchannels mimic *in vivo* drug administration, enhancing drug testing accuracy and showing potential for personalized cancer therapy and immunotherapy outcome prediction. While the integration of this approach may enable personalized cancer therapies, the current systematic review highlights a notable scarcity of studies specifically exploring organ-on-a-chip technology. Future research could prioritize comparing organ-on-a-chip findings with clinical outcomes, alongside enhancing the mechanisms and logistics of high-throughput models.

This systematic review has limitations. The exclusion of non-English articles constrained the scope of insights into PLC organoids, introducing a language bias. However, eligible non-English articles, though limited in number (n = 5), were accounted for in the PRISMA diagram, enhancing this study's reproducibility. Notably, the diversity of organoid culturing systems, alongside the advent of emerging technologies such as microfluidic chip platforms, hydrogel capsules, and novel branching cholangiocyte organoids pose challenges for direct comparisons. The variability in culture techniques and complexity, such as isolating single cell types *vs*. multiple cell types, adds further challenges. This inherent heterogeneity influenced the depth of analysis, urging caution in interpreting the findings. To address this, this study extensively identifies organoid PLC types utilized across all examined studies. Additionally, a data extraction table delineates etiology of organoid culturing systems and presents data as reported by the authors (Table 2), intending to serve as a reference point throughout the review. Finally, as with all systematic reviews, the articles and interpretations are subject to the biases of the reviewers. Using two independent reviewers can help mitigate this but cannot entirely eliminate such biases.

This review underscores the increasingly impressive utility of PLC organoid cultures in advancing biomarker discovery, disease modeling, and therapeutic exploration. Encouraging advances, such as organoid-on-a-chip and co-culturing systems, show promise in revolutionizing PLC treatment strategies. Standardizing and validating *in vitro* protocols remain critical, as do ongoing comparisons of *in vitro* findings with clinical outcomes.

Affiliations

[1]*Nationwide Children's Hospital, Abigail Wexner Research Institute, 575 Children's Crossroad, Columbus, OH, 43215, USA;* [2]*Transplantation Center, Cleveland Clinic, OH, USA;* [3]*CIR Centre for Inflammation Research, University of Edinburgh, 5 Little France Drive Edinburgh, EH16 4UU, UK;* [4]*Department of Immunology, Lerner Research Institute, Cleveland Clinic, Cleveland, OH, USA;* [5]*Cleveland Clinic Foundation, Lerner Research Institute, Center for Immunotherapy and Precision Immuno-Oncology, Cleveland, OH, USA;* [6]*The University of Akron, Department of Chemical, Biomolecular, and Corrosion Engineering, Akron, OH, USA;* [7]*Cleveland Clinic Foundation, Taussig Cancer Institute, Cleveland, OH, USA;* [8]*Translational Hematology & Oncology Research, Cleveland Clinic, Enterprise Cancer Institute, Cleveland, OH, USA*

Abbreviations

BMEs, basement membrane extracts; CCA, cholangiocarcinoma; CCOs, cancer cell line-derived organoids; CHC, combined hepatocellular-cholangiocarcinoma; eCCA, extrahepatic cholangiocarcinoma; FLC, fibrolamellar carcinoma; HANs, high-affinity neoantigens; HBL, Hepatoblastoma; HCC, hepatocellular carcinoma; HiHeps, reprogrammed human hepatocytes; iCCA, intrahepatic cholangiocarcinoma; LICOB, liver cancer organoid biobank; MCCs, mouse cancer cell-derived organoids; MDCOs, mouse derived cancer organoids; MSCs, mesenchymal stem cells; PDO, patient-derived organoids; PLC, primary liver cancer; TKIs, tyrosine kinase inhibitors.

Financial support

The authors did not receive any financial support to produce this manuscript.

Conflict of interest

The authors of this study declare that they do not have any conflict of interest.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

This article was conceptualized and conducted under the direction of Dr. Andrea Schlegel. Manuscript drafting was performed by AAQ, CJW and AS. Critical manuscript review was performed by all authors. AAQ and CJW contributed equally as shared first authors.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhepr.2024.101164.

References

[1] Shek D, Chen D, Read SA, et al. Examining the gut-liver axis in liver cancer using organoid models. Cancer Lett 2021;510:48–58.

[2] Chen YK, Liu YJ, Chen SM, et al. Liver organoids: a promising three-dimensional model for insights and innovations in tumor progression and precision medicine of liver cancer. Front Immunol 2023;14:15.

[3] Khawar MB, Wang YJ, Majeed A, et al. Mini-organs with big impact: organoids in liver cancer studies. Oncol Res 2023;31:677–688.

[4] Xie CY, Gu AC, Khan M, et al. Opportunities and challenges of hepatocellular carcinoma organoids for targeted drugs sensitivity screening. Front Oncol 2023;12:11.
[5] De Siervi S, Turato C. Liver organoids as an in vitro model to study primary liver cancer. Int J Mol Sci 2023;24.
[6] Manduca N, Maccafeo E, De Maria R, et al. 3D cancer models: one step closer to in vitro human studies. Front Immunol 2023;14:1175503.
[7] Zhou B, Feng Z, Xu J, et al. Organoids: approaches and utility in cancer research. Chin Med J (Engl) 2023;136:1783–1793.
[8] El-Khobar KE, Sukowati CHC. Updates on organoid model for the study of liver cancer. Technology Cancer Res Treat 2023;22.
[9] Broutier L, Mastrogiovanni G, Verstegen MMA, et al. Human primary liver cancer-derived organoid cultures for disease modeling and drug screening. Nat Med 2017;23:1424–1435.
[10] Cho SY, Hwang H, Kim YH, et al. Refining classification of cholangiocarcinoma subtypes via proteogenomic integration reveals new therapeutic prospects. Gastroenterology 2023;164:1293–1309.
[11] Dong HJ, Li ZQ, Bian SC, et al. Culture of patient-derived multicellular clusters in suspended hydrogel capsules for pre-clinical personalized drug screening. Bioact Mater 2022;18:164–177.
[12] Fan Z, Gao Y, Zhang W, et al. METTL3/IGF2BP1/CD47 contributes to the sublethal heat treatment induced mesenchymal transition in HCC. Biochem Biophys Res Commun 2021;546:169–177.
[13] Ji S, Feng L, Fu Z, et al. Pharmaco-proteogenomic characterization of liver cancer organoids for precision oncology. Sci Transl Med 2023;15.
[14] Koch M, Nickel S, Lieshout R, et al. Label-free imaging analysis of patient-derived cholangiocarcinoma organoids after sorafenib treatment. Cells 2022;11:15.
[15] Konopa A, Meier MA, Franz MJ, et al. LPA receptor 1 (LPAR1) is a novel interaction partner of Filamin A that promotes Filamin A phosphorylation, MRTF-A transcriptional activity and oncogene-induced senescence. Oncogenesis 2022;11.
[16] Lee HS, Han DH, Cho K, et al. Integrative analysis of multiple genomic data from intrahepatic cholangiocarcinoma organoids enables tumor subtyping. Nat Commun 2023;14.
[17] Li L, Knutsdottir H, Hui K, et al. Human primary liver cancer organoids reveal intratumor and interpatient drug response heterogeneity. Jci Insight 2019;4:16.
[18] Lieshout R, Faria AVS, Peppelenbosch MP, et al. Kinome profiling of cholangiocarcinoma organoids reveals potential druggable targets that hold promise for treatment stratification. Mol Med 2022;28:15.
[19] Liu T, Tan JZ, Wu MH, et al. High-affinity neoantigens correlate with better prognosis and trigger potent antihepatocellular carcinoma (HCC) activity by activating CD39+CD8+ T cells. Gut 2021;70:1965–1977.
[20] Narayan NJC, Requena D, Lalazar G, et al. Human liver organoids for disease modeling of fibrolamellar carcinoma. Stem Cell Rep 2022;17:1874–1888.
[21] Nuciforo S, Fofana I, Matter MS, et al. Organoid models of human liver cancers derived from tumor needle biopsies. Cell Rep 2018;24:1363–1376.
[22] Saito Y, Muramatsu T, Kanai Y, et al. Establishment of patient-derived organoids and drug screening for biliary tract carcinoma. Cell Rep 2019;27:1265–1276.e1264.
[23] Saltsman JA, Hammond WJ, Narayan NJC, et al. A human organoid model of aggressive hepatoblastoma for disease modeling and drug testing. Cancers 2020;12:1–18.
[24] Van Tienderen GS, Willemse J, Van Loo B, et al. Scalable production of size-controlled cholangiocyte and cholangiocarcinoma organoids within liver extracellular matrix-containing microcapsules. Cells 2022;11:14.
[25] Wang W, Yuan T, Ma L, et al. Hepatobiliary tumor organoids reveal HLA class I neoantigen landscape and antitumoral activity of neoantigen peptide enhanced with immune checkpoint inhibitors. Adv Sci (Weinh) 2022;9:e2105810.
[26] Wang YH, Lu H, Sun LC, et al. Metformin sensitises hepatocarcinoma cells to methotrexate by targeting dihydrofolate reductase. Cell Death Dis 2021;12:13.
[27] Wang ZW, Guo YH, Jin Y, et al. Establishment and drug screening of patient-derived extrahepatic biliary tract carcinoma organoids. Cancer Cell Int 2021;21:13.
[28] Xin HY, Sun RQ, Zou JX, et al. As sociation of BRAF variants with disease characteristics, prognosis, and targeted therapy response in intrahepatic cholangiocarcinoma. JAMA Netw Open 2023;6:E231476.
[29] Xu MJ, Liu Y, Wan HL, et al. Overexpression of nucleotide metabolic enzyme DUT in hepatocellular carcinoma potentiates a therapeutic opportunity through targeting its dUTPase activity. Cancer Lett 2022;548:12.
[30] Zhang R, Noordam L, Ou X, et al. The biological process of lysine-tRNA charging is therapeutically targetable in liver cancer. Liver Int 2021;41:206–219.
[31] Zhang Y, Zhang Y, Tao H, et al. Targeting LINC01607 sensitizes hepatocellular carcinoma to Lenvatinib via suppressing mitophagy. Cancer Lett 2023;576:216405.
[32] Zhao Y, Li ZX, Zhu YJ, et al. Single-cell transcriptome analysis uncovers intratumoral heterogeneity and underlying mechanisms for drug resistance in hepatobiliary tumor organoids. Adv Sci 2021;8:13.
[33] Zou Z, Lin Z, Wu C, et al. Micro-engineered organoid-on-a-chip based on mesenchymal stromal cells to predict immunotherapy responses of HCC patients. Adv Sci (Weinheim, Baden-Wurttemberg, Germany) 2023;10:e2302640.
[34] Cho K, Ro SW, Lee HW, et al. YAP/TAZ suppress drug penetration into hepatocellular carcinoma through stromal activation. Hepatology 2021;74:2605–2621.
[35] Gulati R, Johnston M, Rivas M, et al. β-catenin cancer-enhancing genomic regions axis is involved in the development of fibrolamellar hepatocellular carcinoma. Hepatol Commun 2022;6:2950–2963.
[36] Oz O, Iscan E, Batur T, et al. 3d organoid modelling of hepatoblast-like and mesenchymal-like hepatocellular carcinoma cell lines. Hepatoma Res 2021;7.
[37] Peng SW, Ngo MHT, Kuo YC, et al. Niclosamide revitalizes sorafenib through insulin-like growth factor 1 receptor (IGF-1R)/Stemness and metabolic changes in hepatocellular carcinoma. Cancers 2023;15.
[38] Roos FJM, van Tienderen GS, Wu HY, et al. Human branching cholangiocyte organoids functional bile duct formation. Cell Stem Cell 2022;29:776-+.
[39] Wang Y, Takeishi K, Li Z, et al. Microenvironment of a tumor-organoid system enhances hepatocellular carcinoma malignancyrelated hallmarks. Organogenesis 2017;13:83–94.
[40] Zhu YY, Chen BW, Yan JY, et al. BNIP3 upregulation characterizes cancer cell subpopulation with increased fitness and proliferation. Front Oncol 2022;12:9.
[41] Bai PY, Ge C, Yang H, et al. Screening a redox library identifies the anti-tumor drug Hinokitiol for treating intrahepatic cholangiocarcinoma. Front Biosci 2022;27:13.
[42] Fujiwara H, Tateishi K, Misumi K, et al. Mutant IDH1 confers resistance to energy stress in normal biliary cells through PFKP-induced aerobic glycolysis and AMPK activation. Scientific Rep 2019;9:18859.
[43] Cao WL, Li M, Liu JY, et al. LGR5 marks targetable tumor-initiating cells in mouse liver cancer. Nat Commun 2020;11:16.
[44] Chen K, Sheng J, Ma B, et al. Suppression of hepatocellular carcinoma by mycophenolic acid in experimental models and in patients. Transplantation 2019;103:929–937.
[45] Pang X, Wan LF, Yang J, et al. Steroidal saponins from Trillium tschonoskii rhizome repress cancer stemness and proliferation of intrahepatic cholangiocarcinoma. Bioorg Chem 2022;121.
[46] Sun LL, Wang YQ, Cen J, et al. Modelling liver cancer initiation with organoids derived from directly reprogrammed human hepatocytes. Nat Cell Biol 2019;21:1015-+.
[47] Rüland L, Andreatta F, Massalini S, et al. Organoid models of fibrolamellar carcinoma mutations reveal hepatocyte transdifferentiation through cooperative BAP1 and PRKAR2A loss. Nat Commun 2023;14:2377.
[48] Vandana JJ, Manrique C, Lacko LA, et al. Human pluripotent-stem-cell-derived organoids for drug discovery and evaluation. Cell Stem Cell 2023;30:571–591.
[49] Nikokiraki C, Psaraki A, Roubelakis MG. The potential clinical use of stem/progenitor cells and organoids in liver diseases. Cells 2022;11.
[50] Singal G, Miller PG, Agarwala V, et al. Association of patient characteristics and tumor genomics with clinical outcomes among patients with non-small cell lung cancer using a clinicogenomic database. Jama 2019;321:1391–1399.
[51] Passaniti A, Kleinman HK, Martin GR. Matrigel: history/background, uses, and future applications. J Cell Commun Signal 2022;16:621–626.

Keywords: organoid; 3D cell culture; liver cancer; hepatocellular carcinoma; cholangiocarcinoma; diagnosis; biomarker; gene expression; drug; therapy.
Received 22 April 2024; received in revised form 23 June 2024; accepted 26 June 2024; Available online 1 July 2024