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Liver metastatic cancer organoid models: From mechanistic insights to precision medicine

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Abstract

Metastasis is a primary cause of cancer-related mortality, and the liver is the most common site of tumor metastasis. The molecular heterogeneity and complex tumor microenvironment of liver metastases remain major barriers contributing to clinical treatment failure. The dearth of accurate metastatic liver tumor models leads to a paucity of understanding regarding the mechanisms of liver metastasis and limits the exploration of novel therapeutic approaches. Patient-derived organoids provide a three-dimensional, tissue-engineered, cell-based *in vitro* model that reproduces the complex structure and function of the corresponding *in vivo* tissue. The advent of this personalized paradigm, tailored to the specific needs of individual patients, has enabled the translation of foundational research into clinical applications. This review provides a comprehensive summary of various methods for culturing liver metastatic cancer organoids, highlighting the novel findings and clinical applicability of organoids in liver metastasis research. It also discusses current research achievements and recent advances in liver metastatic cancer organoids.

Keywords: Liver metastatic cancer; Organoids; Mechanisms; Clinical applications

1. Introduction

Despite current treatments for liver metastatic cancer, which combine radiotherapy, chemotherapy, and surgery, the prognosis remains unfavorable.[1] Furthermore, the fusion of tumor cells with macrophages leads to the manifestation of tumor heterogeneity, thereby promoting tumor metastasis.[2] Therefore, there is an urgent clinical need for a tumor model that can replicate the genetic heterogeneity and complex microenvironment of tumors in patients with metastatic cancer.

Patient-derived organoids (PDOs), which are derived from human tissue, can help overcome some of these challenges.[3] This model is based on an *in vitro* three-dimensional (3D) cell culture system that can be derived from tissue-resident adult stem cells, induced pluripotent stem cells, or directly from patient biopsy samples.[4-6] Compared to traditional two-dimensional (2D) cell cultures or animal models, organoids recapitulate the *in vivo* tissue structure and function *in vitro*,[7] providing a highly physiologically relevant model for understanding human biology and significantly advancing the development of personalized medicine.[8]

Although PDO technology remains in its early stages in the field of liver metastasis research, its potential for deciphering the spatiotemporal mechanisms of metastasis and accelerating clinical therapeutic discoveries should not

be underestimated. This review summarizes construction strategies for liver metastasis organoids derived from different cancer types—including colorectal cancer (CRC), pancreatic cancer, breast cancer, and other cancers—highlights breakthrough findings in elucidating metastasis mechanisms, such as "seed–soil" interactions and immune evasion, and systematically evaluates organoid applications in drug screening, biobanking, and personalized medicine. It also outlines the combined application of liver metastasis organoids with modern high-technology approaches (Figure 1). Ultimately, this review aims to provide novel perspectives and methodologies for investigating tumor metastasis mechanisms and therapeutic interventions for liver metastatic tumors.

2. Culturing liver metastasis organoids from different primary tumor origins

The metastatic cascade of a tumor comprises three primary phases: Dissemination, dormancy, and colonization. Over the years, numerous hypotheses have been proposed to elucidate the mechanisms underlying malignant tumor metastasis, with the "seed and soil" hypothesis—first posited by Stephen Paget in 1889—remaining the most influential. This theory suggests that disseminated cancer cells ("seeds") detach from the primary tumor microenvironment (TME; the primary "soil") and invade, entering the circulatory system. Before colonization, the primary soil undergoes remodeling in distant metastatic target organs to create a more conducive TME (the secondary "soil") for cancer cell colonization.[9] This is consistent with recent findings that non-genetic dynamic adaptation may occur in cells leaving the primary tumor to different metastatic stages.[10]

While the "seed and soil" theory profoundly explains the organotropism of metastasis, a crucial transformation must occur within the "seed" before it can detach from the "primary soil." Epithelial–mesenchymal transition (EMT) serves as the core driver of this initial step.[11] Within the primary tumor, multifactorial triggers such as gene deletions, cell cycle abnormalities, and hypoxia induce EMT.[12-14] This results in the loss of epithelial polarity and intercellular junctions in tumor cells, leading to the breach of the basement membrane barrier. Consequently, the cells detach from the primary site, either collectively or individually, and invade blood vessels, forming circulating tumor cells (CTCs).

Metastatic cancer organoids can be derived from primary tumor tissues, metastatic lesions, CTCs, or concentrated tumor cells from effusions.[15] The organoid culture system primarily comprises two key components: A supportive extracellular matrix and a tailored culture medium. The matrix, often Matrigel—a protein-rich hydrogel rich in laminin and collagen—provides a 3D structural support for *in vitro* organoid culture. The organoid culture medium delivers essential nutrients and growth factors, establishing a TME model that closely mimics the *in vivo* tissue or organ of origin.

However, a standardized method for selecting the culture medium components to favor either "primary soil" or "secondary soil" remains elusive. Therefore, establishing liver metastasis organoid culture systems is paramount for advancing the field.

Here, we summarize the components of various expansion media detailed in published articles over the past decade that have successfully cultivated liver metastasis organoids, as presented in Table 1.

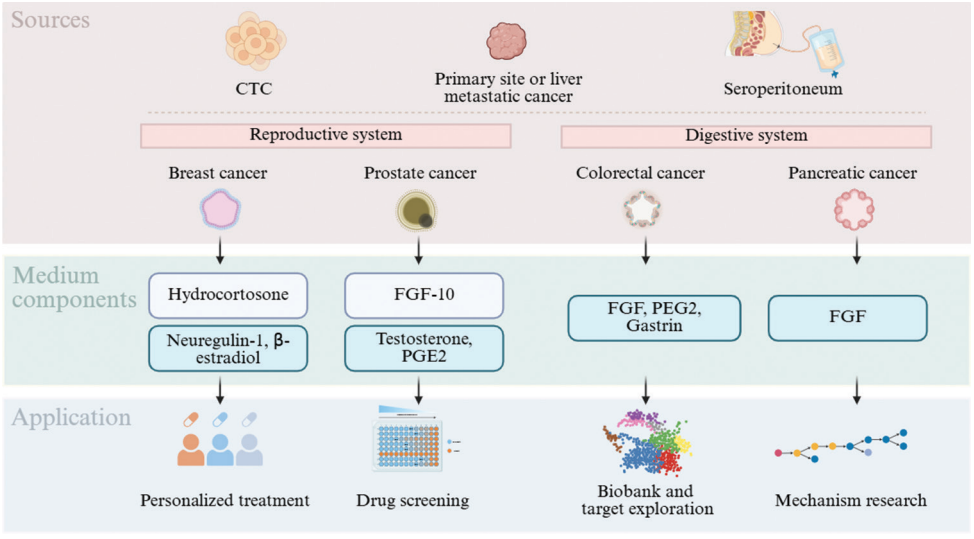

Figure 1. Sources of liver metastatic cancer organoids, their associated nutrient molecules, and corresponding clinical applications. Created with BioRender by Sun T. (2025). https://app.biorender.com/profile/template/details/t-694115604389e1d4f3dcc0c8-sources-of-liver-metastatic-cancer-organoids.
Abbreviations: CTC: Circulating tumor cell; FGF: Fibroblast growth factor; PEG2: Prostaglandin E2.

Table 1. Hepatic metastatic organoids from different tumor origins and their culture medium compositions

Origin	Sample source	Basic ingredients	Liver-specific additions (soil factors)	Primary tumor additions (seed factors)	Success rate (%)	References
Prostate cancer	Liver needle biopsy	Advanced DMEM/F12, 10% FBS, 1% penicillin–streptomycin, primocin, GlutaMax, B-27, N-acetylcysteine, EGF, nicotinamide	FGF-10	Testosterone, PGE2	16	16
Colorectal cancer	Liver tumor resection	Advanced DMEM/F12, Noggin, B-27, N-acetylcysteine, EGF, R-spondin1	-	Gastrin	76	17
Colorectal cancer	Liver tumor resection	Advanced DMEM/F12, 1% penicillin–streptomycin, Normocin, Gentamicin/ Amphotericin B HEPES, GlutaMax, Noggin, B-27, N-acetylcysteine, R-spondin-1, EGF, nicotinamide	-	PGE2, gastrin	75	18
Colorectal cancer	Paired primary/met	Advanced DMEM/F12, Primoicin, GlutaMax, B-27, N2 supplements, Nicotinamide, N-acetylcysteine, EGF	-	FGF	75	19
Breast cancer	Liver tumor resection	Advanced DMEM/F12, Wnt-3a, R-spondin 1, Noggin, EGF, neuregulin-1, N-acetyl-cysteine, HEPES, B27, Glutamax	Hydrocortisone	Neuregulin-1, β-estradiol	65	20
Pancreatic ductal adenocarcinoma	Primary tumor	Advanced DMEM/F12, 1% penicillin–streptomycin, B-27, N-acetylcysteine, Wnt-3a, R-Spondin 1, Noggin, EGF	-	FGF	-	21

Abbreviations: CTC: Circulating tumor cell; DMEM: Dulbecco's Modified Eagle Medium; EGF: Epidermal growth factor; FGF: Fibroblast growth factor; PEG2: Prostaglandin E2.

A thorough analysis of the existing culture medium formulations for liver metastatic cancer organoids revealed a conspicuous contradiction. The prevailing liver metastatic organoid culture medium is characterized by two major issues: enhanced "seed" memory and deficient "soil" signaling. For instance, the medium used to cultivate CRC liver metastasis organoids typically contains a Wnt agonist (R-spondin1), Noggin, and additional intestinal stem cell factors.[10,22,23] Similarly, the medium used to cultivate breast cancer organoids must retain estrogen, human epidermal growth factor receptor 2 (HER2), and other factors of the mammary microenvironment.[24] These formulations are effective in maintaining the stemness of the tumor cells; however, they lack the critical liver-derived signals. For instance, oncostatin M and hepatocyte growth factor are absent in the majority of formulations, and the latter is a pivotal factor for liver metastases.[25,26] Furthermore, these formulations are deficient in their ability to replicate the metabolic and immune stresses experienced by the liver "soil." The characteristic high-lactate environment and glutamine dependence are not reproduced,[27] and immunosuppressive factors such as transforming growth factor-β (TGF-β) and interleukin (IL)-6 secreted by Kupffer cells are not integrated.[28] It is comparable to planting rice seeds in a desert and cultivating them under the same conditions as traditional rice cultivation.

The central question guiding this inquiry is: How can we effectively address this particular issue? The addition of hepatic factors should be implemented gradually to preserve tumor stemness. In the long term, the objective is to establish metastatic niche organoids that integrate vascular endothelium, hepatic stellate cells, and immune components. First, we can simulate metastatic evolution in stages. For example, days 0 to 7 can be defined as stage 1. In this stage, a primary foci medium is used; for instance, R-spondin1 is added to a CRC liver metastasis organoid medium to maintain cell survival. Stage 2, from days 8 to 14, involves the gradual addition of hepatic "soil" factors, such as hepatocyte growth factor, oncostatin M, and 10 mM lactate, to induce metastatic adaptation. In this way, there is a potential for achieving dual objectives through a single action, thereby yielding a dual-purpose outcome.

Furthermore, the low success rate in culturing liver cancer organoids is largely attributed to the absence of epithelial stem cell characteristics in hepatocellular carcinoma cells, which are crucial for their proliferation within organoid culture systems.[29] The plasticity of EMT has been demonstrated to regulate stem cell self-renewal. Therefore, replicating or inducing the EMT program is crucial for establishing organoid models of liver metastatic cancer with high culture success rates and for simulating early metastatic events. Deficiency of the chromatin remodeling factor ATRX induces typical EMT

transformation characteristics. PDOs established with ATRX knockout exhibited downregulation of colonic epithelial factors (e.g., HNF4A, CDX1) and upregulation of squamous markers (e.g., KRT5), accompanied by enhanced TGF-β–induced EMT. This validates its role in driving lineage confusion and transformation potential in human CRC.[30]

In summary, the key to successfully developing a liver metastasis organoid model lies in precisely mimicking its *in vivo* evolutionary pathway. The phased culture strategy proposed herein—transitioning from simulating the primary tumor environment to the metastatic environment—represents a critical experimental approach to address the current deficiency of "soil" signals in culture media. Simultaneously, the recreation of critical biological processes such as EMT provides a vital foundation for ensuring the model can simulate early metastatic events. These cultivation principles establish a fundamental technical framework for the specific operational procedures of liver metastasis organoids. The subsequent section details the generalized culture process for liver metastatic cancer organoids (Figure 2).

3. Organoids as a platform for studying the mechanisms of cancer metastasis

3.1. Investigating the mechanisms of liver metastasis using organoid models

Deciphering the mechanisms governing the evolution of tumor metastasis is crucial for addressing the clinical challenges posed by metastatic cancers.

Liver metastasis in cancer is a multifaceted process, involving diverse cellular components and signaling pathways. Advanced 3D culture techniques offer a more physiologically relevant environment compared to conventional 2D cultures. By manipulating organoids or their microenvironment, researchers can gain valuable insights into the signaling pathways implicated in liver metastasis.

Recent studies have elucidated several key cancer metastatic pathways using organoids. For instance, metabolic profiling and metabolic inhibitor interventions on PDOs derived from primary tumors and liver metastases revealed that the ubiquitin-specific protease 3 antisense RNA–MYC–glycolysis regulatory axis promotes liver metastasis by enhancing H3K18 lactylation and CDC27 expression in CRC.[23] Wang *et al.*[22] utilized $APC^{min/+}$ transgenic mice-derived colorectal tumor organoids to investigate the influence of lentiviral infection, revealing that casein kinase 1 isoform ε, through phosphorylation of the amino-terminal enhancer of split at Ser121, suppressed CRC metastasis. Moorman *et al.*[31] established organoids from specimens of patients undergoing colon resection and metastatic tumor resection, discovering that PROX1 drives cells into an atypical differentiation state, thereby promoting tumor metastasis. The aforementioned study demonstrates that patient-derived liver metastatic organoids provide a flexible *in vitro* cancer model for validating driver mutation pathways and drug resistance mechanisms in tumor metastasis.

Despite these breakthroughs, challenges remain. The liver's dual blood supply is a primary factor contributing

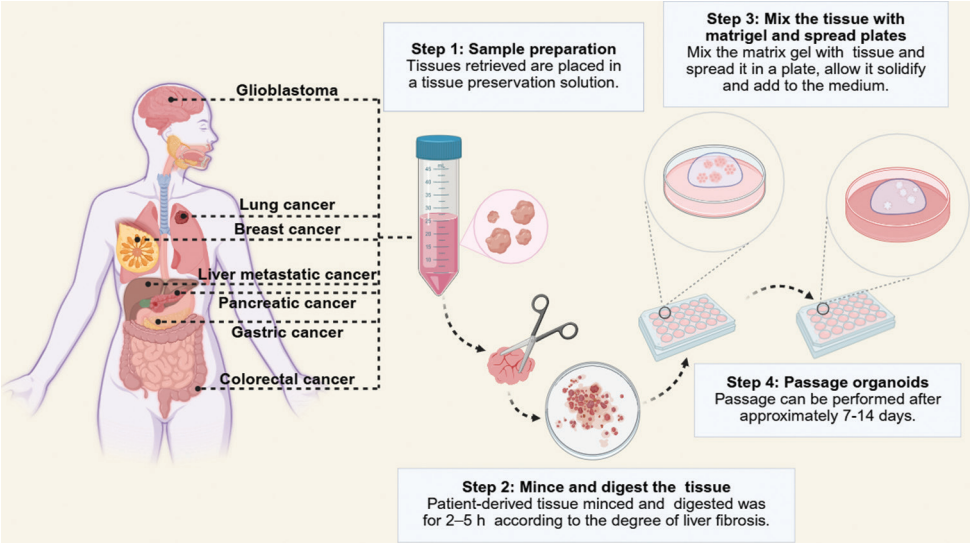

Figure 2. Establishment of organoids from different sources that have been modified to model the characteristics of liver metastases, along with the culture procedure. The general workflow for culturing liver metastatic cancer organoids involves isolating tumor cells, embedding them in a matrix, and maintaining them under customized culture conditions that promote proliferation. Created with BioRender by Sun T. (2025). https://app.biorender.com/profile/template/details/t-6941152869b30f84ec76e161-.

to its status as the predominant target organ for tumor metastasis. However, a co-culture system that incorporates both liver cancer tissue and blood vessels has yet to mature. Future advancements should prioritize the development of co-culture models that incorporate vascular, stromal, and immune elements to better simulate the hepatic metastatic microenvironment and facilitate a deeper mechanistic understanding.

3.2. Liver metastasis organoids and the tumor microenvironment

Reconstitution of the immune TME by incorporating components such as cancer-associated fibroblasts (CAFs), T cells, B cells, and natural killer (NK) cells into the organoid media allows for the exploration of complex crosstalk among various cellular populations.[32] For instance, Neal *et al.*[33] established air–liquid interface PDOs from 100 patient tumors and identified $CD8^+$ (Tc) and $CD4^+$ (Th) T cells, B cells, NK cells, and $CD3^+$ T cells within these PDOs. Nan *et al.*[34] established a co-culture system of pancreatic cancer PDOs and CAFs, demonstrating that TGF-β–activated kinase 1 in CAFs promotes tumor invasion. Esposito *et al.*[35] developed patient-derived immune organoids (PD-IOs). Notably, they utilized immune components derived from patients' peripheral blood rather than TME components, thereby circumventing impaired immune activation in immunodeficient tumors caused by low T-cell content or exhaustion. These studies suggest that a deeper understanding of the immune microenvironment's role within tumor PDOs and its interactions with tumor epithelial cells could provide novel insights for immunotherapeutic strategies.

Tumor-associated macrophages (TAMs) are present throughout all stages of hepatocellular carcinoma progression. Macrophages, key components of the immune system, are generally classified into two subsets: Classically activated (M1) macrophages and alternatively activated (M2) macrophages. Macrophages exert either pro- or anti-tumor effects through polarization into distinct subtypes. The morphology and metabolic characteristics of TAMs are closely associated with prognosis: Smaller TAMs correlate with a favorable prognosis, whereas larger TAMs are linked to immunosuppression and poor prognosis.[36] Employing organoids to investigate CRC liver metastases (CRLM), researchers demonstrated that macrophages play a pivotal role in promoting tumor cell invasion and angiogenesis through the secretion of IL-6 and monocyte chemoattractant protein 1.[31]

The $EEF1G^+$ macrophage subset enriched within liver metastases has been demonstrated to correlate with metastatic progression and immune evasion. These cells may promote tumor invasion through the secretion of pro-angiogenic factors, such as sphingosine-1-phosphate (SPP1).[37-39] Macrophages cooperate with other cells within the liver metastasis microenvironment, thereby promoting tumor metastasis. Specifically, M2-like macrophages are highly abundant in advanced-stage tumors.[40] Tumor organoids provide *in vivo* evidence for tumor metastasis mechanisms. $CD163^+$ M2 macrophages in colorectal liver metastases jointly promote the formation of an immunosuppressive microenvironment by releasing TGF-β, thereby supporting tumor proliferation and metastasis.[41]

In pancreatic cancer liver metastasis, $SPP1^+$ macrophages accelerate the colonization and growth of metastatic foci by modulating the intrahepatic fibrotic microenvironment.[39,42] Using gene knockout mouse organoids, we found that recipient mice harboring NAT10-KO organoids exhibited fewer liver metastatic nodules compared to the control group.[43] Similarly, upon modification of the prostate cancer model mouse organoids and subsequent implantation into the prostates of WT C57BL/6 mice, macrophages and neutrophils were identified as the most prominent immune cell subsets interacting with invasive tumor cells in liver metastasis[44] (Figure 3). These findings provide valuable tools for investigating TAM heterogeneity and immunosuppression.

Traditional organoid co-culture systems can incorporate immune cells or stromal cells. However, accurately simulating the direct spatial competition between tumor cells and hepatocytes within metastatic foci remains challenging. The recent development of a matrix-free microtissue model by Lamprou *et al.*[45] overcomes this issue by promoting tumor cell proliferation within hepatocyte scaffolds and inducing apoptosis through short-range interactions. Despite substantial progress, current organoid models still face limitations in fully replicating the intricate hepatic TME, particularly regarding dynamic immune cell recruitment, vascular remodeling, and hypoxia-driven adaptation.

Abilez *et al.*[46] developed a vascularized liver-like organ (hVO) to recapitulate for the first time *in vitro* the hepatic sinusoidal endothelial phenotype ($LYVE1^+$/$THBD^+$) and pericyte–vessel interactions. Despite the utilization of normal liver-like organs as a model in the study, the introduction of hepatocellular carcinoma cells into hVO co-cultures facilitated the examination of how tumor cells exploit the vascular network. Recent breakthroughs in vascularized organoid-on-chip platforms provide unprecedented resolution for studying tumors–endothelial crosstalk. Du *et al.*[47] developed a personalized vascularized tumor organoid system with self-assembled hierarchical vasculature, demonstrating that highly metastatic tumor cells actively migrate toward blood vessels via Notch-pathway activation. These models confirm the biological behavior of tumor cells actively migrating toward blood vessels and provide preliminary insights into the underlying molecular drivers.

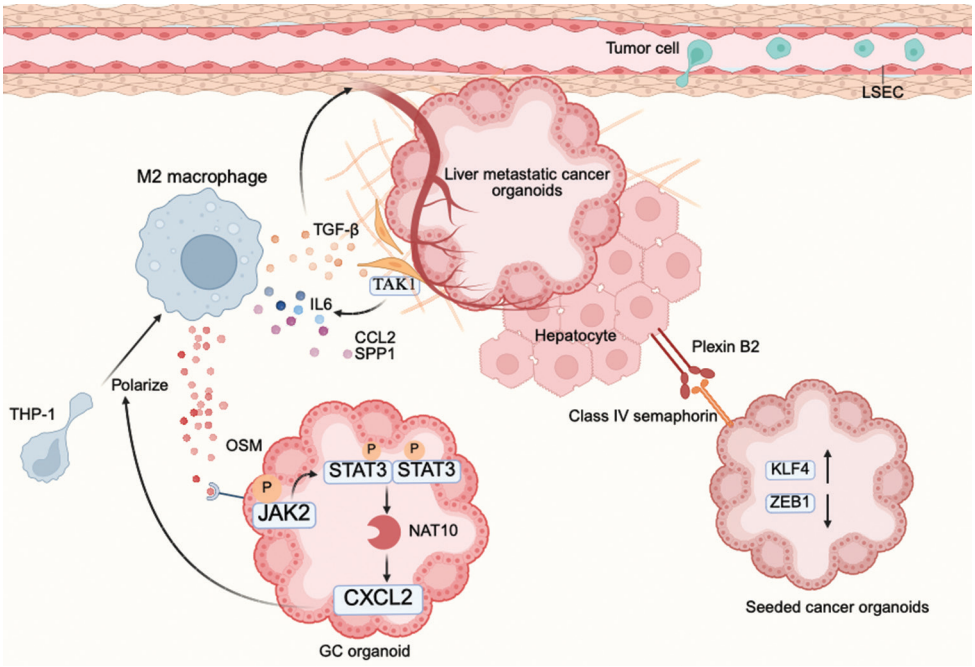

Figure 3. Interconnections between liver metastatic cancer-like organs and the tumor microenvironment. Created with BioRender by Sun T. (2025). https://app.biorender.com/profile/template/details/t-69411865efbf0d596c8be645-pathway.
Abbreviations: CCL2: C-C motif chemokine ligand 2; CXCL2: Chemokine (C-X-C motif) ligand 2; GC: Gastric cancer; IL: Interleukin; JAK2: Janus kinase 2; LSEC: Liver sinusoidal endothelial cell; OSM: Oncostatin M; SPP1: Sphingosine-1-phosphate; STAT3: Signal transducer and activator of transcription 3; TAK1: Transforming growth factor-β–activated kinase 1; TGF-β: Transforming growth factor-β.

4. Clinical applications: Organoid models in liver metastatic cancers

The establishment of metastasis models commonly employs animal models. Murine models, in particular, more accurately recapitulate the complexity of the tumor ecosystem in a comprehensive and "human-like" manner compared to other animal models. Currently utilized murine models encompass orthotopic models, allograft models, cell line-derived xenograft models, patient-derived xenograft (PDX) models, and CTC-derived xenograft models. While murine models have been instrumental in identifying and validating oncogenic drivers, cancer stem cell tumorigenesis, and mechanisms of tumor drug resistance, they also have several limitations, including low metastatic efficiency and constraints within the TME.[48]

The lethality of liver metastasis stems from its heterogeneous evolution and microenvironmental adaptation. This characteristic poses a challenge for traditional animal models, such as murine PDX, due to their time-consuming nature, high cost, and species-specific discrepancies in capturing the spatiotemporal dynamics of human tumors. Furthermore, animal models often present economic, temporal, and ethical limitations, thereby restricting their applicability in research.[49] The advent of organoid technology has addressed this gap. Organoids, which are multicellular *in vitro* structures derived from adult or embryonic stem cells, can be generated from patient tumor tissues. They retain genetic and phenotypic characteristics, closely resembling actual human organs.[7,50]

In this section, we will categorize metastatic hepatocellular carcinomas by their primary site of origin. We will also discuss the construction of organoid models of these liver metastases and their application in clinical studies (Figure 4).

4.1. CRC liver metastasis

CRC represents the third most prevalent malignancy worldwide.[51] The liver is the primary target organ for CRC metastasis.[52] The incidence of liver metastasis is as high as 50–60%, with a higher rate observed in right-sided CRC compared to left-sided colon cancer and rectal cancer.[53] CRC cells remodel the TME, evade immune surveillance, and invade the underlying matrix to access the liver. Subsequently, they reprogram the hepatic microenvironment, establishing a niche that is conducive to CRC cell proliferation.[54]

In metastatic CRC management, it is crucial to consider the unique characteristics of the tumor, including molecular alterations, microsatellite instability status, primary tumor laterality, prior therapies, and the extent of tumor involvement.[51] Patient-derived CRC organoids, which largely recapitulate the heterogeneity of the primary

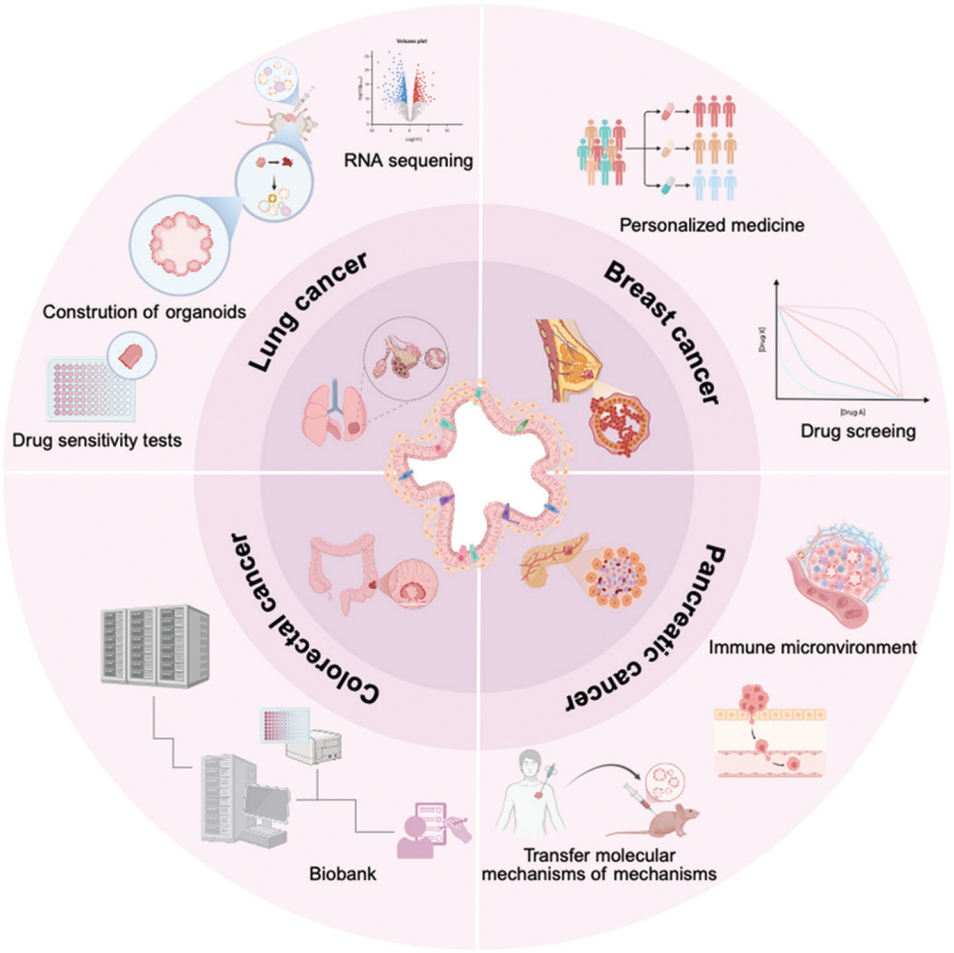

Figure 4. Establishment of organoids from various cancer sources and their clinical applications. Created with BioRender by Sun T. (2025). https://app.biorender.com/profile/template/details/t-694115ba15c13df98ce0435d-summary.

tumor, have emerged as a pivotal tool for investigating the mechanisms of CRC liver metastasis and evaluating therapeutic strategies.

Utilizing primary CRC surgical specimens and matched liver metastasis samples, Mo *et al.*[18] successfully established a biobank of CRLM PDOs. Their study revealed the sustained activation of the yes-associated protein/transcriptional co-activator with PDZ-binding motif pathway within metastatic lesions and the dynamic selection of chemoresistant clones, providing a molecular rationale for targeted therapies. Utilizing CRC PDOs, Cioce *et al.*[55] identified chemotherapeutic agents and investigated the molecular determinants of CRC metastasis to the liver. Screening of CRLM PDOs revealed that the combination of pentoxifylline and 5-fluorouracil inhibited organoid growth, whereas activation of the IL-6–signal transducer and activator of transcription 3 axis promoted metastasis by maintaining an aldehyde dehydrogenase-high, drug-resistant cell population. Investigation on the sensitivity of CRCLM PDOs to 38 clinically relevant drugs, revealing that multidrug-resistant PDOs exhibited resistance to epidermal growth factor receptor inhibition and sensitivity to mitogen-activated protein kinase inhibition within a RAS-mutant background.[17,56]

These findings collectively demonstrate that liver metastasis organoids derived from CRC, owing to their high genetic fidelity and ease of modeling, have become the benchmark model in the field of liver metastasis organoids for predicting the efficacy of clinical drugs and elucidating the mechanisms of cancer cell metastasis.

4.2. Pancreatic ductal adenocarcinoma (PDAC)

PDAC is projected to become the second leading cause of cancer-related death in the next decade.[57] The vast majority of pancreatic cancer patients are diagnosed with liver metastases, which are the leading cause of death in PDAC, with a median overall survival of <6 months.[58] This renders patient-derived pancreatic cancer organoids of particular significance in elucidating the metastatic mechanisms of PDAC.

Yan *et al.*[21] used patient-derived PDAC organoids for *in vivo* xenotransplantation and confirmed that extracellular signal-regulated kinase signaling is a key driver of PDAC liver metastasis. Jeong *et al.*[59] evaluated the transcriptome characteristics of PDOs in invasive and non-invasive PDAC without treatment. They found that differentially expressed genes (e.g., TGF-β pathway members) in invasive PDAC organoids were related to TME remodeling. Laura *et al.*[60] utilized the oxidant hydrogen peroxide to induce the transformation of pancreatic organoids, exploring the pathogenic role of oxidative stress in PDAC. Their study revealed that oxidative stress drives the progression from precancerous lesions to malignant tumours through the nuclear factor erythroid 2-related factor 2/enhancer of zeste homolog 2 signaling axis.

The largest prospective study of PDO-based precision therapy for advanced refractory PDAC was reported in France.[61] The sample source included 50 metastatic liver cancer biopsies. For each PDO, 25 single-drug phenotyping assays were performed. Approximately 91% of patients were matched to the effective drug, with a chemosensitivity rate of 83.3% and specificity of 92.9%. This clinical relevance highlights the potential efficacy of organoids in precision medicine and supports further studies on PDO-based functional precision medicine in the clinic.

In summary, patient-derived pancreatic cancer organoids have emerged as a precise tool for investigating the mechanisms of liver metastasis. These mechanistic insights have been translated into a highly predictive pre-clinical platform, providing robust evidence for large-scale functional precision medicine studies and closing the loop from fundamental mechanisms to clinical decision-making. Both

4.3. Breast cancer liver metastasis

Globally, breast cancer accounts for approximately 30% of all cancers diagnosed in women and remains the second leading cause of cancer-related mortality among women, with a mortality-to-incidence ratio of approximately 15%.[62] However, metastatic breast cancer is considered incurable with existing therapeutic modalities. The most common sites of metastasis for breast cancer include the bone, liver, lung, and brain. The liver is the third most common site of metastasis and is associated with a relatively poor prognosis compared to breast cancers without liver metastases.[1,63,64] The occurrence of liver metastases in breast cancer is likely associated with molecular typing.

A review of clinical data indicates that triple-negative breast cancer (TNBC) and HER2-positive subtypes are more prone to early liver metastases, with an incidence ranging from 30% to 50%. In contrast, luminal A/B liver metastases are more likely to manifest at advanced stages.[65] This discrepancy is closely linked to the inherent characteristics of tumor cells and their ability to adapt to the microenvironment. Different organoid types require distinct culturing conditions, contingent on their specific characteristics. For instance, luminal organoids require the supplementation with estrogen and progesterone, while HER2-positive organoids necessitate the addition of HER2-Ig. TNBC organoids, on the other hand, require a serum-free medium.[66]

Previous studies suggest that the success rate of establishing liver metastasis-like organoids is influenced by the type of organ and hormone levels, reflecting their key role in regulating specific microenvironments.[67] Concomitantly, the evolution of heterogeneity and drug resistance in breast cancer has become a central challenge in clinical treatment. Chen *et al.*[20] developed a patient-derived breast cancer organoid platform comprising 132 primary and metastatic breast cancer samples and successfully established 99 organoids. In most cases, the prediction of trastuzumab resistance in a HER2-positive liver metastasis-like organ has been shown to correspond precisely with the patient's clinical progression. In another case, a TNBC liver metastasis-like organ was unexpectedly sensitized to the proteasome inhibitor bortezomib, and subsequent imaging confirmed a 40% reduction in the size of the liver metastasis. This finding underscores the significance of type-specific organoids in the context of "new uses for old drugs." While bortezomib has been approved solely for the treatment of hematologic tumors, its potential mechanism of action involves the inhibition of the nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) pathway, which is known to be overactive in TNBC.[68]

Based on 51 samples from metastatic breast cancer patients, Lin *et al.* successfully established 46 organoids—including 12 from patients with liver metastases—to guide treatment decisions and evaluated the outcomes of advanced breast cancer patients who received organoid-guided therapy (OGT) compared to treatment of the physician's choice (TPC). They found that the progression-free survival in the PDO prediction group was significantly better than that in the empirical treatment group, verifying the clinical guidance efficacy of organoids in complex drug-resistance scenarios. However, OGT results for three patients were incongruent with their TPC outcomes, and these patients exhibited rapid disease progression following OGT. Notably, all three patients had TNBC. The observed discrepancy in clinical response may be attributable to high tumor burden, declining health status, and the absence of the TME in the PDO model, suggesting a significant role for the TME in malignant progression and drug resistance in TNBC.[69] This finding aligns with the "organoid-informed trials" concept proposed by other studies:[70,71] Organoid predictions must be combined with molecular typing to create differentiated decision-making frameworks.

Although current models still have limitations in fully simulating the TME, particularly in subtypes such as

TNBC that are highly dependent on microenvironmental interactions, OGT regimens have already significantly improved patients' progression-free survival. These findings demonstrate that breast cancer organoid models serve as a functional bridge connecting molecular subtyping with clinical decision-making, representing a critical step in advancing breast cancer precision medicine from "genetic mapping" to "functional validation."

4.4. Lung cancer liver metastasis

Lung cancer is generally categorized into two primary forms: Non-small cell lung cancer (NSCLC), which accounts for 85% of total diagnoses, and small cell lung cancer (SCLC), which accounts for 15% of total diagnoses. In the classification system for NSCLC, lung adenocarcinomas are the most prevalent subtype, with squamous-cell carcinomas ranking second.[72] Distant metastasis is the primary driver of mortality.[73,74] Among metastatic sites, the liver is commonly involved, with liver metastases occurring in approximately 33–40% of patients with lung cancer.[75] Notably, liver metastases from lung cancer are often asymptomatic, with few clinical indications prompting early imaging surveillance.[76]

However, current organoid models of lung cancer liver metastasis predominantly capture late-stage disease phenotypes. Unlike other tumors, liver metastasis in lung cancer is highly dependent on the migratory capacity of CTCs. Most liver metastases from lung cancer arise from hematogenous dissemination, where CTCs traverse the hepatic portal circulation and become trapped within the capillary beds of hepatic sinusoids, initiating colonization of liver tissue.[77,78] Thus, the development of models that can mimic early metastatic events is necessary, including the visualization of the retention, extravasation, and colonization processes of CTCs within the hepatic ecotone. Zheng *et al.*[79] recently developed holographic acoustic tweezers technology and applied it to organoid-organoid systems, including the visualization and preservation of the hepatobiliary system. Incorporating CTCs into this system could enable the simulation of early transfer colonization.

Moreover, most existing models lack critical components of the hepatic microenvironment, such as parenchymal cells and Kupffer cells, limiting their ability to fully recapitulate the complex interplay between CTCs and the liver sinusoidal barrier. Future advancements should focus on integrating live-cell imaging to trace early metastatic events, utilizing liquid biopsy approaches to capture viable CTCs, and incorporating liver-on-chip systems to dynamically reconstruct the pre-metastatic liver microenvironment, thereby improving early diagnostic capabilities.

Fortunately, organoid models provide new opportunities to elucidate the mechanisms underlying metastasis and to predict therapeutic responses. In NSCLC, a lung adenocarcinoma PDO platform identified differential expression of FGFR1 and CDKN1B in metastasis-derived organoids from the liver (MDO-liver) and brain (MDO-brain) compared to other metastatic sites. The inhibition of FGFR1 enhanced the sensitivity of MDO-liver and MDO-brain organoids to sotorasib treatment *in vitro* by 3.1-fold,[80] highlighting the unique value of lung cancer liver metastasis organoids in uncovering site-specific vulnerabilities and guiding precision therapy.

Conversely, SCLC is a highly aggressive subtype with limited targeted therapies. Recent studies have highlighted that targeting periostin/NOTCH1 signaling leads to a significant reduction in liver metastasis in both SCLC PDOs and xenograft models.[81]

In summary, lung cancer liver metastasis organoid models precisely reveal fundamental differences in the molecular mechanisms driving liver metastasis among distinct subtypes within the same major tumor category. However, existing models still struggle to simulate critical early events such as the retention and extravasation of CTCs within hepatic sinusoids, and they generally lack a complete liver microenvironment.

While substantial progress has been made in constructing organoid models of liver metastases from CRC, PDAC, breast cancer, and lung cancer, several challenges remain, particularly regarding the simulation of a fully functional microenvironment.

In addition, organoid research for liver metastases originating from melanoma, gastrointestinal stromal tumors (GISTs), and neuroendocrine tumors (NETs) remains underdeveloped. Melanoma liver metastases exhibit a paucity of histiocytes, primarily due to the limited number of tumor cells present in liver lesions and the inherent plasticity of melanoma cells, which undergo rapid dedifferentiation *in vitro*.[82] For GISTs, a high resistance rate to tyrosine kinase inhibitors (e.g., imatinib)[83] and the lack or low expression of LGR5—a critical stem cell marker required for traditional organoid expansion protocols[84-88]—pose significant barriers to culture establishment. Liver metastases of NETs, which are often associated with severe endocrine symptoms,[84] present additional challenges due to their pronounced cellular heterogeneity and the requirement for specialized hormonal support, such as somatostatin analogs. Standardized culture protocols for NET liver metastases have yet to be developed.

4.5. Practical barriers to the clinical translation of liver metastasis organoids

Despite the significant potential of liver metastasis organoids in basic and pre-clinical research, these models face multiple challenges in transitioning to routine clinical practice. First, lengthy turnaround times pose a primary

obstacle. The process from sample acquisition to drug sensitivity reporting is usually protracted, often taking several weeks. In the case of highly aggressive cancers, patients' conditions may rapidly deteriorate during this period, compelling physicians to initiate treatment immediately based on standard guidelines or experience rather than waiting for results. This limitation restricts their application in urgent first-line therapies.[85] Second, high costs and limited scalability hinder their widespread adoption. The cost of culture media, substrates, and specialized personnel can be a significant barrier to their implementation in healthcare institutions with limited resources. Furthermore, organoid generation success rates and patient accessibility remain suboptimal. Variations in sample quality and tumor types mean that not all patients can successfully generate organoids. The dearth of globally consistent cultivation and testing standards, compounded by the absence of rigorous regulatory approval processes, necessitates the validation of their clinical efficacy and reliability through large-scale prospective clinical trials.[86,87]

Overcoming these obstacles requires a multi-pronged approach: optimizing and streamlining processes through cutting-edge modern technologies, conducting forward-looking clinical studies to accumulate high-level evidence, and actively collaborating with regulatory authorities. It is only through concerted efforts that this technology can realize its full potential in the field of precision medicine.

5. Integration of organoids with emerging technologies

Recent technological advancements are propelling organoid systems to a new level of sophistication. The integration of novel tools, such as single-cell omics, genetic engineering, organ-on-a-chip technologies, and artificial intelligence (AI), is transforming organoid platforms into comprehensive systems for elucidating disease pathogenesis and supporting clinical decision-making (Figure 5).

5.1. Single-cell sequencing technology

It is possible to reveal the underlying mechanisms that drive tumor plasticity through single-cell sequencing,[88,89] which has been widely used to identify the mechanisms of metastasis in various cancers.[90-94] Combining organoids with single-cell sequencing to map the cellular heterogeneity of liver metastases can visually reveal the organoids of liver metastases. Mo *et al.*[18] had an interesting experiment in the process of building a biobank of primary CRC and paired liver metastases from CRC liver metastasis. They used scVelo to perform cell trajectory analysis. They found that a large number of stem cells in primary CRC organoids showed a tendency to differentiate into maturation-like cells. The mature cells in liver metastasis organoids showed a tendency to revert to stem cell-like cells. Stem cell-like tumor cells exhibit excellent self-renewal potential in liver metastasis.

In summary, the integration of single-cell omics technologies with organoid models offers a powerful approach to unravel cellular communication events and the evolutionary dynamics that underpin liver metastasis. This synergistic strategy holds significant potential for identifying new therapeutic targets and optimizing treatment strategies for metastatic disease.

5.2. CRISPR-Cas9 technology

While clustered regulatory interspaced short palindromic repeats (CRISPR)-Cas9 technology facilitates the construction of disease models, most models may not fully recapitulate the diverse disease phenotypes observed across all patients. Organoid models, which preserve tumor heterogeneity and TME components (e.g., macrophages, fibroblasts), combined with gene editing, offer a dynamic platform for dissecting metastatic mechanisms and circumventing specific ethical concerns. CRISPR-Cas9 technology enables single-base editing with high precision and efficiency in organoids, allowing for the accurate simulation of patient-specific mutations. Matano *et al.*[95] demonstrated that targeting tumor suppressor genes (e.g., *APC*, *SMAD4*, and *TP53*) and oncogenes (e.g., *KRAS* and *PIK3CA*) in healthy human intestinal organoids using CRISPR-Cas9 can simulate the development of CRC. Subsequent transplantation experiments demonstrated that the edited organoids could form micrometastases and latent tumor-initiating cells, confirming that the technology enhances the efficiency of tumor organoid modeling. The CRISPR-organoid fusion strategy opens up new avenues for precision medicine targeting metastatic progression.

5.3. Organ-on-a-chip and microfluidic engineering

Although PDOs have shown significant advantages in preserving tumor heterogeneity and microenvironment interactions, traditional organoids are still limited by static culture conditions and face challenges in reproducing dynamic physiological characteristics *in vivo* (e.g., blood flow shear stress, oxygen gradient, and intercellular mechanical stress). To address these limitations, organ-on-a-chip technology has emerged as a transformative approach, combining microfluidic engineering and tissue engineering to construct highly biomimetic models of metastasis.

To evaluate the metastatic potential of mucosal melanoma PDO and plantar melanoma PDO, Chen *et al.*[96] used tumor PDO and polycarbonate membranes to simulate tumor invasion and internalization within the vascular system. To simulate the formation of the premetastatic TME and study the mechanism of breast cancer-derived extracellular vesicles (EVs) in liver metastasis, Kim *et al.*[97]

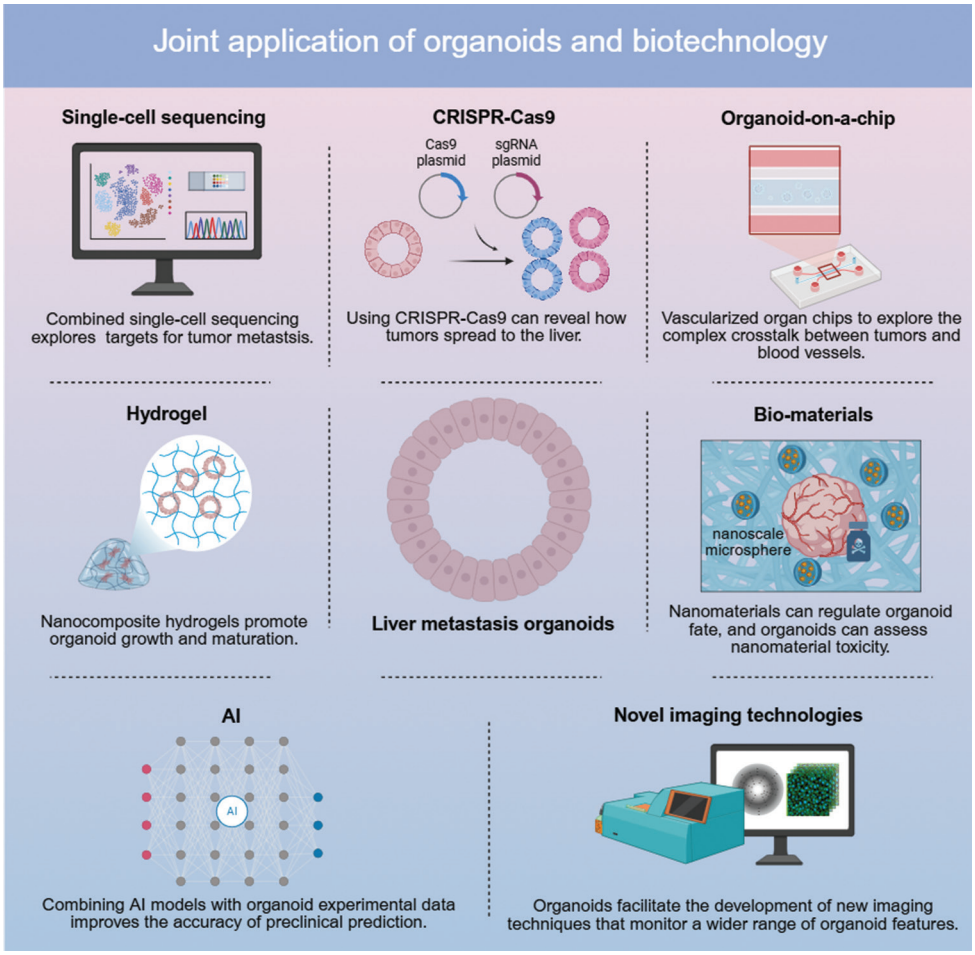

Figure 5. Combined use of organoids from liver metastatic cancer patients and new technologies. Created with BioRender by Sun T. (2025). https://app.biorender.com/profile/template/details/t-6929624b1f28ab3c2c91b850-figure4.
Abbreviations: AI: Artificial intelligence; CRISPR: Clustered regularly interspaced short palindromic repeats; sgRNA: Single guide RNA.

developed a human liver chip, demonstrating that breast cancer-derived EVs activate liver sinusoidal endothelial cells (LSECs), hepatocytes, and liver fibroblasts in the liver microarray to create conditions conducive to metastasis of breast cancer cells to the liver microenvironment. Breast cancer-derived EVs also increase fibronectin on LSECs to promote adhesion of breast cancer cells to the liver microenvironment. This liver chip provides a potential platform for studying the mechanisms of liver-targeted metastasis. In future studies, it will be necessary to integrate fluid shear stress and oxygen gradient to simulate hepatic sinusoidal blood flow.

This interdisciplinary integration provides unprecedented tools to accurately analyze the mechanism of metastasis, optimize drug screening platforms, and develop individualized treatment strategies, marking a paradigm shift from "*in vitro* simulation" to "biomimetic reconstruction" in tumor research.

6. Future perspectives

Compared to conventional cell lines and PDX, tumor organoids better preserve the molecular and tissue phenotypes of the original tumors, thereby maximizing patient-specific heterogeneity. Notably, the global shift toward reducing reliance on animal models highlights the increasing significance of organoid technology. In July 2025, the European Commission announced its "Chemical Industry Action Plan," which provides a roadmap for phasing out animal testing by promoting innovative alternatives, such as organoids and organ-on-a-chip systems. Similarly, the United States National Institutes of Health declared in July 2025 that it will no longer fund projects relying solely on animal experiments and emphasized the adoption of new approach methodologies. These include computational modeling, AI, and organoid technologies. These policies highlight the potential of organoids to transform medical research by offering more ethical, cost-effective, and human-relevant models.

Nevertheless, current models only partially replicate the complex characteristics and evolutionary trajectories of metastatic tumors, and several critical challenges remain. For example:

(i) Biomimetic microenvironmental enhancement is urgently needed, incorporating LSECs, Kupffer cells, and hemodynamic parameters to simulate the spatiotemporal evolution of the premetastatic niche, including hypoxic gradients and immunometabolic interactions.

(ii) Standardization and scalability must be achieved through the establishment of international culture consensus guidelines (e.g., for medium composition and passage protocols) and the integration of AI-driven high-throughput screening platforms to enhance data comparability and application efficiency.

(iii) Clinical translational innovation should be prioritized, including the development of "organoid-informed trials" and the incorporation of organoid-based drug susceptibility data into clinical guidelines such as the National Comprehensive Cancer Network recommendations. In addition, the establishment of linkage platforms connecting liquid biopsy technologies (e.g., circulating tumor DNA and CTCs) with organoid models could enable dynamic monitoring of drug resistance evolution.

7. Conclusion

Organoid models of liver metastases, by closely mimicking tumor heterogeneity and dynamic microenvironmental interactions, have become valuable tools for elucidating metastatic mechanisms and guiding precision therapies. These models have demonstrated significant clinical translational potential in common cancers such as CRC, pancreatic cancer, and breast cancer. As pre-clinical predictive platforms, liver metastasis organoids are widely utilized for mechanistic studies, biobanking, drug screening, clinical decision support, and the development of personalized treatment strategies.

The integration of emerging technologies—such as single-cell sequencing, spatial omics, gene editing, microfluidics, and organ-on-a-chip platforms—has further expanded the analytical capabilities of liver metastasis organoids, addressing many limitations associated with traditional culture systems.

Liver metastasis organoid models are reshaping the paradigm of precision therapy through their capacity to decipher the metastatic mechanism and microenvironmental interactions. Future studies are encouraged to address existing challenges and promote standardized clinical translation, thereby enabling stratified interventions and closed-loop management of long-term survival.

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Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Conceptualization: Tong Sun, Hao Lin
Visualization: Tong Sun
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Writing–review & editing: All authors

Ethics approval and consent to participate

Not applicable.

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