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Review article

Organoids derived from metastatic cancers: Present and future

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ABSTRACT

Organoids are three-dimensional structures derived from primary tissue or tumors that closely mimic the architecture, histology, and function of the parental tissue. In recent years, patient-derived organoids (PDOs) have emerged as powerful tools for modeling tumor heterogeneity, drug screening, and personalized medicine. Although most cancer organoids are derived from primary tumors, the ability of organoids from metastatic cancer to serve as a model for studying tumor biology and predicting the therapeutic response is an area of active investigation. Recent studies have shown that organoids derived from metastatic sites can provide valuable insights into tumor biology and may be used to validate predictive models of the drug response. In this comprehensive review, we discuss the feasibility of culturing organoids from multiple metastatic cancers and evaluate their potential for advancing basic cancer research, drug development, and personalized therapy. We also explore the limitations and challenges associated with using metastasis organoids for cancer research. Overall, this review provides a comprehensive overview of the current state and future prospects of metastatic cancer-derived organoids.

1. Introduction

Despite significant efforts to advance cancer research and therapeutics, cancer is still projected to remain the leading cause of death in the coming decades [1]. Metastasis is the most common cause of cancer-related mortality, with a rate exceeding 90 % [2]. Systemic treatments, including chemotherapy, targeted therapy, and immunotherapy, are the primary approaches used to cure metastatic cancers [3–5]. However, patients frequently develop acquired drug resistance after several cycles of therapy or face native resistance at the beginning, resulting in disease progression and eventual mortality [6]. Treating metastasis remains a great challenge, and developing new drugs, identifying biomarkers of the therapeutic response, and validating resistance mechanisms in ex vivo models are key to benefiting patients with metastatic cancers.

Over the past few decades, many drugs that are expected to be effective in theory and that have performed well in cancer models have ultimately failed in clinical trials [7]. One of the major obstacles is the poor recapitulation of human tumors by conventional cancer models [8]. For instance, two-dimensional (2D) cell line cultures and patient-derived tumor xenografts (PDTXs) have long been employed as tumor models and have made numerous contributions to cancer research [9,10]. However, these methods have several limitations that hinder their clinical application (Table 1). Cell lines are difficult to establish from solid tumors; for primary breast cancers, the success rate is between 1 and 10 % [11], while prostate cancer is represented by fewer than 10 cell lines [12]. Models from tumor-derived cell lines are inefficient at simulating stromal compartments and interactions between cells and the extracellular matrix

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(ECM), which are critical to tumor proliferation, differentiation, and survival, leading to a mismatch between the cell line model and the in vivo response [13]. Moreover, models from cell lines lack the genetic heterogeneity of the original tumors after many passages of cancer cell lines [14]. Additionally, cell line models lack immune cells to validate immunotherapy, which is an essential supplement for standard therapy [15]. Tumor stem cell cultures are valued for their simplicity, allowing easy genetic modifications and rapid growth for high-throughput drug tests [16]. They are cost-effective, not needing the complex setups required for three-dimensional cultures, making them suitable for basic research and initial screenings. Their high self-renewal capacity supports large-scale experiments from small samples. However, these cultures don't replicate the tumor's complex structure and microenvironment, potentially limiting their effectiveness in mimicking true tumor behavior and predicting clinical outcomes. PDTXs models, in which tumor cells or tissues are transplanted in immunodeficient mice, are used to identify the most effective agents [17]. This model allows vascularization of the engrafted tumor and assembly in a realistic tumor structure and environment, thus validating the drug response in a more complex in vivo environment. However, PDTXs are expensive and time-consuming, and high-throughput screening is impossible [18]. Furthermore, their environment will be gradually replaced by stroma of the mice [19]. Organ-on-a-chip technology, which employs microfluidic platforms to simulate the structure and function of human organs on microchips, represents a revolutionary tool for biomedical research and drug development [20,21]. It offers highly accurate simulations of the human microenvironment, reduces the need for animal testing, accelerates drug screening and toxicity testing, supports disease modeling research, and facilitates personalized medicine [22,23]. This model facing challenges such as technical complexity, cost, scalability, and cellular diversity [20]. Humanized mouse are genetically modified mice that have been engrafted with human tissues, cells, or genes, making them capable of mimicking human immune responses and disease processes [24]. The advantages of humanized mice include their potential to evaluate the effectiveness and safety of therapeutics, vaccines, and immunotherapies before clinical trials in humans [25–27]. However, the limitations include the high cost, technical complexity of creating and maintaining these models, potential differences in tissue microenvironments between mice and humans that can affect the interpretation of results, and ethical considerations regarding the use of genetically modified organisms [28,29]. Recently, researchers have focused on a novel cancer model: organoids.

Organoids are a novel three-dimensional (3D) culture model prepared in a dish that can be defined as a collection of organ-specific cell types that develop from stem cells or organ progenitors and self-organize through cell sorting and spatially restricted lineage commitment in a manner similar to that in vivo [31]. Organoid technology has increasingly drawn attention in cancer research because of its advantages (Table 1). First, organoids are easily cultured, with a success rate of over 70 % in certain cancer types [32,33]. Second, compared with traditional cell lines, organoids more faithfully recapitulate the genetic and histological characteristics of tumor [34]. Third, intra- and interpatient heterogeneity is well captured in this in vitro model [35]. Fourth, organoids technology could be exploited as a low-throughput platform for clinical trials and patient-specific treatments [32,36]. Finally, organoids are easily biobanked once established, allowing further study [37]. Metastatic cancer organoids, with their origin from more aggressive cancer stages, offer an unparalleled model for advanced tumor studies, significantly aiding translational therapy efforts [38]. Their rapid establishment and ability to be passaged over long periods make them particularly suitable for patient-specific chemotherapy and targeted drug screenings. These organoids stand out by closely mimicking the genetic and partly microenvironmental complexities of late-stage diseases, thus providing a crucial platform for personalized medicine. The expedited process of drug testing enabled by these organoids accelerates the discovery of effective treatments for patients battling advanced cancers. Additionally, the unique genetic insights they offer into metastasis-specific mutations and pathways open up new avenues for targeted therapy development [39]. Moreover, as metastatic cancers frequently develop resistance to treatments, organoids derived from such cancers serve as prime models for investigating drug resistance mechanisms and devising strategies to counteract them, thereby enhancing the effectiveness of cancer therapies [40].

In this review, we explore the feasibility of culturing organoids from multiple metastatic cancers; evaluate the potential role of organoid biobanks in basic research, drug development and personalized medicine; and discuss the current limitations and potential of using metastasis organoids for cancer research. This review provides valuable insights into the challenges and opportunities associated with this innovative approach to cancer research.

Table 1
Comparison of the preclinical cancer models.

Feature	Cell Lines	Cancer Stem Cell-derived Organoids	PDTX
Initiation Success Rate	Low	High	Moderate
Maintenance Ease	High	Moderate	Low
Resource Consumption	Low	Medium	High
Expansion Speed	Fast	Moderate	Slow
3D Growth Capability	No	Yes	Yes
In Vitro Phenotypic Feature Retention	No	Partial	Optimal
In Vitro Genetic Feature Retention	Limited	High	High
Cancer Spectrum Representation	Partial	Broad	Broad
Tumor–Stroma Interactions	Absent	Limited	Present
Immune System Incorporation	Absent	Possible with Coculture	Absent
Low-throughput Drug Screens	Optimal	Optimal	Challenging
High-throughput drug screens	Optimal	Challenging	Very Challenging
Biobanking	No	Yes	No

Table adapted and updated with permission from Ref. [30].

2. Biobanks of organoids derived from metastatic cancer

To date, organoids have been established from primary colon [41–43], stomach [44,45], esophageal [46,47], lung [48], pancreatic [49,50], prostate [51,52], endometrial [53], and breast cancer tissues [54,55]. These cancer organoids can be derived from primary tumor tissues [32,56], metastatic tissues [57], circulating tumor cells [58], and tumor cells enriched from effusion fluid [35]. Regarding the processing of tumor tissues postcollection, two primary protocols are predominantly followed: the first involves the mechanical and/or enzymatic dissociation of complete tissue specimens into single cells [32], while the second maintains the structural integrity of the tissue, segmenting it into millimeter-scale fragments for culture [59] (Fig. 1). Currently, most organoids are generated from primary cancer tissues, as detailed in Table 2. Several studies have successfully established organoids from metastatic tissues of various cancer types, including prostate [58,60,61], colorectal [62–66], breast [67], pancreatic [56], and gastric cancer [32] tissues.

PDO methodologies involve the collection of tumor tissues from various origins and stages, including primary and metastatic sites, circulating tumor cells, and cells derived from pleural effusions, to create cancer organoids that are specific to individual patients. This process involves a range of tissue preparation techniques, such as cutting tissues into smaller fragments, fully dissociating tissues into single cells, and/or sorting specific cell types, which are crucial for the successful development of cancer organoids within 3D scaffolds. Once these PDOs have been established, their genetic and phenotypic traits, including their structure, immunohistochemical properties, and genomic variations such as mutations, are thoroughly assessed to ensure that they accurately mirror the characteristics of the source tumors.

These organoids have utility in a wide array of fields, notably in drug development, drug screening, and preclinical models, among other applications (see Fig. 2). Metastasis organoids can be generated from metastatic sites such as the liver, lymph nodes, bones, peritoneum, and ascites [38,58]. Paired organoids, derived from primary cancers and their matched metastases, have been successfully cultured using identical systems [38,67]. Different cancer types may lead to differences in organoid production efficiency (see Tables 2 and 3), and the establishment rate doesn't differ between primary and metastatic organoids. Some researchers have noted that the complete failure of growing organoids derived from primary prostate cancers, most likely due to overgrowth by the normal prostate epithelium present within each sample [61]. In addition to differences in cancer types, tumor cellularity is another key factor in culturing organoids [38]. Previous studies have shown that organoids derived from normal tissue can counterintuitively grow faster than their matching tumor organoids [72], possibly due to higher rates of mitotic failure and subsequent cell death in the latter. This result could explain the low establishment rate of organoids from advanced prostate cancer [58]. Using selective culture conditions to establish organoids may effectively prevent the overgrowth of normal tissue in cancer organoids with low cellularity [73]. In 2016, Fujii et al. cultured metastatic colorectal cancer (CRC) with an excellent success rate of 100 % [63]. The improved establishment rate is a result of culturing organoids in eight different culture systems based on three niche factor combinations: Wnt activators, oxygen concentration, and a p38 inhibitor [63]. These factors are essential for certain subsets of CRC organoids, whereas others show adverse or no sensitivity to these factors [63]. This finding indicates that genetic alterations involved in carcinogenesis may explain the progressive loss of the niche factor requirements of organoids during tumorigenesis and diverse niche factor requirements in different cancer subtypes [74].

When PDOs from metastatic CRC were tested before and after several months of continuous culture, high concordance was observed in mutational, copy number alterations (CNA), and transcriptomic profiling over successive passages (passage range: 5–13) [38]. It was observed that organoid establishment could occur as swiftly as approximately 10 days, with some lines continuing to be passaged for up to 14 months (P57). Additionally, the process of screening each organoid against a panel of 37 anti-cancer drugs was efficiently completed in under two weeks [32]. The successful establishment of organoid lines from metastatic prostate cancer patients have been maintained in culture for over six months, noting that the majority were established within a one-month timeframe [58]. These findings indicate that the establishment and passaging times of organoids are acceptable, making them a viable platform for

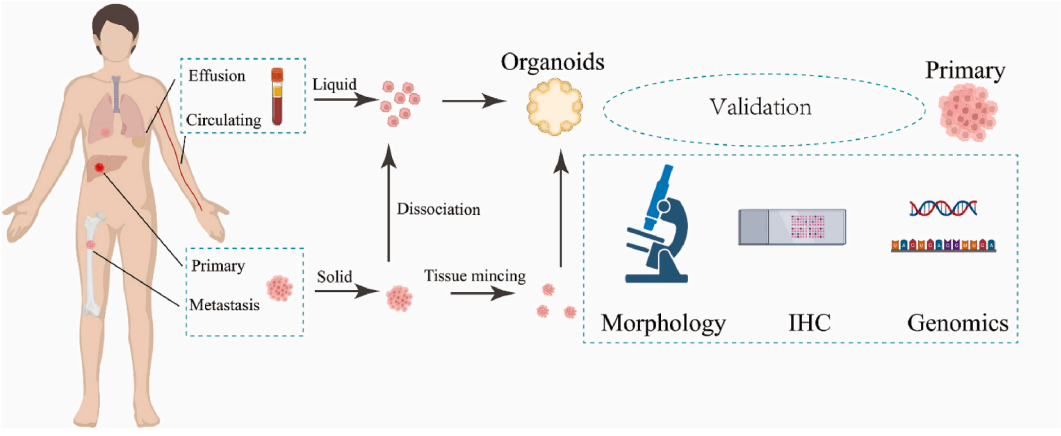

Fig. 1. PDO methodologies.

Table 2
Overview of some available cancer organoid biobanks.

Cancer type	Source	Organoid number	Success rate of establishment	References
Head and neck cancer	Primary cancer tissue	31 cancer organoids from 40 patients	60.0 %	[68]
Lung cancer	Primary cancer tissue	80 cancer organoids	87.0 %	[48]
Colorectal cancer	Primary cancer tissue	22 cancer organoids	90.0 %	[69]
Pancreatic cancer	Primary and metastatic cancer tissues	114 cancer organoids	75.0 %	[56]
Breast cancer	Most from primary tumors	95 cancer organoids	80 %	[67]
Bladder cancer	Primary cancer tissue	56 cancer organoids	85.0 %	[70]
Liver cancer	Primary cancer tissue	27 cancer organoids	~27 %	[71]

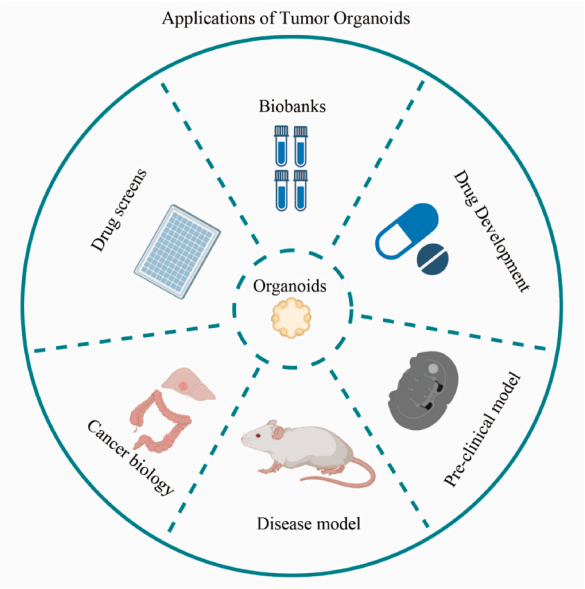

Fig. 2. Current applications of organoids derived from cancer cells, including research on cancer biology, drug screening, disease modeling, and preclinical modeling.

Table 3
Overview of organoids derived from metastatic sites.

Cancer type	Metastatic sites	n	efficiency	Refs
Colorectal cancer	Liver	10	71 %	[62]
	NR	13	76	[75]
	Metastatic or recurrence sites	17	77 %	[76]
	Liver	6	100 %	[63]
	Peritoneum and ascites	5	NR	[66]
	NR	NR	60 %	[77]
Gastrointestinal cancers (CRC, GOC, and cholangiocarcinoma)	Liver, pelvis, peritoneum, and lymph nodes	29	70 %	[38]
Pancreatic cancer	NR	NR	75 %	[56]
Gastric Cancer	Lymph nodes	NR	Over 50 %	[32]
Prostate cancer	NR	NR	15–20 %	[61]
	NR	6	15–20 %	[58]
	Bone	>4	NR	[60]
Breast cancer	Lymph nodes	12	>80 %	[67]
Esophageal cancer	Brain	NR	NR	[78]

NR, not reported; Refs., references.

preclinical drug screening.

Metastasis organoids maintain morphological and genetic characteristics similar to those of parental tissues. Ruth et al. cultured colorectal cancer organoids from liver metastases, and some of them presented lumen structures consistent with their corresponding parental tissue [79]. Other studies have also shown that CRC organoids can histologically maintain the morphological features of the corresponding specimens, as well as immunohistochemical expression of differentiation markers [75]. Importantly, the histopathological grade and differentiation status of their parental tumors are reproduced even after xenotransplantation [63]. Molecularly, the

mutation landscape of metastatic PDOs largely overlaps with that of corresponding biopsies [38]. Although some somatic mutations are not common in metastatic CRC organoids and their corresponding biopsies, none of them are mutations of driver genes [62]. Moreover, the DNA copy number profiles of the organoids and the corresponding biopsies are strongly correlated, as are the inherent CpG island methylation patterns [62,63]. Interestingly, organoids from paired primary and metastatic CRC exhibit concordant niche factor requirements, indicating that niche independence per se may not be essential for tumor invasion or metastasis [63]. Within malignancies, cancer stem cells are a niche population of cells that possess the capacity for self-renewal and resistance to chemotherapy and radiation. Markers such as CD24, CD44, CD133, and CD166 are commonly used to identify these cells [80]. Metastatic colorectal cancer (CRC) organoids are enriched in cancer stem cells [75]. As a result, they provide an ideal model for investigating cancer stem cells and identifying effective drugs to treat them.

In 2018, Sachs et al. generated massive breast cancer (BC) organoids from primary and metastatic cancers [67]. Compared with those of the BC panel, the BC organoids showed a similar distribution in terms of histological type and grade [67]. Importantly, the most valuable and prevalent BC receptors, such as estrogen receptor (ER), progesterone receptor (PR), and HER2, were well retained in most BC organoids. ER and PR expression are valuable predictors of the outcome of hormone/endocrine therapy, while the HER2 status has predictive value for systemic chemotherapy and is itself a target for targeted therapies [81]. Similarly, in metastatic castration-resistant prostate cancer (CRPC), a broad spectrum of androgen receptor (AR) levels was also observed in organoids [58]. BC organoids recapitulate the diverse genomic landscape of BC, including CNAs, mutational load, and signatures, as well as cancer gene mutations. While the total number of mutations, as well as the relative contribution of individual signatures, greatly differed between patients, driver mutation loads and types, such as BRCA, were largely preserved in matching organoid-tissue pairs.

PDO cultures revealed unique gene expression programs that divide PDAC into two distinct molecular classes. In 2018, Tuveson and colleagues generated 114 organoids from primary and metastatic pancreatic cancer, including both classical and basal-like subtypes [56]. This advance is significant, as few cell line models of the classical PDAC subtype are available [82]. In addition to being able to efficiently culture organoids from every stage of pancreatic cancer, this culture method enables the propagation and study of PDO cultures from both classical and basal-like PDAC subtypes. The PDO transcriptomes revealed unique gene expression programs that divide PDAC into two distinct molecular classes (C1/C2). The classifications were largely concordant, with 83 % of the basal-like PDO cultures falling in the C1 classifier and 93 % of the classical PDO cultures falling in the C2 cluster [56]. Spatial intrapatient heterogeneity and temporal evolution of PDO chemosensitivity were observed in metastatic PDAC, highlighting the possibility that patients with metastasis may possess different cancer subclones that will require novel therapeutic regimens to achieve the best clinical outcome [56]. Further studies are needed to investigate the biological mechanisms underlying these observations and to develop effective therapies for pancreatic cancer.

2.1. Metastasis organoids for basic research

Metastasis organoids have emerged as valuable tools in cancer research because they allow the study of tumor heterogeneity and metastasis in a controlled environment [65]. Despite the challenges in culturing metastasis organoids, they hold great promise for identifying novel therapeutic targets and elucidating the mechanisms of tumor spread.

Organoids provide a valuable tool to bridge the gap between gene transcription, gene expression, and cell behavior, which can

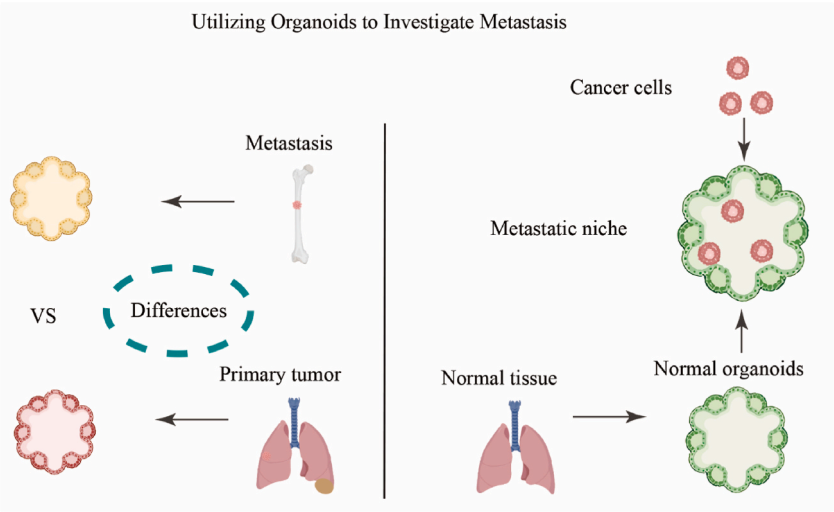

Fig. 3. Two approaches to studying tumor metastasis using organoids. The first approach generates organoids from paired primary and metastatic cancers separately, elucidating potential mechanisms through a comparison of the differences between these two types of organoids [85]; however, this method lacks the interaction between tumor cells and the microenvironment [57]. The second approach involves creating organoids from normal tissue, followed by coculturing these organoids with tumor cells and organoids derived from normal tissue to study the metastatic niche.

sometimes be inconsistent. Compared with primary cancer organoids, metastasis organoids exhibit a greater proportion of tumor cells positive for Ki-67, CEA, and SOX2 [75]. Interestingly, CRC liver metastasis organoids exhibit more aggressive phenotypes, tumorigenesis, and metastatic capacity than those from primary lesions, despite comparable gene expression and cell morphology [65]. Compared with primary cancer organoids, metastatic cancer organoids also exhibit a greater growth rate and volume both in vitro and in vivo [62,63,65]. However, healthy epithelial organoids unexpectedly exhibit greater proliferation than organoids cultured from primary cancer, creating an interesting contradiction [83]. Further studies are needed to understand and reconcile the contrasting growth rates observed between normal epithelial cells, metastatic cells, and primary cancer cells, which may help to address the question of whether normal epithelial cells can replace cancer cells in organoid cultures.

The use of organoids derived from primary tumors and matched metastases has allowed researchers to elucidate the mechanisms of tumor spread and identify new potential therapeutic targets. Whole-genome sequencing revealed that driver gene mutations are common between the organoids of primary and metastatic CRCs, suggesting that driver gene mutations precede metastasis development [63]. This result is consistent with recent findings suggesting that mutations specific to metastasis are not the cause of cancer spread but rather are associated with drug resistance [75]. In 2014, Nadauld and colleagues utilized organoids to investigate the genetic heterogeneity and metastatic differentiation of diffuse gastric cancer [79]. In contrast, researchers induced metastatic diffuse gastric cancer in a primary murine gastric organoid model through TGFBR2 knockdown in the context of CDH1 and TP53 loss [79]. Thus, this study revealed the potential role of TGFBR2 loss of function in promoting metastasis [79]. More studies are needed to further investigate and clarify the controversial results regarding the role of specific mutations in promoting cancer metastasis. These findings will help us better understand the underlying mechanisms of metastasis and identify potential therapeutic targets for preventing and treating metastatic disease.

Organoids derived from primary tumors and matched metastases have provided powerful tools for studying tumor evolution [84]. For instance, under selection pressure from treatment, metastatic CRPC is a highly heterogeneous disease in which AR expression is surprisingly diverse from patients' initial biopsies of the primary tumors [85]. Most organoids derived from metastatic CRPC harbor losses of PTEN and TP53, which are common in CRPC samples but rare in primary cancers (Fig. 3, left panel) [58]. Therefore, organoids derived from metastatic CRPC have the potential to be used to research tumor evolution and corresponding castration resistance mechanisms [58]. This finding suggests the therapeutic potential of drugs targeting dysfunctional RB and TP53 pathways in advanced prostate cancer. However, direct comparisons of genotype and phenotype characteristics should be performed between organoids derived from paired primary and advanced prostate cancers to investigate the mechanisms of tumor heterogeneity and tumor evolution.

Cancer cells and normal tissues can work together to form metastases, as observed in a study where normal cells were induced by metastatic niche factors to become cancer cell-associated parenchymal cells that support tumor growth [39]. However, the lack of preclinical models for studying the complex interactions between metastatic tumors and surrounding normal tissues hinders the development of effective strategies to prevent tumor metastasis [86]. Metastasis organoids offer a valuable tool for this purpose. For instance, lung metastasis organoids derived from colon cancer generated from primary lung organoids cocultured with primary colon cancer cells mimic the architecture of metastatic tumors in the lung, including angiogenesis (Fig. 3, right panel) [57]. These organoids can predict the outcomes of chemotherapy and targeted therapy and can be rapidly generated in just 3–5 days, which is much faster than other organoids generated from metastatic cancer stem cells [69]. This model revealed that tumor exosomes isolated from cancer cells can enhance cancer cell colonization in primary lung organoids [57]. Additionally, the ability of cancer cells to colonize normal organoids is reduced after chemotherapeutic agents are used to treat the organoids or tumor cells alone. Thus, rapidly generated organoids not only directly reveal the complex interactions between metastatic cancer cells and normal cells through metastatic niche factors in vitro but also indicate that chemotherapeutic drugs can prevent tumor metastasis by affecting these interactions.

2.2. Metastasis organoids and translational research

High-throughput sequencing has been widely used in precision medicine to identify somatic mutations for cancer treatment and drug development [87]. However, the limitations of mutation signatures in predicting the response to targeted therapies, as well as the shortcomings of preclinical models used for drug validation, pose significant challenges to the success of personalized medicine [38]. PDOs have potential for use as preclinical predictive models because they exhibit different responses to various therapeutic agents and can acquire chemoresistance [75]. Importantly, even after xenotransplantation through the kidney capsule of immunodeficient mice, organoids maintain the histopathological features of the primary tumor, suggesting that PDOXs can validate drug responses in a more

Table 4
Overview of the use of metastasis organoids for predicting drug responses.

Cancer type	Therapy (TP:FP:FN:TN)	Sensitivity	Specificity	PPV	NPV	Refs
CRC	7:1:0:13	100 %	93 %	88 %	100 %	[38]
BC	1:0:0:1	100 %	100 %	100 %	100 %	[67]
PC	5:1:0:2	100 %	66.6 %	83.3 %	100 %	[56]
GC	2:0:0:1	100 %	100 %	100 %	100 %	[32]
Prostate cancer	1:0:0:0	100 %	100 %	–	–	[88]
Total	16:2:0:17	100 %	89 %	88 %	100 %	

CRC, colorectal cancer; BC, breast cancer; PC, pancreatic cancer; GC, gastric cancer; TP, true positive; FP, false-positive; FN, false-negative; TN, true negative; PPV, positive predictive value; NPV, negative predictive value; Refs., references.

complex in vivo environment [63]. Small-scale drug screens of organoids derived from metastatic cancer have yielded promising results, further emphasizing the potential of organoids as preclinical models for predicting drug responses (Table 4) [38,56,58,66].

Chemotherapy can help treat cancer and prolong the life of patients with advanced disease. However, it can also damage cancer cell DNA and cause mutations that make the cells more aggressive and resistant to treatment [89]. Preclinical models are needed for drug screening that specifically targets cancer cells to minimize harm to healthy cells and reduce toxicity. Organoids derived from liver metastasis of CRC have shown a comparable response to chemotherapy (including TAS-102) when comparing the parental biopsy and clinical patients, suggesting that organoids can accurately recapitulate tumor heterogeneity [75]. Moreover, organoids derived from different metastases of the same patient can also exhibit different sensitivities to drugs but maintain consistency with the parental tumor, indicating that organoids could generalize the spatial heterogeneity of the parental tumor [75]. Furthermore, organoids derived from the same metastatic lesion at baseline are sensitive to chemotherapy agents, but PDOs derived from the same lesion at later stages of progression become resistant to chemotherapy, indicating that organoids can exhibit temporal heterogeneity [38]. Another team has also documented the potential of organoids as a promising model for preclinical studies treating colorectal peritoneal metastases [38]. Metastasis organoids are also promising preclinical models for exploring drug combinations. For example, Ubink used organoids from colorectal peritoneal metastases as a platform for improving hyperthermic intraperitoneal chemotherapy (HIPEC) and found that ataxia telangiectasia and Rad3-related protein kinase (ATR) inhibition could increase the sensitivity of all peritoneal metastasis-derived organoids to mitomycin C (MMC), the most commonly used drug for HIPEC [66]. Moreover, CRC liver metastasis organoids can develop chemotherapy resistance after multiple rounds of chemotherapy, indicating their potential as a platform for studying chemoresistance [75].

Targeted therapy is more effective and less toxic than traditional chemotherapy, but cancer cells can sometimes become resistant to drugs over time [90]. Additionally, not all cancer types have a clear molecular target that can be targeted by these drugs [56,77], limiting the potential applications of targeted therapy. The use of organoids can supplement the inaccuracy of molecular profiling in predicting the effectiveness of targeted therapy. Mutations in the RAS pathway can be used as biomarkers to select patients for anti-EGFR therapy. Organoids can be used to predict the drug response based on the mutation status, but not all predictions are accurate [38]. Hugo et al. [72] observed the expected sensitivity of organoids harboring wild-type (WT) KRAS to afatinib and the insensitivity of KRAS mutant organoids. Metastasis organoids from breast cancer (BC) patients with high levels of the gene signature associated with BRCA mutations were sensitive to drugs called PARPis, while those with low levels of the signature were not [67]. However, organoids cannot always achieve the corresponding expectations based on their driver gene mutation status [58]. Organoids can also be used to identify new therapeutic targets, such as ERBB2, for colorectal cancer treatment. Research has shown that organoids with ERBB2 amplification derived from liver metastasis strongly respond to lapatinib, a dual ERBB2/EGFR inhibitor, while organoids with EGFR amplification but without ERBB2 amplification do not respond [38]. Organoids can also be used to identify new therapeutic targets, such as ERBB2 for colorectal cancer. Research has shown that organoids with ERBB2 amplification derived from liver metastasis strongly respond to lapatinib, a dual ERBB2/EGFR inhibitor, while organoids with EGFR amplification but without ERBB2 amplification do not respond [38]. This outcome once again confirms that lapatinib mainly targets ERBB2 and suggests that ERBB2 could be a promising therapeutic target for colorectal cancer [91]. Overall, a large drug screen has 100 % sensitivity, 93 % specificity, 88 % positive predictive value, and 100 % negative predictive value for predicting the responses of patients with metastatic gastrointestinal cancer [38]. These findings suggest that PDOs have the potential to predict clinical outcomes more accurately than molecular pathology alone, which underscores the value of PDOs in personalized cancer treatment.

Prostate cancer is the second most common malignancy and a major cause of cancer-related death in men worldwide [92]. However, propagating prostate cancer cells in vitro has been challenging, which limits the potential of preclinical models [58]. Organoids derived from CRPC cells are promising therapeutic agents. Androgen deprivation therapy (ADT) is a standard treatment for patients with locally advanced disease or metastatic PCa, but the cancer can become insensitive to ADT, leading to disease progression or relapse of CRPC. Reactivation of AR signaling is a key mechanism of CRPC [93]. Enzalutamide is a second-generation AR inhibitor that can improve progression-free survival (PFS) and overall survival (OS) in men with CRPC but has limited use as a preclinical predictor [94,95]. Studies of organoids derived from CRPC have shown that AR-amplified organoids are sensitive to enzalutamide, whereas organoids without AR amplification are resistant [58]. Organoids harboring both PTEN loss and PIK3R1 mutation are sensitive to two PI3-kinase pathway inhibitors, everolimus and BKM [58]. Furthermore, everolimus significantly increases the tumor response to enzalutamide, which may point to a new agent combination method for treating CRPC [58]. CHD1 loss sensitizes prostate cancer to DNA damage therapy, and organoids derived from metastatic CRPC achieve similar sensitivity to olaparib and carboplatin ex vivo as the patient's response in vivo [88]. This result provides a rationale for treating patients with CRPC harboring CHD1 loss in prospective clinical trials of PARP inhibitors or DNA damaging agents. These results indicate that organoids provide opportunities for exploring drug combinations for CRPC treatment.

Brain metastases are a frequent consequence of various cancer types, but patient-derived brain metastases are notoriously challenging to culture in vitro using traditional methods [96]. Recently, organoids derived from brain metastases have successfully exhibited growth and invasion abilities [78]. Organoids thus provide opportunities to explore the mechanisms and therapeutic potential of brain metastases. Additionally, organoids derived from metastatic gastric cancer have been used as a model to study tumor evolution and heterogeneity and predict drug sensitivity [32]. Organoids derived from metastatic BC have been shown to successfully simulate patients' responses to endocrine therapy, such as tamoxifen, providing an opportunity for in vitro drug response testing [58]. Overall, the use of PDOs for drug screening in current trials has shown 100 % sensitivity, 88 % specificity, 89 % positive predictive value (PPV), and 100 % negative predictive value (NPV) in predicting the response to chemotherapy, endocrine therapy, or targeted therapy in patients (as summarized in Table 4). This high predictive value suggests that PDOs could be a valuable tool for drug screening, enabling the more accurate and efficient selection of treatments for individual patients. And organoids have been a disease

model to evaluate drug response in more basic research [97]. The use of PDOs in drug screening may help to accelerate the development of effective cancer therapies and improve patient outcomes.

2.3. Limitations and perspectives

In this review, we discuss the current state of organoids derived from metastatic cancer and their potential applications in various areas such as modeling cancer heterogeneity [32], exploring novel drug combinations [58], and predicting therapeutic responses [38,69]. However, despite significant efforts, the field is still in its early stages and several challenges remain.

One of the main limitations is the lack of mature culture protocols for metastasis organoids. Although current studies suggest that culture conditions may be similar to those for primary cancer, it is challenging to culture organoids from patients' metastases when the primary cancer is unknown [69]. Fine needle biopsy can be used to identify cancer types, but the small amount of tissue obtained from these biopsies poses a challenge [58]. Establishing and expanding organoids from these biopsies for pathological diagnosis and genetic assessments would benefit patients with a cancer of an unknown primary origin [98].

Another challenge associated with culturing metastasis organoids is the potential overgrowth of fast-growing normal epithelial cells, which can replace cancer cells [58]. One approach to address the potential replacement of cancer cells with fast-growing normal epithelial cells is to remove specific niche factors that are crucial for normal cells but not necessary for cancer cells [57]. Furthermore, most metastasis organoids develop from epithelial cancers, and few develop from sarcomas, which have a low mutation burden [99]. This characteristic can make it challenging to establish organoids from sarcomas by selectively removing niche factors [69]. Further research is needed to develop novel techniques that can specifically distinguish cancer cells from mesenchymal cells and establish organoids from sarcomas.

Furthermore, the ability of organoids to fully mimic the complexity of in vivo tissues and organs is still limited, as they lack many of the diverse cell types and extracellular matrix components present in native tissues [100]. Their size is also limited due to the lack of a vascular network [101]. Recent studies focusing on developing coculture systems incorporating additional cellular and microbial elements in organoids have shown promising potential [73,102]. Coculturing organoids with other cell types, such as fibroblasts, immune cells and even specific bacteria, could better mimic the complex tumor microenvironment.

Matrigel, a mouse-derived extracellular matrix substitute, has long been used to support organoids derived from various cancer types [103]. However, it exhibits substantial batch-to-batch variability and contains ill-defined and xenogeneic impurities that can unpredictably influence the organoid phenotype [104,105]. This unpredictability leads to a lack of reproducibility in cell culture experiments and prevents researchers from determining causative mechanisms of matrix-induced cancer cell behavior [106]. Moreover, its cost prohibits its use in large-scale drug screens and clinical use. Hence, chemically and mechanically well-defined natural and synthetic scaffolds are needed for organoid culture. Recently, synthetic scaffolds derived from polyacrylamide (PAM) and polyethylene glycol (PEG) have shown potential as alternatives to Matrigel [107,108]. The approaches used to customize synthetic scaffolds could result in materials that outperform naturally derived scaffolds [104].

Despite the limitations and challenges associated with organoids derived from metastatic cancers, they have emerged as highly relevant in vitro models for translational applications in studying cancer metastasis and predicting drug responses for the development of personalized cancer treatments. Recently, a study designed to predict drug responses using organoids from metastatic gastrointestinal cancer, which we referred to above, showed encouraging results [38]. Furthermore, extensive clinical trials are currently underway to evaluate the role of organoids as a platform for drug testing [109–111]. The potential dominance of organoids over PDXs and other factors, such as genetic alterations, as predictors of drug efficacy may be gradually confirmed.

3. Conclusions

Tumor-derived organoids have shown significant promise because they effectively replicate the morphology and genetics of the original tissue, establishing themselves as a highly pertinent model for translational research and the creation of personalized treatment approaches for metastatic cancers. The efficient generation and expansion of patient-derived organoids are crucial for facilitating drug screening within a clinically relevant timeframe. Furthermore, coculture with immune cells could lay the groundwork for future predictions of immunotherapy responses.

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CRediT authorship contribution statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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