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Review

Development of Liver Cancer Organoids: Reproducing Tumor Microenvironment and Advancing Research for Liver Cancer Treatment

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Abstract

Liver cancer a leading cause of cancer-related deaths worldwide, yet understanding of its development mechanism remains limited, and treatment barriers present substantial challenges. Owing to the heterogeneity of tumors, traditional 2D culture models are inadequate for capturing the complexity and diversity of tumor biology and understanding of the disease. Organoids have garnered considerable attention because of their ability to self-renew and develop functional structures *in vitro* that closely resemble those of human organs. This review explores the history of liver organoids, their cellular origins, techniques of constructing tumor microenvironments that recapitulate liver cancer organoids, and the biological and clinical applications of liver and liver cancer organoids and explores the current challenges related to liver cancer organoid applications and potentially valuable solutions, with the aim of facilitating the construction of *in vitro* clinical models of liver cancer therapeutic research.

Keywords

liver cancer, liver organoid, tumor organoids, tumor microenvironment

Abbreviations:

2D, two-dimensional; 3D, three-dimensional; ALGS, Alagille syndrome; ALI, air-liquid interface; ARPKD, autosomal recessive polycystic kidney disease; CAF, Cancer-associated fibroblasts; Cas9, CRISPR-associated protein 9; CRISPR, clustered regularly interspaced palindromic repeats; ECM, extracellular matrix; ESC, embryonic stem cells; FADS2, fatty acid desaturase 2; GDP, Gross Domestic Product; HBV, hepatitis B; HCC, hepatocellular carcinoma; HCV, hepatitis C; HDI, Human Development Index; HE, hepatic endoderm; hiPSC-LO, human induced pluripotent stem cell liver organoid; HOT, homology-independent organoid transgenic; HUVECs, human umbilical vein endothelial cells; ICB, immune checkpoint blockade; iPSCs, induced pluripotent stem cells; Lgr5, leucine-rich-repeat-containing G-protein-coupled receptor 5; LT, Liver transplantation; MSC, Mesenchymal stromal cells; MSCs, mesenchymal stem cells; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; PBMC, peripheral blood mononuclear cells; PD-1, programmed death protein 1; PDL-1, programmed cell death ligand 1; PDOs, patient-derived organoids; PLC, primary liver cancer; PSCs, pluripotent stem cells; RB, retinoblastoma; TICs, tumor initiating cells; TME, tumor microenvironment

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Introduction

Worldwide, the annual incidence and mortality rates of cancer are increasing, with approximately 900,000 people being diagnosed with liver cancer worldwide, of which over 800,000 die from the disease,[1] Making it one of the major causes of cancer-related deaths. Liver cancer is categorized into primary and secondary types, which are further classified into hepatocellular, intrahepatic cholangiocellular, combined hepatocellular, and cholangiocellular carcinoma and dry blast carcinoma and undifferentiated carcinoma. Hepatocellular carcinoma is the most common, accounting for more than 90% of all liver cancers. The risk factors for primary liver cancer (PLC), especially hepatocellular carcinoma (HCC), include viral infections (hepatitis B [HBV] and hepatitis C [HCV]), alcohol abuse, metabolic disorders (overweight, obesity, elevated cholesterol), and socioeconomic factors (GDP [Gross Domestic Product], and HDI [Human Development Index].[2] The distribution of these etiologies differs geographically; Asian countries have a higher prevalence of viral hepatitis B, whereas in Western countries, chronic HCV infection and alcoholic cirrhosis are the main causes. Recent studies have indicated non-alcoholic steatohepatitis (NASH) to be a significant contributor to the rising annual incidence of liver cancer.[3] These differences may be attributed to environmental factors, genetic and cultural differences, or dietary habits. However, with the development of vaccination and antiviral therapies, the main cause of PLC has gradually shifted from viral infections to NASH, which may be owing to the global increase in number of patients with obesity.[4] Traditional animal models and two-dimensional (2D) cell cultures are commonly used for human biological studies, including cancer biology. The self-updating and generation of functional structures, including the accurate cell type patterns found in the original tissue, is a defining characteristic of organoids, allowing the histological characteristics, morphological features, and functional characteristics to be extremely similar to the bodily organs. In addition to normal organoids, patient-derived tumor organs have been established from various human tumors including pancreatic,[5] colorectal,[6] breast,[7] liver cancer,[8] and prostate cancer.[9]

This disease model can help in drug screening for potential targeted therapies and precision medicine. In this study, we collected research articles on liver cancer organoids from PubMed, Web of Science, Google Scholar, and other databases published in the past 5 years. We summarized the existing research results for possible omissions and improvement details in the research, tumor immune microenvironment replication through various techniques which can help construct liver cancer organoids, those that provide theoretical support and new ideas for the subsequent basic research on liver cancer organoids, and those which explored the mechanism for the clinical diagnosis and treatment of liver cancer with organoid technology. This will provide theoretical support and new perspectives for future basic research on liver cancer organoids as well as an investigation into the mechanisms of organoid technology in the clinical diagnosis and treatment of liver cancer.

Development of Liver Organoids

In the early stages of research in liver organoid development focused on generating 3D structures that mimic the structural architecture of the liver, which often contain hepatocytes, the primary functional cells of the liver embedded in artificial scaffolds or hydrogels. However, these early attempts lacked the complexity and functional features required to accurately reproduce liver tissue.[10] Researchers then focused on hepatocyte aggregates. By culturing hepatocytes in hanging drops or non-adherent plates, they successfully achieved cell aggregation, which promoted self-organization and improved liver-specific functions. Although these aggregates demonstrate increased functionality, they still lack the realistic *in vivo* tissue architecture of the liver.[11] To improve the structural integrity of liver organoids, researchers began using extracellular matrices (ECMs) generated from natural and/or synthetic sources. ECMs provide a more realistic microenvironment, facilitating cell-cell interactions, and mimicking liver tissue organization. These advances allowed for improved hepatocyte survival and function within the organoids.[12] Recognizing the importance of non-parenchymal cells in liver function and the tumor microenvironment (TME), researchers then incorporated additional cell types into liver organoids. Co-culturing hepatocytes with endothelial cells, stellate cells, and immune cells allows the reproduction of a more representative liver microenvironment, promoting greater organoid functionality and improved disease modeling capabilities.[13] In addition to hepatocytes, research has focused on the inclusion of liver progenitor cells to liver organoid cultures. Liver progenitor cells are capable of differentiation into hepatocytes and other liver cell types, making them valuable for studying liver development, regeneration, and pathological processes. Their inclusion in liver organoids further enhanced the complexity and functionality of these models.[14] The advent of induced pluripotent stem cells (iPSCs) has marked a fundamental shift in stem cell research and regenerative medicine, bringing up new options for cancer treatment. By reprogramming adult cells, such as skin cells, into a pluripotent state, researchers induced in these cell types the ability differentiate into any cell type, including hepatocytes. Human iPSC-derived hepatocytes have demonstrated promise in development of liver organoid, offering patient-specific models for disease research and drug testing.[15] Advances in biofabrication techniques like 3D bioprinting and microfluidics have accelerated liver organoid development by enabling precise cell placement and arrangement, and when combined with biomaterials, can create more complex and functional liver organoids with improved vascularization.[16]

Thus, liver organoids have evolved from simple 3D structures to complex and functional models that closely resemble liver tissues and the TME. Researchers are utilizing advanced biomaterials, cell types, and biofabrication techniques to refine liver organoids, thereby unlocking their potential for advancing liver cancer research.

Cells of Origin for Liver Organoids

Two-dimensional *in vitro* models of HCC have proven effective for biomarker and drug screening; however, they have limitations like cross-contamination with other cell lines and susceptibility to genetic drift and bias after prolonged cultivation.[17,18] Two-dimensional cultured cells are restricted by adherence to rigid surfaces and adopt a flat morphology. Hepatocytes, which exhibit polarity *in vivo*, lose polarity when forced to adopt a flat morphology *in vitro*, they gradually lose polarity, a feature closely related to their functional properties.[18,19] This affects their normal cellular functions such as signaling, proliferation, migration, and apoptosis.[20,21] Additionally, ECM components, and cell-cell and cell-ECM interactions are negatively affected by 2D conditions.[20] Three-dimensional models are increasingly utilized in cancer research owing to their ability to accurately represent *in vivo* conditions, developmental processes,[22] and microenvironmental changes during tumor presence and progression.[23] Three-dimensional culture systems can bridge the gap between 2D cultures and animal models, providing more relevant preclinical models for cancer drug discovery and testing.[24]

The liver is a highly complex organ with a rich vascularization network that influences various metabolic processes. Early liver organoid models had several disadvantages, the most obvious being the lack of multiple structures that form complex cellular networks, such as the stroma, blood vessels, nervous innervation, and immune cells. Currently, liver organoids are primarily composed of pluripotent stem cells (PSCs), adult stem cells, primary hepatocytes, and other cells. (Figure 1)

Pluripotent Stem Cell-Derived Liver Organoids

Pluripotent stem cells (PSCs), which include embryonic stem cells (ESCs) and iPSCs, have an unlimited capacity for self-renewal, can imitate embryogenesis, and generate various adult tissue cell types. PSCs are also easily modifiable via gene editing. Because of these properties, PSCs are favored for generating human organ models for studying disease mechanisms and development of new therapies . Human embryonic hepatocytes that pass through stages involving fibroblast growth factor 2, human activin-A, hepatocyte growth factor, and dexamethasone were utilized to differentiate into cells possessing several characteristics of primary human hepatocytes.[25] Although Song et al's[26] pioneering work demonstrated the potential of iPSCs to efficiently differentiate into hepatocyte-like cells, the creation of a fully functional 3D vascularized liver remains an unsolved challenge. Takebe et al[13] explored the use of organ buds as a new approach for liver generation, which showed promise for future developments in regenerative medicine. The researchers established a vascularized and functional human liver model by co-culturing iPSC-derived hepatic endoderm (HE) cells with human mesenchymal stem cells (MSCs) and human umbilical vein endothelial cells (HUVECs). This was achieved through the formation of self-organized aggregates, referred to as liver buds, with characteristics similar to mature human hepatocytes, and developed vascular networks following in vivo implantation into immune-deficient mice. The cells differentiated into mature hepatocyte-like cells through direct contact with MSCs and HUVECs.[27] Additionally, co-culture with liver-specific non-parenchymal cells, such as hepatic stellate cells and liver sinusoidal endothelial cells, resulted in better hepatic function, allowing liver parenchymal cells to form a 3D construct.[28] Wu et al[29] developed a system for generating *in vitro* functional hepatobiliary organoids using human-iPSCs, without exogenous cells or genetic modifications. The benefit of utilizing PSC-derived liver organoids lies in their capacity as an unlimited cell source and the ability to produce donor-derived cells non-invasively.

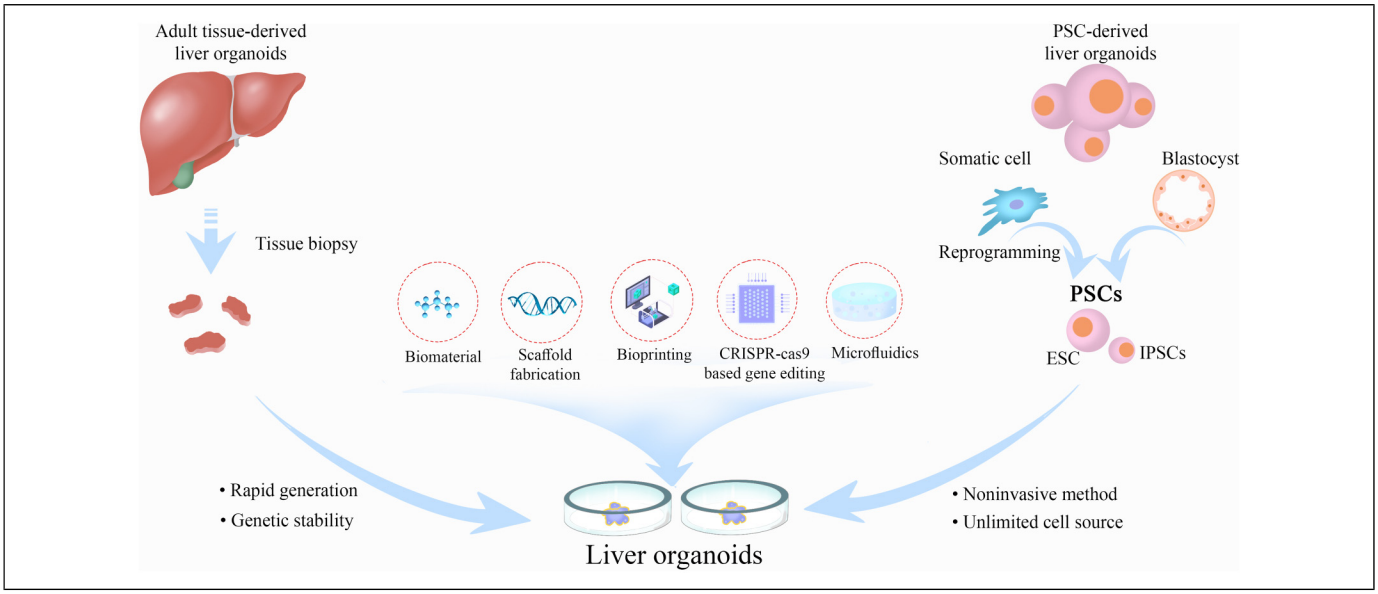

Figure 1. Schematic diagram depicting the establishment of liver organoids derived from adult tissue and pluripotent stem cells (PSCs). iPSCs, induced PSCs; ESC, embryonic stem cells, CRISPR, clustered regularly interspaced palindromic repeats/Cas9, CRISPR-associated protein 9.

Adult Liver Tissue-Derived Liver Organoids

Liver organoids can be created from adult liver tissues. Previous studies have demonstrated that leucine-rich-repeat-containing G-protein-coupled receptor 5 (*Lgr5*), a Wnt target gene, identifies actively dividing stem cells in Wnt-driven, self-renewing tissues.[30] This method first described by Huch et al[14] in 2015, in which a 3D culture system allows the long-term clonal expansion of single *Lgr5*(+) stem cells into transplantable organoids (budding cysts) that retain many characteristics of the original epithelial architecture. Furthermore, *Lgr5* positive liver stem cells possess bipotent liver progenitor cells that can be effectively converted into functional hepatocytes *in vivo* and *in vitro*.[14] They also observed that the cytogenetic features of these expanded cells were stably maintained. However, the reproducibility and scaling-up of current organoid systems continue to be significant challenge for their clinical application.[31] Some studies have demonstrated improved oxygenation in spinner flasks, leading to rapid organoid proliferation compared to traditional static culture methods.[32] Organoids also demonstrate advantages in cell proliferation and liver differentiation. Primary hepatocytes have been shown to be more precise *in vivo* hepatocyte model. Primary hepatocytes exhibit a higher rate of base replacement compared to hepatocytes produced by iPSC induction. Primary hepatocytes retain the characteristic features of the donor and can thus, serve as an ideal research model.[33] The novel approach of using clustered regularly interspaced palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9) and CRISPR- homology-independent organoid transgenic (HOT) techniques for generating human fetal hepatocyte organoids represents a significant advancement in liver research.[34] This technology offers a novel approach for the long-term expansion of primary liver cells *in vitro*. However, because of the challenge of procuring fresh specimens, this strategy has not yet become mainstream. Furthermore, primary healthy human hepatocytes have been used to produce long-term expanded organoids as a scaffold for constructing liver cancer organoids.[8] This system enables the long-term amplification of tumor tissues and subtypes under similar medium conditions. It preserves the original tumor's histological architecture, gene expression, and genomic landscape while distinguishing between tumor tissues. Additionally, this system is also advantageous in identifying biomarkers and conducting drug screening tests, making it relevant to liver cancer biology and the development of disease-specific personalized therapeutic approaches. Organoids with liver structure and function were created through direct reprogramming of human hepatocytes.[35] To simulate the early changes in human liver cancer, *p53* and retinoblastoma (*RB*) genes were inactivated and it was found that overexpressing c-Myc led to the development of HCC, suggesting that excessive mitochondrion-endoplasmic reticulum coupling could be a target for preventive treatment. Organoids have also generated using cells obtained from tumor needle biopsies of patient with liver cancer.[36] These HCC organoids retain the morphology and expression pattern of HCC tumor markers as well as the genetic heterogeneity of the original tumor. Thus, organoid models can be created from needle biopsy cell or of liver tumors, providing a tool for developing personalized therapeutics for patients with liver cancer by identifying drug sensitivity. Adult liver tissue-derived organoids have an advantage over PSCs because they require less time to produce organoids and genetic features can be stably maintained over passages, providing a superior platform for modeling patient-specific diseases.

The advancements in development of organoid construction techniques, bioengineering techniques such as biomaterial and scaffold fabrication[37]; bioprinting[38]; CRISPR-Cas9 based gene editing[39]; and microfluidics[40] has led to the creation of more mature and complex organoids and miniature tissues *in vitro*. Computational biology have been applied to the organoid research and have advanced the field of stem cell and organoid biology by increasing structural complexity, cellular diversity, and tissue maturity of human organoids, as well as by facilitating the use of organoid technology in the development of new drugs and regenerative medicine.

Reproducing the Tumor Microenvironment in Liver Cancer Organoids

The development, invasion, and metastatic behavior of tumors are closely related to direct and indirect interactions between cells and the cellular environment.[41] Currently, liver cancer organoid models are limited by a lack of complexity and immunological microenvironment that promotes tumor growth . Previously, HCC organoids were constructed using only tumor epithelial cells, which lacked a complete tumor immune microenvironment. Conversely, patient-derived organoids (PDOs) have distinct advantages when investigating the interplay between tumor cells, stroma, and immune cells. PDOs maintain their histopathological and genomic features, including tumor-infiltrating lymphocytes and stromal components, making them highly representative of the patient's original tumor. However, PDOs have low transplantation rates, long processing times, are expensive, and are not suitable for large-scale drug sensitivity testing.[42–44]

Submerged Matrigel culture involves culturing isolated tumor cells within 3D Matrigel, either on a dome or on a plane situated beneath the tissue culture medium. Growth factors and/or pathway inhibitors are added according to the tissue type,[45–47] which replicates the genetic and phenotypic diversity of the original tumor. This culture method has the potential to mimic the functional patients' response to clinical treatment, aiding cancer disease modeling and drug screening.[48–54] However, the cancer cells isolated from this culture type contain only epithelial cancer cells and lack cell matrix components.[55] Consequently, TME modeling requires the introduction of pertinent immune cells from an external source, which are devoid of innate immune and stromal elements. (Figure 2)

Another type of culture method is the air-liquid interface (ALI), whereby tumor organoids are grown from minced

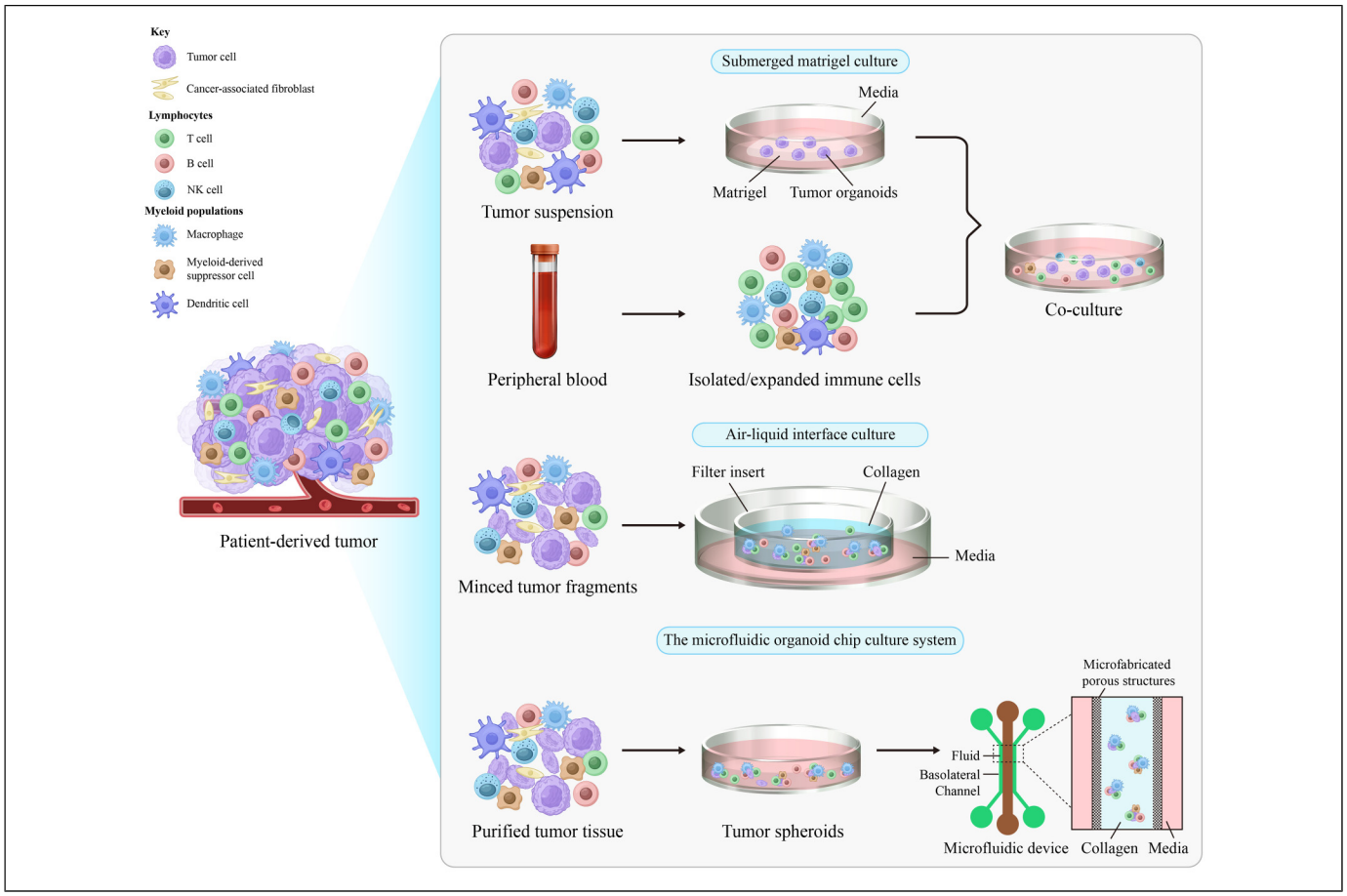

Figure 2. Methods for the construction of various reproducible tumor microenvironments.

primary tissue fragments and subsequently embedded in a collagen matrix, where the top of the gel is exposed to air. This method facilitates the incorporation of organoids comprising epithelial and stromal cells.[56,57] ALI approach has been used to construct PDOs) using tumor epithelial cells and native immune cells (T, B, natural killer [NK], and macrophages). The successful modeling of immune checkpoint blockade (ICB) with anti-programmed death protein 1 [PD-1] and/or anti-programmed cell death ligand 1 [PDL1] expands and activates tumor antigen specificity and cytotoxicity across the complexity of cancer organoids. This promotes individualized immunotherapy testing.[25] However, this culture method has a limitation in that it only includes natural tumor immune-infiltrating cells and does not include circulating tumor immune cells. This may generate inaccuracies in establishing a microenvironment conducive for tumor growth *in vivo* (Figure 2).

Microfluidic systems have the advantage of being customized according to specific requirements and providing a high degree of control. Microfluidics can simulate biomechanical forces, including electrical and shear stress and stretch, which can serve as useful stimuli for cell maturation and development.[58] Organs-on-chips integrate various tissues into a 3D system to reconstruct the structure and function of tissues and organs *in vitro* and accurately replicate the biological and physical forces of tissues and organs *in vivo*.[59] Conventional 3D cell cultures have been employed to analyze the effects of pharmaceuticals on the growth of tumor cells; however, they fail to offer insights into the intricate interplay between cancerous cells, their corresponding matrix constituents, and the physicochemical microenvironment within human organs where active tumors develop. Microfluidic human organs-on-chip can emulate the complexity of the 3D organization of living organs and introduce mechanical forces that affect cancer cell behavior, such as fluid shear stress, hydrostatic pressure, and tissue deformation. Microfluidic devices are used to create 'tissue chips' that mimic the microphysiological environment of the liver and internal blood vessels. This is achieved either by directly connecting porous ECM-coated membranes or by ECM gel separation. Tissue chips may consist of a single channel lined with cells from a single tissue type or a more complex structure that contains two or more tissue types. This offers a notable advantage over static models, such as spheroids, transwell, and organoid cultures.[60] Neovascularization, invasion, and dissemination of cancer cells, as well as maintaining cell viability and functionality over extended periods, are crucial factors for the culture systems of cancer organoids. The peristalsis of colon tumor organoids has been replicated by employing a microfluidic chip system that uses pressure channel cyclic contraction.[61] However, this culture system is more challenging to operate than alternative 3D culture methods, and has drawbacks such as low production capacity and high cost. Owing to the intricacy and limited scale of microfluidic systems that undergo

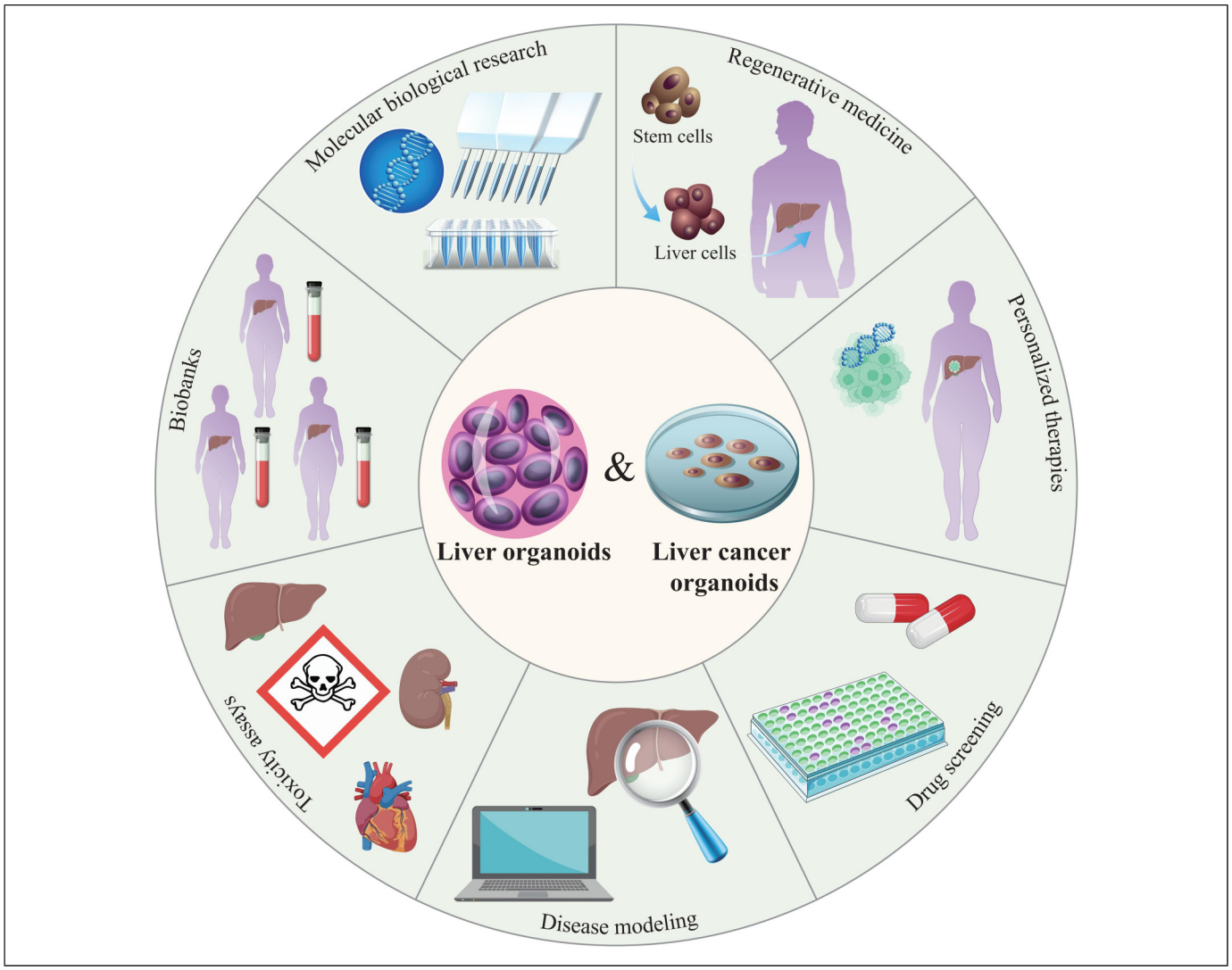

Figure 3. Applications of liver and liver cancer organoids.

controlled fluidic flow, several factors must interact flawlessly to achieve optimal function, resulting in limited applicability of the technique.[62] (Figure 2).

Application Prospects of Liver Cancer Organoids

Liver cancer organoids have a wide range of applications and provide an important tool for the future development of therapeutic interventions for cancer. The applications of liver cancer organoids are listed and described below. (Figure 3)

Liver Organoid Disease Models

Studies conducted on organoid models have helped understand the developmental processes in brain,[63] kidneys,[64] lungs,[65] prostate,[66] colon,[67] pancreas,[68,69] liver,[30] and other organs. Organoid models of liver disease are gradually becoming enriched (Table 1). First liver organoids were developed using green fluorescence labeled human fetal hepatic cells.[86] With the advancements in organoid research, organoid models of liver disease are continuously improving. Human fetal hepatocyte organoids reproduce steatosis in non-alcoholic fatty liver disease (NAFLD), where FADS2 (fatty acid desaturase 2) plays a crucial role in hepatic steatosis. FADS2 could be a possible target for NAFLD therapy.[70] The personalized infection model of the human induced pluripotent stem cell liver organoid (hiPSC-LO) may aid in the development of bespoke treatments for hepatitis. The model retains HBV transmission, exhibits replication potential over an extended duration, enacts the viral life cycle, and causes liver malfunction.[87] Successfully constructed alcoholic liver disease organoids mimic pathophysiological changes associated with the disease, such as oxidative stress, steatosis, release of inflammatory mediators, and fibrosis. This model represents a potentially novel *ex vivo* pathophysiological tool for studying alcoholic liver disease and is a promising cellular source for treating

Table 1. Organoid Models of Liver Diseases.

Disease	Species	Disease Induce	Source	Reference
NAFLD	Human	FFA	Human fetal hepatocyte	70
		Modifiable SIRT1 Metabolism OR PNPLA3	IPSCs	71,72
NASH	Human	FAs treatment	IPSCs	73
Alcohol-related liver disease	Human	ethanol treatment	ESCs + FLMCs	74
Steatohepatitis	Human	FA	PSCs	75
HBV infection	Human	recombinant HBV	Liver tissue	76
HCV infection	Human	HCV infection	PSCs	77
Sars-CoV-2 infection	Human	SARS-CoV-2 Pseudo-entry Virus Infection	PSCs	78
Liver fibrosis	Human	TGF-β, lipopolysaccharide or fetal bovine serum	IPSC-HSCs	79,80
ARPKD	Human	genome editing (PKHDM36 mutations)	PSC	81,82
Wilson's disease	Dog	commd1 deficient canine liver	Liver tissue	83
CTLN1	Human	patient-derived cells	IPSCs	84,85
A1AT deficiency	Human	patient-derived cells	Liver tissue	14
ALGS	Human	patient-derived cells	Liver tissue	14
		genome editing (ALGS inductions/revert)	IPSCs	81
Liver cancer	Human	patient-derived cells	Liver tissue	8

PSCs, pluripotent stem cells; iPSCs, induced PSCs; FLMCs, fetal liver mesenchymal cells; HSCs, hepatic stellate cells; ESCs, embryonic stem cells; iPSC-HSCs, human pluripotent stem cells into hepatic stellate cell-like cells; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; ARPKD, autosomal recessive polycystic kidney disease; CTLN1, citrullinemia type 1; A1AT, Alpha-1 antitrypsin; ALGS, Alagille syndrome; SIRT1 Sirtuin 1; HBV, hepatitis B virus; HCV, hepatitis C virus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TGF-β, transforming growth factor beta.

liver diseases in humans.[74] Liver fibrosis is a crucial factor in the prognosis of chronic liver disease. Coll et al[80] demonstrated that, by effectively differentiating human PSCs into HSC-like cells (iPSC-HSCs), the cells can exhibit transcriptional, cellular, and functional levels similar to those of primary human HSC and possess a gene expression profile intermediate between that of quiescent and activated HSCs. This provides an effective method, comparable to the human physiological state organ fibrosis model, for understanding the mechanisms of liver fibrosis and drug development.[79,80] Guan et al[81] demonstrated that during embryonic development, induced pluripotent stem cells differentiate into 3D human liver organoids that mimicked the human liver. This could be a novel model for describing the impact of varying JAG 1 mutations on liver regeneration. The study determined that mutations in the Notch signaling pathway lead to impaired bile duct formation, resulting in Alagille syndrome (ALGS).Moreover, an autosomal recessive polycystic kidney disease (ARPKD) organoid model was created, which developed abnormal bile ducts and fibrosis—a key feature of ARPKD liver pathology—within 21 days. In addition to elucidating the pathogenesis of inborn and conceivably procured liver fibrosis, ARPKD organoids can be used to ascertain the effectiveness of potential antifibrotic treatments.[82] A long-term canine liver organoid model was established to demonstrate that gene supplementation in the liver organoids of COMMD1-deficient dogs can recover function and be an effective treatment for copper storage disease.[83] Akbari et al utilized human iPSC-derived EpCAM-positive endodermal cells as intermediates to create a liver organoid (eHEPO) culture system that replicated citrullinemia type 1.[84] The ammonia-accumulation phenotype associated with the disease in eHEPOs can be reversed via overexpression of the wild-type *ASS1* gene. This also implies that the model is adaptable for genetic manipulation and can aid in curing this type of disease. The successful establishment of liver organoid models has contributed to a more accurate understanding of the occurrence and progression mechanisms of diverse diseases and has provided positive prospects for disease therapy, exhibiting its potential for future application. (Table 1). However, several important challenges still remain to be addressed.

Biobank and Biomolecular Markers for Liver Cancer

Liver cancer organoids, as a consequence of the advancement of organoid technology, are extremely useful in elucidating the molecular mechanisms underlying liver cancer and facilitating the identification of responsive treatment medicines for this disease. Biobanks have extensive applications in disease modeling and prediction of patient drug responses. A biobank comprising eight patients with primary liver cancer was established.[8] The biobank not only retains the tumorigenic potential, histological features and metastatic properties *in vivo*, but also preserves the genetic alterations found in the original tumor tissue. Compared to the transcriptome of organoids in constructed HCC organoids and healthy liver, we identified 11 genes among the top 30 most upregulated genes that have not been previously associated with PLC. Of these 11 novel genes, four were found to be overexpressed, leading to a poor prognosis. Comparison of the gene expression profiles of liver tumor organoids with healthy liver organoids has enabled identification of novel genes, including *C19ORF48*, *UBE2S*, *DTYMK*, and *C1QBP*, with potential prognostic value in PLC, indicating that liver tumor organoids may serve as prognostic biomarkers for individuals with HCC. This approach has the potential to identify new genes with important roles in the development and progression of human liver cancer. Currently, the Human Cancer Models Initiative is creating a

widely available repository of organoid cultures to provide publicly shared data,[88] to facilitate further exploration of the molecular mechanisms underlying the occurrence of cancer as well as development of precision medicine.

Drug Toxicity Tests

Hepatotoxicity is the primary reason for the restriction of clinical drug use and development, as well as the high turnover rate of drugs.[89,90] Previously, 2D cell cultures were used to study molecular and cellular mechanisms and drug effectiveness. Although 2D cell culture strategies are simple and cost-effective, they fail to mimic the structure, function, and physiology of cells *in vivo*.[17] However, the 3D culture model addresses the limitations of 2D cell culture and allows for more precise detection of drug toxicity. Moreover, leveraging organoid technology has resolved the challenge of medium-to high-throughput drug screening.[91,92] Kostadinova et al[93] reported that a 3D *in vitro* model sustained liver function for up to 3 months and maintained the induction capacity of cytochrome P450. This facilitates long-term assessment of adverse drug reactions and improves the detection of drug-induced toxicity *in vivo*. An organoid-based assay based on organoid technology was developed for precise determination of the viability, cholestasis, and mitochondrial toxicity of 238 commercially available drugs at four concentrations.[94] Human cardiac, kidney, and intestinal organoids have been used in clinical toxicology research; therefore, the organoid model has the potential to evaluate preclinical drugs and provide an effective method for toxicology research that can aid in drug screening.

Precision Medicine and Drug Screening

Previously, drug screenings were predominantly performed using *in vitro* 2D and animal models.[95,96] However, the high heterogeneity of tumors, as well as the complex TME and host tumor cells, play a crucial role in tumor development, progression, drug resistance, and metastasis. Consequently, traditional models must satisfy the requirements of precision treatment. To improve its applicability, a new technology for drug screening is urgently required.[97,98] Organoid culture technology is capable of reproducing the heterogeneity and complex microenvironment of tumors, leading to enhanced applicability in drug screening compared with traditional models. Thus, organoid culture technology has emerged as a crucial platform for assessing drug efficacy.[25,99] A study using a liver cancer organoid model screened 29 anticancer compounds, finding that ERK inhibition could potentially enhance treatment outcomes for patients with PLC. Previous studies have demonstrated the effectiveness of PLC organoids in drug screening.[8] Li et al[100] conducted a study on the drug responsiveness of primary human liver cancer organoids using 27 models and 129 cancer drugs. The results suggest that certain unused drugs are effective in organoid models, potentially forming a basis for tailored treatment. They were able to construct tumor organoids that accelerated tumor growth and provided evidence of drug resistance against clinically established anticancer drugs such as sorafenib,[101] regorafenib, and 5-fluorouracil, thus endorsing organoids as a potential screening tool for drugs.

Immunotherapy is an integral part of precision medicine and has been used as a first-line treatment for various tumors. One of the major drawbacks of tumor organoids in the past was the lack of a TME and low culture success rates, both of which are time-consuming. Neal et al[25] successfully conserved the initial tumor T-cell receptor profile of organoids formed by co-cultivating PDO with native immune cells and created a model of ICB by expanding and activating tumor antigen-specific TILs using anti-PD-1 and/or anti-PD-L1. This approach triggered tumor cytotoxicity studies, which significantly contributes to precision treatment. Mesenchymal stromal cells (MSC) and peripheral blood mononuclear cells (PBMC) co-culture construction has been utilized for drug screening. The results indicated a more accurate potential of this organoid for predicting anti-PD-L1 drugs, allowing for high-throughput drug testing with a multi-layer microfluidic chip. This study provides a new approach for predicting immunotherapeutic responses in liver cancer patients.[99]

Precision medicine has become an increasingly important therapeutic option. Owing to the varying levels of resistance to targeted therapy in patients and the heterogeneity of tumors, the identification of targeted therapeutic drugs suitable for all cancer patients is challenging. Organoid models generated from the tumor cells of patients who are resistant to certain treatments may provide a fresh avenue for individualized therapy. Phosphatidylethanolamine biosynthetic pathway plays a key role in the early stages of liver development. Some studies have shown that inhibiting the rate-limiting enzyme with meclizine in this pathway together with a glycolysis inhibitor in an organoid model inhibits the growth of a human hepatoma cell line. Therefore, phosphatidylethanolamine biosynthesis is a potential pathway for cancer treatment and a new therapeutic strategy for liver cancer.[102] The LGR5 compartment is a crucial cell population that initiates tumors and is a potential therapeutic target. Cancer-associated fibroblasts (CAF) are also closely associated with tumor initiating cells (TICs). Zhang et al[103] demonstrated the promotion of hepatic TIC formation, growth, and metastasis marked by LGR5 by CAF. Combination therapy targeting CAF and cancer stem cells has been suggested for liver cancer.

Regenerative Medicine

Liver transplantation (LT) is an effective treatment for liver disease in patients with PLC and end-stage failure. However, scarcity of donors reveals an urgent need for new approaches to overcome pivotal hindrances in LT.[104,105] Owing to their ability to self-renew and differentiate, iPSCs have the potential

to produce organoid systems. With advances in stem cell and gene-editing technologies, these organoids offer promising possibilities as novel therapeutic strategies for the treatment of liver diseases.[106] Although organoids have significant potential in regenerative medicine, further research is necessary before their clinical applications can be considered.

Co-cultures of hiPSCs, HUVECs, and MSCs have been utilized to self-organize into 3D structures resembling embryonic liver buds (iPS-LB). Once transplanted into nude mice, these liver buds showed rapid and real vascularization of the constructs 48 h after transplantation, as demonstrated by dextran infusion, showcasing functional human vascularization and links between donor and host cells. This study observed that the number of vessels which increased 3 days post-transplantation and the vessel area, paralleled that of the human liver. Additionally, stem cell researchers have successfully cultured liver organoids with successful preservation of mature liver features. These include serum protein production, drug metabolism, detoxification, active mitochondrial bioenergetics, inflammation, and regeneration capabilities.[107] These findings provide new insights for regenerative medicine.

Limitations of Liver Cancer Organoids and Prospects for the Future

Liver cancer remains the leading cause of cancer-related fatalities globally, despite improved treatment. Biological sample libraries and biomolecular markers, along with drug screening and precision medicine, are valuable tools. Multiple organoid and tumor models have been used to investigate molecular mechanisms, but they have limitations. This report discusses these shortcomings and proposes potential solutions to address the limitations of liver cancer organoids.

Tumor organoids have demonstrated favorable outcomes in various tumors.[67] However, obtaining PLC organoids is only possible from poorly or moderately differentiated patient specimens.[8,36,108] Patients with early primary HCC did not benefit significantly from this approach. Presently, compared with other tumor organoids, the success rate of constructing liver cancer organoids remains low.[5,36] The success rate of organoid construction correlates with collection of high-quality tumor specimens. However, HCC specimens tend to have more necrotic tissue, which hinders the separation of viable tumor tissues. Thus, it is advantageous to collect specimens with relatively low necrotic tissue, as this improves specimen quality. Additionally, reducing the duration of specimen collection and processing, along with ensuring optimal preservation conditions, contributes to an improvement in cell viability, leading to an enhanced success rate in organoid construction. Currently, the dominant culture method for organoids relies on the presence of various growth factors and small chemical compounds. However, it lacks hepatic hormone regulation, physical interaction conditions, and complex interplay between multiple growth factors and signaling pathways. These limitations possibly hinder organoids from completely replicating the *in vivo* state and impede the accurate prediction of clinical outcomes and prognosis.[109] Although liver cancer organoids have been the focus of numerous studies, the main hindrance to their ability to simulate the *in vivo* condition lies in the absence of a TME. This article describes the tools used to construct such an *in vivo* environment in liver cancer organoids and provides novel approaches for creating fully mature liver cancer organoids. Moreover, the issue of lengthy construction period of the HCC organoid models remains to be addressed. Culturing of hepatic patient-derived organoids and sufficient drug screening may take between 4 and 12 weeks,[18] risking the exclusion of some patients with liver cancer from optimal treatment. Hence, the culture system for liver cancer organoids needs optimization, considering the advantages and disadvantages of each of the various culture methods.[110] The development of a novel method which can completely and accurately emulate the *in vivo* environment is imperative to enhance the widespread applicability of liver cancer organoids.

Conclusion

This review provides a summary of the classifications of liver organoids and their benefits and drawbacks, focusing on those constructed from various cell types. It examines the techniques used to construct liver cancer organoids, along with the advantages and limitations of constructing TME within these structures. Additionally, this study considers the potential applications of liver cancer organoids and the challenges they face, as well as possible solutions to overcome these obstacles. In summary, although the development and use of liver cancer organoids have been impeded by a low success rate, long culture time, and the absence of a necessary tumor immune microenvironment, they still offer great potential for enhancing our understanding of liver cancer mechanisms and clinical applications. Studies on liver cancer organoids are expected to provide significant benefits to patients in the future.

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Data Availability Statement

The data that supports the findings of this study are available from the corresponding author upon reasonable request.

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The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Statement

Not applicable, because this article does not contain any studies with human or animal subjects.

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References

1. Chidambaranathan-Reghupaty S, Fisher PB, Sarkar D. Hepatocellular carcinoma (HCC): Epidemiology, etiology and molecular classification. *Adv Cancer Res*. 2021;149:1-61. doi:10.1016/bs.acr.2020.10.001.
2. Huang J, Lok V, Ngai CH, et al. Disease burden, risk factors, and recent trends of liver cancer: A global country-level analysis. *Liver Cancer*. 2021;10(4):330-345. doi:10.1159/000515304.
3. Yang J, Pan G, Guan L, et al. The burden of primary liver cancer caused by specific etiologies from 1990 to 2019 at the global, regional, and national levels. *Cancer Med*. 2022;11(5):1357-1370. doi:10.1002/cam4.4530.
4. Piñero F, Pages J, Marciano S, et al. Fatty liver disease, an emerging etiology of hepatocellular carcinoma in Argentina. *World J Hepatol*. 2018;10(1):41-50. doi:10.4254/wjh.v10.i1.41.
5. Boj SF, Hwang CI, Baker LA, et al. Organoid models of human and mouse ductal pancreatic cancer. *Cell.* 2015;160(1–2):324-338. doi:10.1016/j.cell.2014.12.021.
6. Hsu C, Wang Z, Oh S, et al. Microorganospheres as a novel precision oncology platform in colorectal cancer. *J CLIN ONCOL*. 2022;40(4):149. doi: 10.1200/JCO.2022.40.4_suppl.149
7. Liu F, Ma D, Liu Q, Qi X. A novel patient-derived organoid model for triple-negative breast cancer patients with residual disease after neoadjuvant chemotherapy for screening personalized drugs. *J Clin Oncol*. 2022;40(16_suppl):e12625. doi:10.1200/jco.2022.40.16_suppl.e12625.
8. Broutier L, Mastrogiovanni G, Verstegen MM, et al. Human primary liver cancer-derived organoid cultures for disease modeling and drug screening. *Nat Med*. 2017;23(12):1424-1435. doi:10.1038/nm.4438.
9. Pamarthy S, Sabaawy HE. Patient derived organoids in prostate cancer: Improving therapeutic efficacy in precision medicine. *Mol Cancer*. 2021;20(1):125. Published 2021 Sep 29. doi:10.1186/s12943-021-01426-3.
10. Bhatia SN, Balis UJ, Yarmush ML, Toner M. Effect of cell-cell interactions in preservation of cellular phenotype: Cocultivation of hepatocytes and nonparenchymal cells. *FASEB J*. 1999;13(14):1883-1900. doi:10.1096/fasebj.13.14.1883.
11. Miki T, Ring A, Gerlach J. Hepatic differentiation of human embryonic stem cells is promoted by three-dimensional dynamic perfusion culture conditions. *Tissue Eng Part C Methods*. 2011;17(5):557-568. doi:10.1089/ten.TEC.2010.0437.
12. Khetani SR, Bhatia SN. Microscale culture of human liver cells for drug development. *Nat Biotechnol*. 2008;26(1):120-126. doi:10.1038/nbt1361.
13. Takebe T, Sekine K, Enomura M, et al. Vascularized and functional human liver from an iPSC-derived organ bud transplant. *Nature*. 2013;499(7459):481-484. doi:10.1038/nature12271.
14. Huch M, Gehart H, van Boxtel R, et al. Long-term culture of genome-stable bipotent stem cells from adult human liver. *Cell*. 2015;160(1-2):299-312. doi:10.1016/j.cell.2014.11.050.
15. Takayama K, Morisaki Y, Kuno S, et al. Prediction of interindividual differences in hepatic functions and drug sensitivity by using human iPS-derived hepatocytes. *Proc Natl Acad Sci U S A*. 2014;111(47):16772-16777. doi:10.1073/pnas.1413481111.
16. Kurth F, Györvary E, Heub S, et al. Chapter 3 - Organs-on-a-chip engineering. In: *Organ-on-a-chip*. Academic Press; 2020:47-130.
17. Duval K, Grover H, Han LH, et al. Modeling physiological events in 2D vs. 3D cell culture. *Physiology (Bethesda)*. 2017;32(4):266-277. doi:10.1152/physiol.00036.2016.
18. Zeigerer A, Wuttke A, Marsico G, Seifert S, Kalaidzidis Y, Zerial M. Functional properties of hepatocytes in vitro are correlated with cell polarity maintenance. *Exp Cell Res*. 2017;350(1):242-252. doi:10.1016/j.yexcr.2016.11.027.
19. Müsch A. The unique polarity phenotype of hepatocytes. *Exp Cell Res*. 2014;328(2):276-283. doi:10.1016/j.yexcr.2014.06.006.
20. Saydé T, El Hamoui O, Alies B, Gaudin K, Lespes G, Battu S. Biomaterials for three-dimensional cell culture: From applications in oncology to nanotechnology. *Nanomaterials (Basel)*. 2021;11(2):481. Published 2021 Feb 13. doi:10.3390/nano11020481.
21. Leung M, Kievit FM, Florczyk SJ, et al. Chitosan-alginate scaffold culture system for hepatocellular carcinoma increases malignancy and drug resistance. *Pharm Res*. 2010;27(9):1939-1948. doi:10.1007/s11095-010-0198-3.
22. Bartlett R, Everett W, Lim S, et al. Personalized in vitro cancer modeling — fantasy or reality? *Transl Oncol*. 2014;7(6):657-664. doi:10.1016/j.tranon.2014.10.006.
23. Koledova Z. 3D Cell culture: An Introduction. *Methods Mol Biol*. 2017;1612:1-11. doi:10.1007/978-1-4939-7021-6_1.
24. Blidisel A, Marcovici I, Coricovac D, Hut F, Dehelean CA, Cretu OM. Experimental models of hepatocellular carcinoma-A preclinical perspective. *Cancers (Basel)*. 2021;13(15):3651. Published 2021 Jul 21. doi:10.3390/cancers13153651.
25. Neal JT, Li X, Zhu J, et al. Organoid modeling of the tumor immune microenvironment. *Cell*. 2018;175(7):1972-1988.e16. doi:10.1016/j.cell.2018.11.021.
26. Song Z, Cai J, Liu Y, et al. Efficient generation of hepatocyte-like cells from human induced pluripotent stem cells. *Cell Res*. 2009;19(11):1233-1242. doi:10.1038/cr.2009.107.
27. Takebe T, Zhang RR, Koike H, et al. Generation of a vascularized and functional human liver from an iPSC-derived organ bud transplant. *Nat Protoc*. 2014;9(2):396-409. doi:10.1038/nprot.2014.020.

28. Koui Y, Kido T, Ito T, et al. An in vitro human liver model by iPSC-derived parenchymal and non-parenchymal cells. *Stem Cell Rep*. 2017;9(2):490-498. doi:10.1016/j.stemcr.2017.06.010.
29. Wu F, Wu D, Ren Y, et al. Generation of hepatobiliary organoids from human induced pluripotent stem cells. *J Hepatol*. 2019;70(6):1145-1158. doi:10.1016/j.jhep.2018.12.028.
30. Huch M, Dorrell C, Boj SF, et al. In vitro expansion of single Lgr5 + liver stem cells induced by wnt-driven regeneration. *Nature*. 2013;494(7436):247-250. doi:10.1038/nature11826.
31. Huch M, Knoblich JA, Lutolf MP, Martinez-Arias A. The hope and the hype of organoid research. *Development*. 2017;144(6):938-941. doi:10.1242/dev.150201.
32. Schneeberger K, Sánchez-Romero N, Ye S, et al. Large-Scale production of LGR5-positive bipotential human liver stem cells. *Hepatology*. 2020;72(1):257-270. doi:10.1002/hep.31037.
33. Sahu B, Pihlajamaa P, Zhang K, et al. Human cell transformation by combined lineage conversion and oncogene expression. *Oncogene*. 2021;40(36):5533-5547. doi:10.1038/s41388-021-01940-0.
34. Hendriks D, Artegiani B, Hu H, Chuva de Sousa Lopes S, Clevers H. Establishment of human fetal hepatocyte organoids and CRISPR-Cas9-based gene knockin and knockout in organoid cultures from human liver. *Nat Protoc*. 2021;16(1):182-217. doi:10.1038/s41596-020-00411-2.
35. Sun L, Wang Y, Cen J, et al. Modelling liver cancer initiation with organoids derived from directly reprogrammed human hepatocytes. *Nat Cell Biol*. 2019;21(8):1015-1026. doi:10.1038/s41556-019-0359-5.
36. Nuciforo S, Fofana I, Matter MS, et al. Organoid models of human liver cancers derived from tumor needle biopsies. *Cell Rep*. 2018;24(5):1363-1376. doi:10.1016/j.celrep.2018.07.001.
37. Labour MN, Le Guilcher C, Aid-Launais R, et al. Development of 3D hepatic constructs within polysaccharide-based scaffolds with tunable properties. *Int J Mol Sci*. 2020;21(10):3644. doi:10.3390/ijms21103644.
38. Bernal PN, Bouwmeester M, Madrid-Wolff J, et al. Volumetric bioprinting of organoids and optically tuned hydrogels to build liver-like metabolic biofactories. *Adv Mater*. 2022;34(15):e2110054. doi:10.1002/adma.202110054.
39. Artegiani B, Hendriks D, Beumer J, et al. Fast and efficient generation of knock-in human organoids using homology-independent CRISPR-Cas9 precision genome editing. *Nat Cell Biol*. 2020;22(3):321-331. doi:10.1038/s41556-020-0472-5.
40. Rennert K, Steinborn S, Gröger M, et al. A microfluidically perfused three dimensional human liver model. *Biomaterials*. 2015;71:119-131. doi:10.1016/j.biomaterials.2015.08.043.
41. Gkretsi V, Stylianopoulos T. Cell adhesion and matrix stiffness: Coordinating cancer cell invasion and metastasis. *Front Oncol*. 2018;8:145. Published 2018 May 4. doi:10.3389/fonc.2018.00145.
42. Cavalloni G, Peraldo-Neia C, Varamo C, et al. Establishment and characterization of a human intrahepatic cholangiocarcinoma cell line derived from an Italian patient. *Tumour Biol*. 2016;37(3):4041-4052. doi:10.1007/s13277-015-4215-3.
43. Vitale S, Calapà F, Colonna F, et al. Advancements in 3D In Vitro Models for Colorectal Cancer. *Adv Sci (Weinh)*. 2024;11(32):e2405084. doi:10.1002/advs.202405084.
44. Thorel L, Perréard M, Florent R, et al. Patient-derived tumor organoids: a new avenue for preclinical research and precision medicine in oncology. *Exp Mol Med*. 2024;56(7):1531-1551. doi:10.1038/s12276-024-01272-5.
45. Seino T, Kawasaki S, Shimokawa M, et al. Human Pancreatic Tumor Organoids Reveal Loss of Stem Cell Niche Factor Dependence during Disease Progression. *CELL STEM CELL*. 2018;22(3):454-467.e6. doi:10.1016/j.stem.2017.12.009.
46. Fujii M, Shimokawa M, Date S, et al. A colorectal tumor organoid library demonstrates progressive loss of niche factor requirements during tumorigenesis. *Cell Stem Cell*. 2016;18(6):827-838. doi:10.1016/j.stem.2016.04.003.
47. Drost J, van Jaarsveld RH, Ponsioen B, et al. Sequential cancer mutations in cultured human intestinal stem cells. *Nature*. 2015;521(7550):43-47. doi:10.1038/nature14415.
48. van de Wetering M, Francies HE, Francis JM, et al. Prospective derivation of a living organoid biobank of colorectal cancer patients. *Cell*. 2015;161(4):933-945. doi:10.1016/j.cell.2015.03.053.
49. Yan HHN, Siu HC, Law S, et al. A comprehensive human gastric cancer organoid biobank captures tumor subtype heterogeneity and enables therapeutic screening. *Cell Stem Cell*. 2018;23(6):882-897.e11. doi:10.1016/j.stem.2018.09.016.
50. Roerink SF, Sasaki N, Lee-Six H, et al. Intra-tumour diversification in colorectal cancer at the single-cell level. *Nature*. 2018;556(7702):457-462. doi:10.1038/s41586-018-0024-3.
51. Vlachogiannis G, Hedayat S, Vatsiou A, et al. Patient-derived organoids model treatment response of metastatic gastrointestinal cancers. *Science*. 2018;359(6378):920-926. doi:10.1126/science.aao2774.
52. Ganesh K, Wu C, 'Rourke O, et al. A rectal cancer organoid platform to study individual responses to chemoradiation. *Nat Med*. 2019;25(10):1607-1614. doi:10.1038/s41591-019-0584-2.
53. Driehuis E, Kolders S, Spelier S, et al. Oral mucosal organoids as a potential platform for personalized cancer therapy [published correction appears in cancer discov. 2020 mar;10(3):476. Doi: 10.1158/2159-8290.CD-20-0129]. *Cancer Discov*. 2019;9(7):852-871. doi:10.1158/2159-8290.CD-18-1522.
54. Pasch CA, Favreau PF, Yueh AE, et al. Patient-Derived cancer organoid cultures to predict sensitivity to chemotherapy and radiation. *Clin Cancer Res*. 2019;25(17):5376-5387. doi:10.1158/1078-0432.CCR-18-3590.
55. Sato T, Vries RG, Snippert HJ, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature*. 2009;459(7244):262-265. doi:10.1038/nature07935.
56. Ootani A, Li X, Sangiorgi E, et al. Sustained in vitro intestinal epithelial culture within a wnt-dependent stem cell niche. *Nat Med*. 2009;15(6):701-706. doi:10.1038/nm.1951.
57. Li X, Nadauld L, Ootani A, et al. Oncogenic transformation of diverse gastrointestinal tissues in primary organoid culture. *Nat Med*. 2014;20(7):769-777. doi:10.1038/nm.3585.
58. Zheng W, Jiang B, Wang D, Zhang W, Wang Z, Jiang X. A microfluidic flow-stretch chip for investigating blood vessel biomechanics. *Lab Chip*. 2012;12(18):3441-3450. doi:10.1039/c2lc40173h.

59. Saorin G, Caligiuri I, Rizzolio F. Microfluidic organoids-on-a-chip: The future of human models. *Semin Cell Dev Biol*. 2023;144:41-54. doi:10.1016/j.semcdb.2022.10.001.
60. Teng Y, Zhao Z, Tasnim F, Huang X, Yu H. A scalable and sensitive steatosis chip with long-term perfusion of in situ differentiated HepaRG organoids. *Biomaterials*. 2021;275:120904. doi:10.1016/j.biomaterials.2021.120904.
61. Fang G, Lu H, Al-Nakashli R, et al. Enabling peristalsis of human colon tumor organoids on microfluidic chips. *Biofabrication*. 2021;14(1). doi:10.1088/1758-5090/ac2ef9.
62. Sontheimer-Phelps A, Hassell BA, Ingber DE. Modelling cancer in microfluidic human organs-on-chips. *Nat Rev Cancer*. 2019;19(2):65-81. doi:10.1038/s41568-018-0104-6.
63. Lancaster MA, Renner M, Martin CA, et al. Cerebral organoids model human brain development and microcephaly. *Nature*. 2013;501(7467):373-379. doi:10.1038/nature12517.
64. Takasato M, Er PX, Chiu HS, et al. Kidney organoids from human iPS cells contain multiple lineages and model human nephrogenesis [published correction appears in nature. 2016;536(7615):238. Doi: 10.1038/nature17982]. *Nature*. 2015;526(7574):564-568. doi:10.1038/nature15695.
65. Lee JH, Bhang DH, Beede A, et al. Lung stem cell differentiation in mice directed by endothelial cells via a BMP4-NFATc1-thrombospondin-1 axis. *Cell*. 2014;156(3):440-455. doi:10.1016/j.cell.2013.12.039.
66. Chua CW, Shibata M, Lei M, et al. Single luminal epithelial progenitors can generate prostate organoids in culture. *Nat Cell Biol*. 2014;16(10):951-954. doi:10.1038/ncb3047.
67. Sato T, Stange DE, Ferrante M, et al. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and barrett's epithelium. *Gastroenterology*. 2011;141(5):1762-1772. doi:10.1053/j.gastro.2011.07.050.
68. Loomans CJM, Williams Giuliani N, Balak J, et al. Expansion of adult human pancreatic tissue yields organoids harboring progenitor cells with endocrine differentiation potential. *Stem Cell Rep*. 2018;10(3):712-724. doi:10.1016/j.stemcr.2018.02.005.
69. Huch M, Bonfanti P, Boj SF, et al. Unlimited in vitro expansion of adult bi-potent pancreas progenitors through the Lgr5/R-spondin axis. *EMBO J*. 2013;32(20):2708-2721. doi:10.1038/emboj.2013.204.
70. Hendriks D, Brouwers JF, Hamer K, et al. Engineered human hepatocyte organoids enable CRISPR-based target discovery and drug screening for steatosis. *Nat Biotechnol*. 2023;41(11):1567-1581. doi:10.1038/s41587-023-01680-4.
71. Collin de l'Hortet A, Takeishi K, Guzman-Lepe J, et al. Generation of human fatty livers using custom-engineered induced pluripotent stem cells with modifiable SIRT1 metabolism. *Cell Metab*. 2019;30(2):385-401.e9. doi:10.1016/j.cmet.2019.06.017.
72. Tilson SG, Morell CM, Lenaerts AS, et al. Modeling PNPLA3-associated NAFLD using human-induced pluripotent stem cells. *Hepatology*. 2021;74(6):2998-3017. doi:10.1002/hep.32063.
73. Gurevich I, Burton SA, Munn C, et al. iPSC-derived hepatocytes generated from NASH donors provide a valuable platform for disease modeling and drug discovery. *Biol Open*. 2020;9(12):bio055087. Published 2020 Dec 16. doi:10.1242/bio.055087.
74. Wang S, Wang X, Tan Z, et al. Human ESC-derived expandable hepatic organoids enable therapeutic liver repopulation and pathophysiological modeling of alcoholic liver injury. *Cell Res*. 2019;29(12):1009-1026. doi:10.1038/s41422-019-0242-8.
75. Ouchi R, Togo S, Kimura M, et al. Modeling steatohepatitis in humans with pluripotent stem cell-derived organoids. *Cell Metab*. 2019;30(2):374-384.e6. doi:10.1016/j.cmet.2019.05.007.
76. De Crignis E, Hossain T, Romal S, et al. Application of human liver organoids as a patient-derived primary model for HBV infection and related hepatocellular carcinoma. *Elife*. 2021;10:e60747. Published 2021 Jul 30. doi:10.7554/eLife.60747.
77. Lee J, Gil D, Park H, et al. A multicellular liver organoid model for investigating hepatitis C virus infection and nonalcoholic fatty liver disease progression. *Hepatology*. 2024;80(1):186-201. doi:10.1097/HEP.0000000000000683.
78. Yang L, Han Y, Nilsson-Payant BE, et al. A human pluripotent stem cell-based platform to study SARS-CoV-2 tropism and model virus infection in human cells and organoids. *Cell Stem Cell*. 2020;27(1):125-136.e7. doi:10.1016/j.stem.2020.06.015.
79. Bao YL, Wang L, Pan HT, et al. Animal and organoid models of liver fibrosis. *Front Physiol*. 2021;12:666138. Published 2021 May 26. doi:10.3389/fphys.2021.666138.
80. Coll M, Perea L, Boon R, et al. Generation of hepatic stellate cells from human pluripotent stem cells enables in vitro modeling of liver fibrosis. *Cell Stem Cell*. 2018;23(1):101-113.e7. doi:10.1016/j.stem.2018.05.027.
81. Guan Y, Xu D, Garfin PM, et al. Human hepatic organoids for the analysis of human genetic diseases. *JCI Insight*. 2023;8(20):e176034. Published 2023 Oct 23. doi:10.1172/jci.insight.176034.
82. Guan Y, Enejder A, Wang M, et al. A human multi-lineage hepatic organoid model for liver fibrosis. *Nat Commun*. 2021;12(1):6138. Published 2021 Oct 22. doi:10.1038/s41467-021-26410-9.
83. Nantasanti S, Spee B, Kruitwagen HS, et al. Disease modeling and gene therapy of copper storage disease in canine hepatic organoids. *Stem Cell Rep*. 2015;5(5):895-907. doi:10.1016/j.stemcr.2015.09.002.
84. Akbari S, Sevinç GG, Ersoy N, et al. Robust, long-term culture of endoderm-derived hepatic organoids for disease modeling. *Stem Cell Rep*. 2019;13(4):627-641. doi:10.1016/j.stemcr.2019.08.007.
85. Yoshitoshi-Uebayashi EY, Toyoda T, Yasuda K, et al. Modelling urea-cycle disorder citrullinemia type 1 with disease-specific iPSCs [published correction appears in biochem biophys res commun. 2017;488(3):570-571. Doi: 10.1016/j.bbrc.2017.05.055]. *Biochem Biophys Res Commun*. 2017;486(3):613-619. doi:10.1016/j.bbrc.2017.03.037.
86. Takebe T, Sekine K, Suzuki Y, et al. Self-organization of human hepatic organoid by recapitulating organogenesis in vitro. *Transplant Proc*. 2012;44(4):1018-1020. doi:10.1016/j.transproceed.2012.02.007.

87. Nie YZ, Zheng YW, Miyakawa K, et al. Recapitulation of hepatitis B virus-host interactions in liver organoids from human induced pluripotent stem cells. *EBioMedicine*. 2018;35:114-123. doi:10.1016/j.ebiom.2018.08.014.
88. Drost J, Clevers H. Organoids in cancer research. *Nat Rev Cancer*. 2018;18(7):407-418. doi:10.1038/s41568-018-0007-6.
89. Kibar G, Dutta S, Rege K, Usta OB. Evaluation of drug carrier hepatotoxicity using primary cell culture models. *Nanomedicine*. 2023;48:102651. doi:10.1016/j.nano.2023.102651.
90. Kola I, Landis J. Can the pharmaceutical industry reduce attrition rates? *Nat Rev Drug Discov*. 2004;3(8):711-715. doi:10.1038/nrd1470.
91. Zhang CJ, Meyer SR, O'Meara MJ, et al. A human liver organoid screening platform for DILI risk prediction. *J Hepatol*. 2023;78(5):998-1006. doi:10.1016/j.jhep.2023.01.019.
92. Jiang S, Xu F, Jin M, et al. Development of a high-throughput micropatterned agarose scaffold for consistent and reproducible hPSC-derived liver organoids. *Biofabrication*. 2022;15(1). doi:10.1088/1758-5090/ac933c.
93. Kostadinova R, Boess F, Applegate D, et al. A long-term three dimensional liver co-culture system for improved prediction of clinically relevant drug-induced hepatotoxicity. *Toxicol Appl Pharmacol*. 2013;268(1):1-16. doi:10.1016/j.taap.2013.01.012.
94. Shinozawa T, Kimura M, Cai Y, et al. High-Fidelity drug-induced liver injury screen using human pluripotent stem cell-derived organoids. *Gastroenterology*. 2021;160(3):831-846.e10. doi:10.1053/j.gastro.2020.10.002.
95. Winograd B, Boven E, Lobbezoo MW, Pinedo HM. Human tumor xenografts in the nude mouse and their value as test models in anticancer drug development (review). *In Vivo*. 1987;1(1):1-13.
96. Barretina J, Caponigro G, Stransky N, et al. The cancer cell line encyclopedia enables predictive modelling of anticancer drug sensitivity [published correction appears in nature. 2012 dec 13;492(7428):290] [published correction appears in nature. 2019 jan;565(7738):E5-E6. Doi: 10.1038/s41586-018-0722-x]. *Nature*. 2012;483(7391):603-607. Published 2012 Mar 28. doi:10.1038/nature11003.
97. Choi SY, Lin D, Gout PW, Collins CC, Xu Y, Wang Y. Lessons from patient-derived xenografts for better in vitro modeling of human cancer. *Adv Drug Deliv Rev*. 2014;79-80:222-237. doi:10.1016/j.addr.2014.09.009.
98. Tosca EM, Ronchi D, Facciolo D, Magni P. Replacement, reduction, and refinement of animal experiments in anticancer drug development: The contribution of 3D in vitro cancer models in the drug efficacy assessment. *Biomedicines*. 2023;11(4):1058. Published 2023 Mar 30. doi:10.3390/biomedicines11041058.
99. 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 (Weinh)*. 2023;10(27):e2302640. doi:10.1002/advs.202302640.
100. Li L, Knutsdottir H, Hui K, et al. Human primary liver cancer organoids reveal intratumor and interpatient drug response heterogeneity. *JCI Insight*. 2019;4(2):e121490. doi: 10.1172/jci.insight.121490.
101. Cao G, Xian L, Zhao P, et al. Heterogeneity, Inherent and Acquired Drug Resistance in Patient-Derived Organoids Models of Primary Liver Cancer. *CELL ONCOL(Dordr)*. 2022;45(5):1019-1036. doi:10.21203/rs.3.rs-1229184/v1.
102. Guan Y, Chen X, Wu M, et al. The phosphatidylethanolamine biosynthesis pathway provides a new target for cancer chemotherapy. *J Hepatol*. 2020;72(4):746-760. doi:10.1016/j.jhep.2019.11.007.
103. Zhang M, Fang Y, Fu X, et al. Cancer-associated fibroblasts nurture LGR5 marked liver tumor-initiating cells and promote their tumor formation, growth, and metastasis. *Cancer Med*. 2023;12(17):18032-18049. doi:10.1002/cam4.6408.
104. Dwyer BJ, Macmillan MT, Brennan PN, Forbes SJ. Cell therapy for advanced liver diseases: Repair or rebuild. *J Hepatol*. 2021;74(1):185-199. doi:10.1016/j.jhep.2020.09.014.
105. Ceresa CDL, Nasralla D, Pollok JM, Friend PJ. Machine perfusion of the liver: Applications in transplantation and beyond. *Nat Rev Gastroenterol Hepatol*. 2022;19(3):199-209. doi:10.1038/s41575-021-00557-8.
106. Zahmatkesh E, Khoshdel Rad N, Hossein-Khannazer N, et al. Cell and cell-derivative-based therapy for liver diseases: Current approaches and future promises. *Expert Rev Gastroenterol Hepatol*. 2023;17(3):237-249. doi:10.1080/17474124.2023.2172398.
107. Mun SJ, Ryu JS, MO L, et al. Generation of expandable human pluripotent stem cell-derived hepatocyte-like liver organoids. *J Hepatol*. 2019;71(5):970-985. doi:10.1016/j.jhep.2019.06.030.
108. Artegiani B, van Voorthuijsen L, Lindeboom RGH, et al. Probing the tumor suppressor function of BAP1 in CRISPR-engineered human liver organoids. *Cell Stem Cell*. 2019;24(6):927-943.e6. doi:10.1016/j.stem.2019.04.017.
109. Gjorevski N, Sachs N, Manfrin A, et al. Designer matrices for intestinal stem cell and organoid culture. *Nature*. 2016;539(7630):560-564. doi:10.1038/nature20168.
110. Yuki K, Cheng N, Nakano M, Kuo CJ. Organoid models of tumor immunology. *Trends Immunol*. 2020;41(8):652-664. doi:10.1016/j.it.2020.06.010.