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REVIEW ARTICLE

Cancer organoids: A platform in basic and translational research

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Abstract An accumulation of previous work has established organoids as good preclinical models of human tumors, facilitating translation from basic research to clinical practice. They are changing the paradigm of preclinical cancer research because they can recapitulate the heterogeneity and pathophysiology of human cancers and more closely approximate the complex tissue environment and structure found in clinical tumors than *in vitro* cell lines and animal models. However, the potential applications of cancer organoids remain to be comprehensively summarized. In the review, we firstly describe what is currently known about cancer organoid culture and then discuss in depth the basic mechanisms, including tumorigenesis and tumor metastasis, and describe recent advances in patient-derived tumor organoids (PDOs) for drug screening and immunological studies. Finally, the present challenges faced by organoid technology in clinical practice and its prospects are discussed. This review highlights that organoids may offer a novel therapeutic strategy for cancer research.

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Introduction

Cancer is a leading cause of global mortality with a huge impact on health, and social and economic development, necessitating progress toward preventive and treatment options. A total of 28.4 million cases are predicted by 2040 around the globe.[1] Therefore, the establishment of high-fidelity preclinical tumor models is crucial to investigate cancer-related mechanistic studies as well as allow personalized anti-cancer therapy in clinical.

Maintenance of tumor heterogeneity and mimicry of the microenvironment are challenges in establishing preclinical models. Cancer cell lines are limited in applications such as high-throughput drug screening due to the lack of tumor architecture and microenvironment.[2] Mouse models better mimic the *in vivo* situation but have low success rates, long generation cycles, high costs, and dubious clinical applications. Therefore, a three-dimensional (3D) cell culture technique producing "organoids" has been developed.[3] Organoids are multicellular clusters with the *in vitro* capacity for self-renewal and proliferation while maintaining the physiological structure and function of the tissue from which they are derived.[4] Compared with the traditional model, cancer organoids have their unique advantages, including a better ability to simulate the physiological and pathological state of tumor organs, moderate cost, and better combination with some emerging technologies. In addition, compared with traditional 2D cell culture, this 3D model maintains the mutation pattern without genetic changes during long-term culture, which makes it more suitable for studying dynamic processes, such as tumor development. These 3D constructs represent a promising, near-physiological model for human cancers, and exhibit enormous potential for translational studies of solid tumors.[5,6] Thus, our review will discuss various cancer organoid culture strategies and summarize the recent advances in tumor organoids from the basic mechanism and clinical application. Finally, the limitations of this emerging technology and future developments are discussed.

Establishment of organoids

Organoids are miniaturized *in vitro* organ models developed from stem cells or tumor tissues extracted from patients and grown in a specific 3D *in vitro* microenvironment. Organoids mimic the characteristics of real organs *in vivo* and 3D culture systems may be stably expanded.[7] Tissue may be enzymatically and/or mechanically digested and cultured on a basement membrane (BME) or at the air-liquid interface, using Transwell inserts.[8–10] Organoids may be passaged every 1–2 weeks. Crucially, the media in which organoids are cultured varies, often depending on the tissue of origin. For example, small intestinal organoids cultured with epidermal growth factor (EGF), R-spondin, and noggin, while colonic organoids require nicotinamide, the p38 inhibitor SB202190, prostaglandin E2, and the TFG-β inhibitor A83-014.[8]

Cancer organoids (Fig. 1) may be generated through differentiation of iPSCs or directly derived from tumor tissues,[5,11] such as patient surgical specimens or needle biopsies.[12] Gao et al established metastatic prostate cancer 3D organoid cultures from patient biopsies and circulating tumor cells (CTCs).[13] Cancer organoids derived from primary and/or metastatic samples of breast,[14,15] bladder,[16] colons,[17,18] esophagus,[19] stomachs,[20] gallbladders,[21,22] head and neck,[23–25] kidney,[26,27] liver,[28,29] lung,[30,31] ovary,[32,33] pancreas,[34,35] and prostate[13,36] cancers have been described (Table 1). Cancer organoids vary in their growth rate and size.[12] This depends on the culture system but is also influenced by the viability and amount of necrosis of the original tumor tissue, whether samples were taken pre- or posttreatment, and the sample processing time and technique. As with other organoids, PDOs retain many histological, transcriptional, and genetic

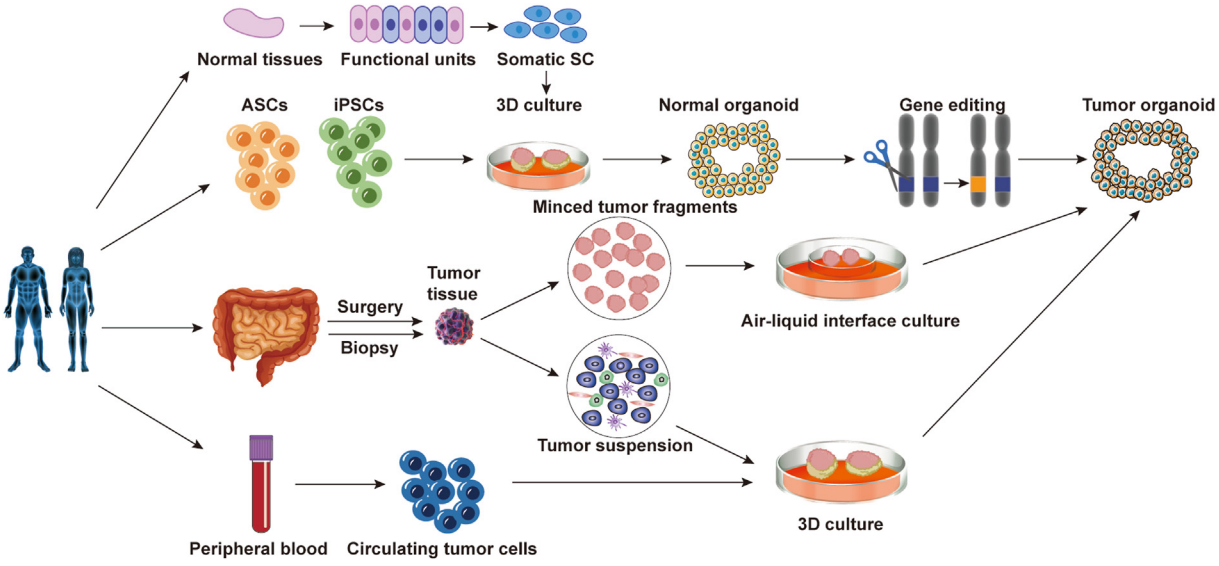

Figure 1 Schematic representation of patient-derived tumor organoids. Patient-derived tumor organoids from surgically resected/biopsied tissues and circulating tumor cells. Non-tumor organoids may be mutated into tumor organoids after gene editing.

Table 1 Culture systems of different cancer organoid models.

Tumoroid type	Extracellular matrix	culture components			Rf.
		Growth factors	Small Molecules	Molecule inhibitors	
Breast cancer	Basement membrane extract	Advanced DMEM/F12 Wnt3A EGF FGF-7/FGF-10 Noggin R-spondin 1	nicotinamide N-acetylcysteine B27 primocine neuregulin 1	A83-01 SB202190 Y-27632	14,15
Bladder cancer	Matrigel or Basement membrane extract	Advanced DMEM/F12 FGF-7/FGF-10/ FGF-2	nicotinamide N-acetylcysteine B27	A83-01 Y-27632	16
Colorectal cancer	Matrigel	Advanced DMEM/F12 Wnt3A EGF FGF-10 Noggin R-spondin 1	nicotinamide PGE2 Gastrin N-acetylcysteine B27	A83-01 SB202190 Y-27632	17,18
Esophageal cancer	BME-2	Advanced DMEM/F12 Wnt3A EGF FGF-10 Noggin R-spondin 1	nicotinamide Gastrin N-acetylcysteine B27	A83-01 SB202190 Y-27632	19
Gastric cancer	Matrigel	Advanced DMEM/F12 Wnt3A EGF FGF-10 Noggin R-spondin 1	nicotinamide Gastrin N-acetylcysteine B27	A83-01 Y-27632	20
Gallbladder cancer	Matrigel	Advanced DMEM/F12 EGF FGF-10 IGF HGF Noggin	nicotinamide Gastrin N-acetylcysteine B27 Forskolin N2 Dexamethasone Primocin	A83-01 Y-27632	21,22
Glioblastoma	Matrigel	DMEM/F12 EGF/bEGF Noggin R-spondin 1	nicotinamide PGE2 Gastrin	CHIR99021 A83-01 SB202190 Y-27632	23,24
Head and neck squamous cell carcinoma	Matrigel	Advanced DMEM/F12 EGF FGF-10/FGF-2 Noggin R-spondin 1	nicotinamide PGE2 N-acetylcysteine B27 Forskolin	CHIR99021 A83-01	25
Kidney cancer	Growth factor-reduced Matrigel or Basement membrane extract	Advanced DMEM/F12 EGF FGF-10 R-spondin 1	N-acetylcysteine B27 Primocin	A83-01 Y-27632	26,27
Liver cancer	Matrigel or Basement membrane extract	Advanced DMEM/F12 Wnt3A EGF, HGF FGF-10 Noggin R-spondin 1	nicotinamide Gastrin N-acetylcysteine B27 Forskolin Dexamethasone	A83-01 Y-27632	28,29
Lung cancer	Matrigel	MBM Wnt3A EGF FGF-4/FGF-7/FGF-10 Noggin R-spondin 1	/	A83-01	30,31
Ovarian cancer	Matrigel	Advanced DMEM/F12 EGF FGF-10 Noggin	nicotinamide N-acetylcysteine B27 forskolin primocine	A83-01 Y-27632	32,33

		R-spondin 1 BMP-4	Heregulinβ-1		
Pancreatic cancer	Matrigel	Advanced DMEM/F12 Wnt3A EGF FGF-10 Noggin R-spondin 1	nicotinamide PGE2 Gastrin N-acetylcysteine B27	A83-01 Y-27632	[34,35]
Prostate cancer	Matrigel	Advanced DMEM/F12 EGF,HGF FGF-2/FGF-10 Noggin R-spondin 1	nicotinamide PGE2 N-acetylcysteine B27 Dihydrotestosterone	A83-01 SB202190 Y-27632	[13,36]

A83—01: Transforming growth factor-beta inhibitor which suppresses organoid proliferation; BME-2: Soluble basement membrane purified from Engelbreth-Holm-Swarm (EHS) tumor; contains laminin, collagen IV, entactin, and heparin sulphate proteoglycan; EGF: Binds EGF receptor to induce cancer cell proliferation; FGFs: A group of pluripotent and promiscuous growth factors; Gastrin: Stimulates proliferation and suppresses apoptosis of cancer cells; HGF: HGF/Met signaling promotes oncogenesis, tumor angiogenesis, and invasion; Nicotinamide: Vitamin B3 required for the long-term culture of organoids; Noggin: Inhibits bone morphogenetic proteins and modulates cellular differentiation, proliferation, and apoptosis; promotes bone metastasis of some cancers and is associated with tumorigenesis of primary bone malignancies; PGE2: Promotes angiogenesis in gastric cancer through up-regulation of vascular endothelial growth factor; R-spondin 1: Binds Lgr5 and a niche factor required for self-renewal of stem cells and activates Wnt signaling; facilitates growth and metastasis of cancer cells; SB202190: p38 inhibitor which suppresses the proliferation and migration of cancer cells; Wnt: Master regulator of cell development, proliferation, differentiation, adhesion, and polarity; aberrant Wnt signaling promotes carcinogenesis and cancer progression; Y-27632: Rho kinase inhibitor that reduces anoikis of dissociated stem cells, improves culture media, and promotes proliferation of tumor epithelial cells.

characteristics of the original tumor, maintaining the high heterogeneity of tumor cells in a simple and time-efficient culture system.[37,38] However, culture stability may vary depending on tumor type and culture conditions. Therefore, characterizing and tracking the genotype and phenotype of organoids over time is essential to avoid culturing only one or a few dominant clones from heterogeneous tumor tissue and to ensure that the model represents the original tumor.

Applications of organoids in various cancers

Organoids are suitable preclinical models due to their ability to recapitulate the genotype, phenotype, and cellular features of their parent tissues. Some applications of patient-isolated organoids are listed in Figure 2.

Breast cancer

Dekkers et al described a protocol for long-term culture of normal human breast and breast cancer organoids from clinical samples[15] and a diverse biobank of normal and breast cancer PDOs with a focus on triple-negative breast cancer (TNBC) has been compiled.[39] In addition, organoids have been used to study rare cancers, such as adenomyoepithelioma,[40] papillary carcinomas,[41] Paget's disease,[42] and TNBC with malignant pleural effusion,[43] and genes mimicking neoplasia have been knocked out by CRISPR/Cas9 in organoids for transplant into mice.[44] Furthermore, patient-specific drug sensitivity may be evaluated *in vitro* organoids to guide treatment.[45,46] Other applications include the identification of agents that reverse the epithelial–mesenchymal transition[47] and response to oncolytic agents for testing of oncolytic viruses.[48]

Bladder cancer

The heterogeneity of bladder cancer causes a high mutation rate, high risk of recurrence, and poor prognosis. Culture systems for bladder cancer organoids have been mentioned in many studies.[16,49,50] Mullonders et al created a live biobank of bladder cancer organoids from 53 patients in which common bladder cancer mutations were identified.[16] In addition, Kong's team used organoid models to identify biomarkers that accurately predict responses to chemotherapy in 77 bladder cancer patients.[51] Yu et al used organoids to assess chimeric antigen receptor (CAR) T cell-mediated cytotoxicity against bladder cancer.[52]

Colorectal cancer

Several groups have successfully established organoids for colorectal cancer (CRC).[53–68] For example, Zhao et al used organoid models to show that cancer stem cells (CSCs) and differentiated cancer cells (non-CSCs) have different metabolic phenotypes. Lactate derived from non-CSCs promoted self-renewal of CSCs, thereby promoting CRC progression.[55]Another study modeled the progression of colorectal tumors and metastases using paired PDOs and found that organoids from metastatic sites exhibited more tumorigenic and metastatic abilities than those from

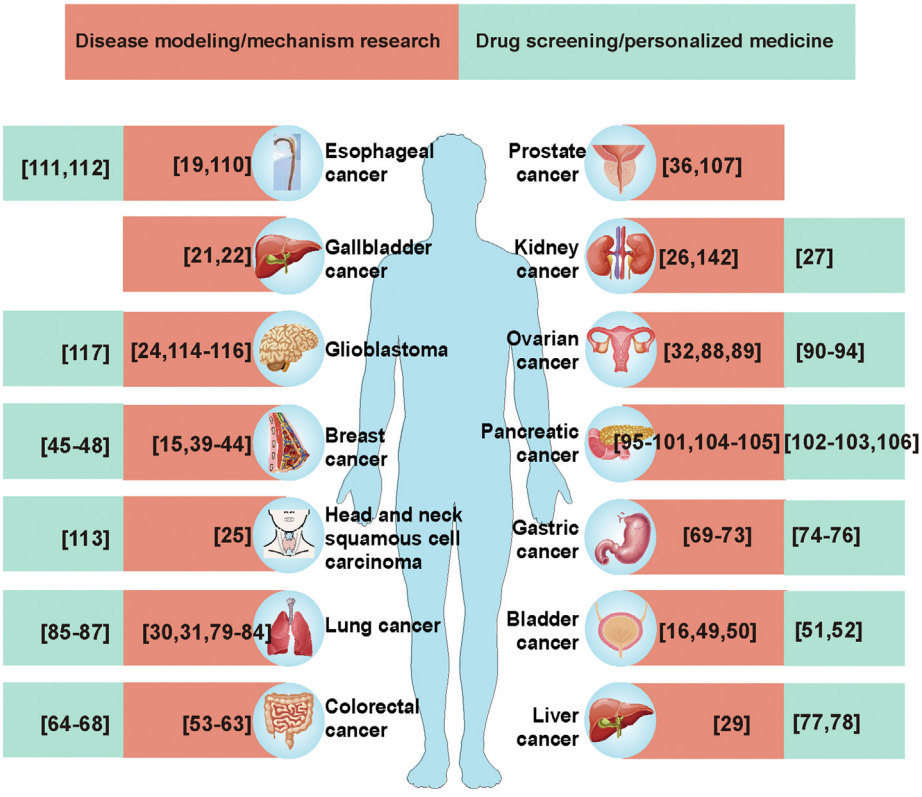

Figure 2 Current applications of various cancer organoid models (references in brackets).

primary lesions.[54] Organoids have also been used to find more targeted therapies for CRC. Bruun et al developed an *in vitro* pharmacogenomic profiling platform using PDOs and found it useful for drug screening to find new therapeutic strategies for metastatic colorectal cancer.[59] Furthermore, the use of locally advanced rectal cancer organoids has demonstrated a strong matching of response to chemoradiotherapy between patients and organoids with an accuracy of 84.43%, a sensitivity of 78.01%, and a specificity of 91.97%.[64]

Gastric cancer

Several studies have described procedures for creating gastric cancer organoids from clinical samples and have been successfully used for mechanistic studies of drug resistance.[69–74] Ukai et al[69] established 5-FU-resistant gastric cancer organoids and Harada et al[70] established oxaliplatin-resistant gastric cancer organoids to explore the mechanism of gastric cancer resistance. In addition, several groups have developed organoid models of *H. pylori* infection for mechanistic studies of *H. pylori*-induced gastric cancer.[75] Organoids have also been used to study the more uncommon gastric cancers, such as Signet ring cell carcinoma.[76]

Liver cancer

Broutier et al first established primary liver cancer organoids from eight surgically resected hepatocellular carcinoma (HCC), cholangiocarcinoma (CC), and combined HCC-CC subtypes in 2017.[29] Resistance to chemotherapy is common in all types of cancer while organoids of liver cancer have been shown to help identify mechanisms of resistance to anticancer drugs.[77] Furthermore, Liu et al established 3D co-culture models of primary liver tumor-derived organoids with CAF to understand their interaction and response to chemotherapeutic agents.[78]

Lung cancer

Biobanks of lung cancer organoids and normal bronchial organoids have been established from primary lung cancer tissues, including adenocarcinoma,[79–82] squamous cell carcinoma,[30] small cell carcinoma,[83] large cell carcinoma,[31] and adenosquamous carcinoma,[30] as well as paired non-neoplastic airway tissues.[84] In addition, Dijkstra et al found a 17% rate of pure non-small cell lung cancer (NSCLC) organoid formation by assessing samples from >70 NSCLC samples, indicating that the methods of establishing pure NSCLC organoids from intrapulmonary lesions require improvement.[85] Lung cancer organoid models can also accurately select anticancer drugs based on drug sensitivity to provide a powerful supplement and validation for gene sequencing.[86,87]

Ovarian cancer

Kopper et al established 56 organoids from 32 patients representing all major subtypes of ovarian cancer, including serous borderline tumor (BT), mucinous BT, low-grade serous BT, mucinous carcinoma, endometrioid carcinoma, clear cell carcinoma, and high-grade serous subtype.[32] In addition, high-grade serous ovarian cancer organoids require a low Wnt environment for long-term growth.[88,89] Some studies have shown that PDOs are physiologically appropriate *in vitro* tumor drug screening models and can use effective personalized drugs to target ovarian cancer.[90–94] De Witte et al assessed the ability of PDOs to predict functional consequences of clinical drug response and tumor heterogeneity and showed that PDO drug screening identified 88% of patients as highly responsive to at least one drug.[92]

Pancreatic cancer

Pancreatic cancer is characterized by elevated intra-tumoral and intertumoral heterogeneity and a highly malignant phenotype which makes it difficult to establish organoids. Pancreatic ductal organoids are an *in vitro* model of pancreatic ductal adenocarcinoma (PDAC) and can be investigated in patients with local, advanced, and malignant metastases by surgical, fine needle aspiration of ascites specimens.[95–103] Furthermore, Huang et al[104] and Beato et al[105] established the intraductal papillary mucinous neoplasms (IPMNs) organoid biobank to investigate the genetic and biological mechanisms of IPMNs. Another study achieved rapid molecular profiling and drug detection by generating PDOs from free DNA which has implications for clinical practice.[106] Briefly, PDOs can mimic the response of primary tumors to drug therapy and therefore have the potential for translational research and drug discovery.

Prostate cancer

Human prostate cancer organoids have also been created. Karkampouna et al developed prostate cancer models with specific biological and genetic landscapes that can be used to investigate tumor growth, metastasis, and drug resistance in the early stages of the disease and to assess response to chemotherapy.[36] Servant et al developed a biobank that included prostate cancer organoids from 81 patients for the construction of stable cell lines.[107] In addition, the use of other biotechnologies in combination with prostate cancer organoids has helped to better investigate the complexity of tumor pathogenesis as well as integrate the tumor microenvironment.[108,109]

Other cancer

In addition to the organoids described in detail above, other organoids have also developed well in recent years. For example, some studies have shown that specific, targeted therapy for individual patients can be guided by PDO produced in biopsies from patients with esophageal cancer, including esophageal adenocarcinoma and esophageal squamous cell carcinoma.[110–112] The head and neck squamous cell carcinoma (HNSCC) organoids established by Driehuis et al have been used to assess the efficacy of targeted photodynamic therapy for HNSCC.[113] Many methods for generating patient-derived glioblastoma organoids have been established.[114–117] Patient-derived glioblastoma organoids have been demonstrated to

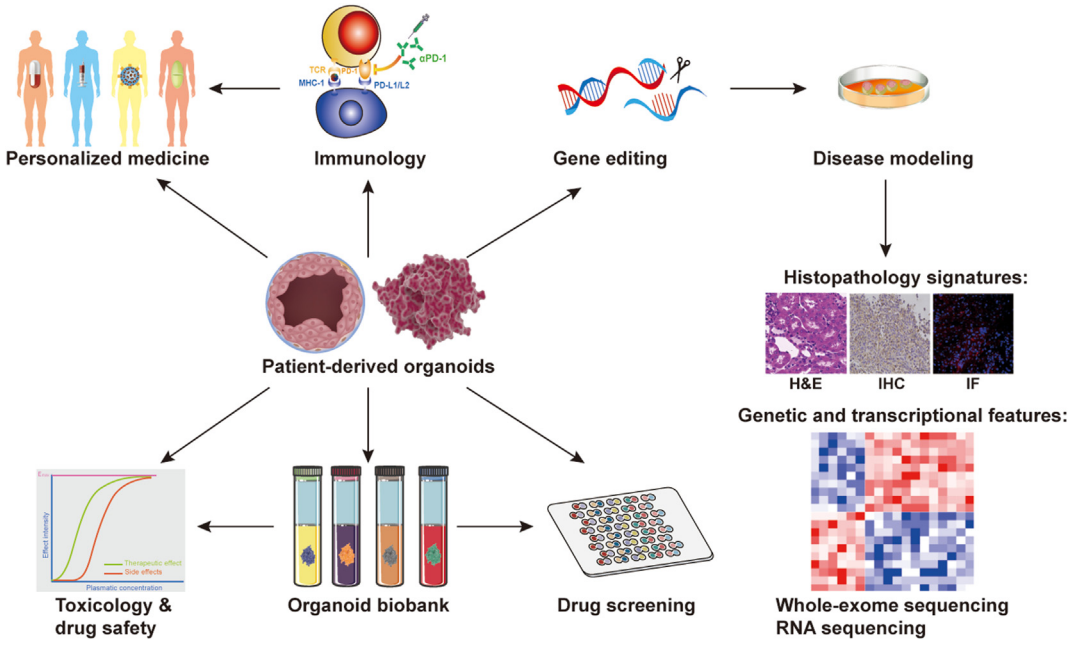

Figure 3 Application of organoids in cancer research. Organoid technology combined with CRISPR/Cas9 gene editing allows the impact of genetic changes on disease development to be studied. Organoid biobanks can be used for drug screening and validation. Patient-derived tumor organoids co-culture with immune cells mimics the mechanism of immunotherapy efficiency and drug resistance. In addition, tumor organoid models may become a tool for personalized medicine validation to optimize treatment selection.

recapitulate key features of the tissue of origin, including their histological features, cellular diversity, gene expression patterns, and mutation profiles.[24]

In brief, organoids have been applied (Fig. 3) to disease model construction, tumor mechanisms, drug screening, testing cancer immunotherapy, and developing new methods for personalized medicine.

Tumor mechanisms

Tumorigenesis

Tumorigenesis is characterized by multistep genetic alterations that lead to the inactivation of tumor suppressor genes and activation of oncogenes which together drive cancer growth.[118,119] Introduction of targeted mutations by CRISPR-Cas9 allows tumorigenesis to be mimicked.[120] Colorectal cancer (CRC) may be mimicked by the CRISPR-Cas9 introduced mutations of KRAS, Apc, p53, and Smad4.[121,122] Organoids with activating mutations in KRAS ($KRAS^{G12D}$) and inactivating mutations in Apc, TP53, and Smad4 grow independently of the stem cell growth factors, EGF, WNT, R-spondin1, and noggin. Primary colonic organoids may be transformed into adenocarcinoid tumors via mutation of Apc, p53, $Kras^{G12D}$, and Smad4, and gastric and pancreatic organoids transformed by p53 loss, $Kras^{G12D}$expression, or both.[10] Naruse et al used four genotoxic chemicals, ethylmethanesulfonate (EMS), acrylamide (AA), diethylnitrosamine (DEN), and 7,12-dimethylbenz [a] anthracene (DMBA) to study chemical carcinogenesis.[123] DMBA-treated mammary tissue-derived organoids with a heterozygous knockout of Trp53 showed tumorigenicity while organoids with wild-type Trp53 did not, consistent with previous reports on corresponding mouse models. Pulmonary organoids with or without Trp53 knockout developed oncogenic histopathological features with oncogenic kinase activity in subcutaneous nodules when treated with EMS or AA and similar changes were found in hepatic organoids treated with DEN. Tumor organoid technology has also been used to investigate pathways involved during tumorigenesis. Mouse colonic organoids have been used to demonstrate that aryl hydrocarbon receptor (AhR) signaling regulates the IL22 response by regulating SOCS3 expression, validating a rationale for using AhR to reduce colon cancer risk.[124] lnc-RP11-536 K7.3 was shown to promote colon cancer (CC) progression in CC-derived organoids through the SOX2/USP7/HIF-1α signaling axis, revealing regulation of chemosensitivity and exposing therapeutic targets related to lncRNAs.[63] All these results demonstrate the utility of gene-editing organoid systems in validating driver pathway mutations in tumorigenesis, thus providing a flexible *in vitro* cancer model for the study of tumorigenesis.

Although CRISPR-Cas9 has been successfully used in organoids, precise integration of exogenous DNA sequences into organoids is lacking robust knock-in approaches. CRISPR-Cas9-mediated homology-independent organoid transgenesis (CRISPR-HOT) enables knock-in human organoids representing different tissues to be generated.[125] CRISPR-HOT simplifies gene editing and has been used to introduce fluorescent labeling of reporter gene products for subcellular visualization in intestinal cells. Moreover, CRISPR-HOT has been used to generate human liver ductal organoids and human fetal hepatocyte organoids within 2—3 months.[126] The application of CRISPR-HOT to organoid

Table 2 Cancer organoid biobanks.

Tumor type	Source		Success rate (%)	Achievement	Rf.
	Type	Quantity			
Breast cancer	Ductal adenocarcinoma, lobular adenocarcinoma	100 patients	>80	Used for cancer research, drug development, and to assess personalized *in vitro* drug responses.	[14]
	invasive ductal carcinoma, invasive lobular carcinoma	33 patients	87.5	New protocol for obtaining patient-derived oganoids from breast cancer	[197]
Bladder Cancer	Urothelial carcinoma Squamous-cell carcinoma	16 patients	70	*In vitro* model of tumor evolution and therapeutic response to precision cancer medicine.	[198]
	Primary tumor	50 patients	50	Bladder organoids biobank for drug testing in the future.	[16]
Colorectal cancer	Primary tumor	20 patients	90	Tumor organoids may fill the gap between cancer genetics and patient trials for drug research as well as personalized therapy.	[17]
	Primary tumor	43 patients	100	Functional links between genetic alterations, niche requirements and biological phenotypes of tumors, providing a multifunctional platform for biomedical research.	[18]
	Metastases	14 patients	71	Personalized screening tool using patient-derived tumor organoids.	[37]
Esophageal cancer	Oesophageal squamous-cell carcinoma, Oropharyngeal squamous- cell carcinoma	21 patients	71.4	Platform to analyze cancer cell heterogeneity, assess personalized drug treatment response and treatment resistance.	[110]
Gastric cancer	Normal, dysplastic, cancer, and lymph node metastases	34 patients	>50 (tumor)/ >90 (normal)	Identified potential targeted drugs to guide patient drug selection.	[199]
Glioblastoma	IDH1 mutant tumors, recurrent tumors	53 patients	91.4	The establishment a large cohort of unique organoids and patient-derived orthotopic xenografts of various glioma subtypes.	[24]
	IDH1 mutant IV glioblastoma (GBM), IDH1 mutant II-III gliomas	173 patients	79/68	The establishment a large cohort of unique organoids and patient-derived orthotopic xenografts of various glioma subtypes	[117]
Head and neck squamous cell carcinoma	Primary tumor	40 patients	65	Comparison of organoids with normal epithelium applied to *in vitro* drug screening.	[25]
Kidney cancer	Wilms tumors, malignant rhabdoid tumors (MRTK), renal cell carcinomas (RCC), and congenital mesoblastic nephromas	50 children	100(normal)/ 75 (Wilms tumors)/ 100(MRTK)/75(RCC)	Captures heterogeneity of pediatric renal tumors; well-characterized model for basic cancer research, drug screening and personalized medicine.	[27]
Lung cancer	Non-small cell lung cancer	14 patients	71.43	A living biobank of patient-derived organoids from non-small cell lung cancer patients was established.	[200]
	adenocarcinoma, squamous cell carcinoma, adenosquamous carcinoma, large cell carcinoma, and small cell carcinoma	36 patients	87	Successfully construct biobank of lung cancer organoids	[30]

(*continued on next page*)

Table 2 (*continued*)

Tumor type	Source		Success rate (%)	Achievement	Rf.
	Type	Quantity			
Liver cancer	Hepatocellular carcinoma	10 patients	26	Hepatocellular carcinoma (HCC) organoids generated from needle biopsies of patients with liver cancer; response to sorafenib treatment.	201
ovarian cancer	Borderline tumors, Clear-cell carcinoma, Endometrioid carcinoma, Mucinous carcinoma, Serous carcinoma	32 patients	65	Ovarian cancer organoids can be used for drug-screening assays and different tumor subtype responses to platinum-based chemotherapy.	32
Pancreatic cancer	Ductal adenocarcinoma (primary tumor and Metastases)	138 patients	75	Predict drug responses in pancreatic cancer patients and provide a rational for prioritizing therapeutic regimens.	202
	pancreatic ductal adenocarcinoma (PDAC)	39 patients	/	Revealed functional heterogeneity in Wnt niche independence in PDAC.	203
	Intraductal papillary mucinous neoplasms (IPMNs)	8 patients	/	Potential drivers of IPMN tumor development were identified.	104
	Intraductal papillary mucinous neoplasms (IPMNs)	15 patients	81(tumor)/87(normal)	The mechanism of IPMN invasion was revealed.	105
Prostate Cancer	Adenocarcinoma metastases Circulating tumor cells	7 patients	15—20	Recapitulated the molecular diversity of prostate cancer subtypes, providing an *in vitro* model for understanding disease pathogenesis and response to therapy.	13
Neuroendocrine tumors	Gastroenteropancreatic (GEP) neuroendocrine neoplasm (NEN)	39 patients	/	Understanding of GEP-NEN and its genetic and biological phenotypes.	204

construction allows universal reproduction of the process of tumorigenesis.

Tumor metastasis

Cancer metastasis involves the spread of cancer cells from the primary site to other organs and is the leading cause of death in cancer patients.[127] Metastasis is a complex molecular process and maintenance of tumor pathophysiological features by organoids renders this a suitable platform for study. For example, Fujii et al generated four independently matched sets of organoids for primary colorectal cancer and metastatic lesions. Exome sequencing confirmed that they share similar genetic profiles and niche factor requirements and metastasis-derived organoids exhibit higher metastatic capacity.[18] Cancer organoids can also help identify critical targets that inhibit tumor metastasis. SOX4 has been shown to promote the maintenance of an undifferentiated and proliferative state in breast cancer organoids. Compared to SOX4-positive breast cancer organoids, SOX4-knockdown breast cancer organoids contained more differentiated cells, had intraluminal or basal gene expression patterns and lower levels of cell cycle genes, and showed the impaired ability of tumor metastasis and growth.[128] Another study revealed that SOX2 was identified as having pro-metastatic activity from primary tumor-derived organoids matched with liver metastases of CRC patients. Thus, SOX2 is thought to be associated with CRC invasion and proliferation as well as liver metastasis.[54] In addition, organoids were generated from primary and metastatic breast cancer tissues and accurately replicated histopathology, hormone receptor status, HER2 status, and DNA copy number changes in a previous study conducted by Sachs N et al.[14] This suggests that organoids can accurately mimic native as well as metastatic tumors and provide a better understanding of cancer biology. In short, cancer organoids provide an effective tumor model to investigate the mechanisms of promotion and inhibition of tumor invasion.

Drug assays

Most cytotoxic agents are more effective in tumor cell lines than in patients, in whom tumor responsiveness varies greatly according to tumor type.[129] Voskoglou-Nomikos et al established that cell line *in vitro* models predict cytotoxic responses of non-small cell lung cancer but not of colon cancer under a disease-oriented approach.[130] Organoids are closer to physiological structures and more realistically reproduce drug responses filling a gap between drug screening and clinical trials. Several tumor organoid biobanks (Table 2) have been established for the identification and testing of new drugs and toxicology assessments.[14,16,18,30,32]

Drug efficacy testing

As an *in vitro* individualized preclinical model, organoids have great potential in susceptibility testing of individualized drugs. Organoids from metastatic gastrointestinal cancer (colorectal and gastroesophageal) have been tested for their ability to predict a patient's treatment response. It accurately reproduced the patient treatment response of gastrointestinal cancer with high sensitivity (100%), specificity (93%), positive predictive value (88%), and negative predictive value (100%).[131] Li et al used signet ring cell carcinoma (SRCC) and non-SRCC organoids to observe treatment responses to 5-fluorouracil, oxaliplatin, docetaxel, and irinotecan. The SRCC organoid system had a lower IC_{50} value for docetaxel than the non-SRCC. No further differences were found between the two types of organoids for other drugs.[76] The sensitivity of 40 drugs and gene expression profiles of 38 CRC liver metastases from 22 patients were tested using PDOs. Sensitivity to several anticancer drugs and antimetabolites that have not previously been used to treat CRC was revealed while some drugs without clear genomic markers (alisetidine b and navitoclax) showed heterogeneity in anticancer activity.[59] In addition, cancer organoids are also effective tools to interrogate gene—drug associations. A study showed that TP53 mutant organoids were insensitive to nutlin-3a in the biliary tract and CRC organoids.[132] This result is consistent with clinical findings in cancer patients with TP53 mutations. Cancer organoids have also facilitated research into mechanisms of therapeutic resistance.[30,70,133,134] Among oxaliplatin-resistant GCOs, the presence of myoferlin was shown to be strongly associated with the acquisition of oxaliplatin resistance.[70] In another study, expression of atypical cyclin P was found to promote the stem-like phenotype of intestinal cancer organoids, which often leads to tumor recurrence, metastasis, and treatment resistance.[134]

Personalized treatment options have been explored via organoid predictions. Combination therapy with RAS pathway inhibitors and drugs (HER2 inhibitor selafatinib, MEK inhibitor selumetinib, and ERK inhibitor SCH772984) in CRC organoids with wild-type or mutant RAS were evaluated and further oncogenic mutations were introduced by CRISPR. The combination of afatinib with selumetinib and SCH772984 with selumetinib both inhibited mutant organoid growths, demonstrating the potential of patient-derived CRC organoid repertoires for preclinical assessment of inhibitors and drug combinations.[135] A blinded study cultured 77 samples from 57 patients with stage IV colorectal cancer to generate organoids and evaluate the predictive accuracy of chemotherapy responses. Organoids and metastatic tissues were very consistent with the original cancer tissues, preserving histological features and marker expression. The sensitivity, specificity, and precision of patient-derived tumor organoid models for chemotherapy response prediction were 63.33%, 94.12%, and 79.69%, respectively.[136] These studies have shown that for patients, organoid sensitivity testing technology can quickly detect the most appropriate drugs, develop the best and most effective drug treatment regimen, and reduce the probability of drug side effects, drug resistance, and tumor recurrence, so as to obtain the best treatment.

Cancer organoid biobanks can serve as critical models for new drug discovery. This may shorten the preclinical trial cycle, reduce development costs and risks, improve the success rate, and facilitate drug discovery. Plocabulin is a novel microtubule-disrupting antitumor agent of marine origin currently undergoing phase II clinical trials. Costales-Carrera et al used 3D tumor organoids from 3 CRC patients to show that plocabulin is strongly cytotoxic to CRC.[137] Organoids represent a reproducible platform for personalized drug screening which addresses inter- and intra-tumoral heterogeneity despite the small sample sizes of most studies to date although there is more work to be done before they are a predictive tool for clinical decision-making.

Drug toxicity testing

Tissue-derived organoids allow the selection of drugs that specifically target cancer cells but do not damage healthy cells. At present, general cell screening and animal model screening often fail to accurately predict adverse reactions in humans. The organoid model plays a vital role in drug toxicity screening. Indeed, hepatotoxicity, cardiotoxicity, and nephrotoxicity are the main causes of drug clinical trial failure. Liver organoids have been used to assess hepatotoxicity.[138—140] For example, Mekky et al used human liver organoids to test the toxicity of aspartic acid-coated magnesium oxide nanoparticles and valproate showing that these drugs decrease cell viability, decrease ATP, and increase reactive oxygen species in hepatocytes.[139] Similarly, cardiac organoids were used to demonstrate that hypoxic cardiac injury aggravates the cardiotoxicity of doxorubicin.[141] Cardiac organoids from mouse embryonic stem cell-derived embryoid bodies[142] and human iPSCs have also been used for drug testing,[143] as have iPSC-derived kidney organoids for nephrotoxicity screening.[144] These organoids provide patient-specific models for investigating the toxicity of anticancer drugs in personalized medicine.

Immunological studies

Immunotherapy involves tumor recognition by the immune system and initiation or enhancement of immune responses. Non-specific immunotherapy introduces adjuvants, such as cytokines or other cell signaling molecules, to enhance overall immune activity while specific immunotherapy induces antigen-specific immune responses to one or more tumor targets. Anti-cancer vaccines, dendritic cell therapy, or oncolytic viruses may be used. T-cell and antibody-based therapies administer immune system components to act directly on the tumor or enhance existing immunity.[145—147] However, most patients are insensitive to immunotherapy due to poor tumor immunogenicity and immune escape mediated by the tumor microenvironment.[148] Immunotherapy cannot simulate the complex tumor microenvironment of pericytes and fibroblasts, resident or infiltrating vascular structures (endothelial cells), and immune cells because cancer cell lines do not reproduce the heterogeneity of tumor epithelial cells and the mouse immune system is too different from the human. However, cancer organoids preserve the characteristics of

the human immune system and are gradually becoming a powerful tool for studying cancer immunotherapy.

Two conceptually different approaches have been described to construct the tumor microenvironment. (i) In reconstitution models, cancer organoids are cultured in an extracellular matrix, such as Matrigel or BME-2, and immersed in a tissue culture medium; exogenous immune cells, from autologous peripheral blood or tumors, are co-cultured with the organoids.[50,61,72,78,97,149] (ii) In the overall native organoid model, the innate immune microenvironment (endogenous immune cells and other non-epithelial cell types) of tumor specimens is preserved without reconstitution. Organotypic tumor spheroids (40—100 μm) have been cultured in a microfluidic device containing collagen for 5—9 days, preserving tumor cells and endogenous immune cells, such as lymphocytes and medullary cell populations.[150] Alternatively, primary tissue fragments containing tumor cells and immune components were embedded in collagen gels within Transwell dishes for air-liquid interface (ALI) culture. The top of the collagen gel is exposed to air, allowing the cells to obtain an adequate supply of oxygen. Many different primary tumors, including colon, lung, pancreas, and kidney have been cultured for up to 30 days. The complex histological tumor microenvironment is preserved with tumor parenchyma and stroma, including functional and tumor-infiltrating lymphocytes (TILs).[151,152]

The lack of functional blood vessels may lead to immature organoids, necrotic kernels, and premature differentiation, and vascularization would support the growth of more viable organoids. Organoid angiogenesis may be achieved by implanting organoids into highly vascular animal tissues, such as chicken chorion allantois membrane, where host blood vessels penetrate the organoid.[153] Mesodermal precursor cells have been incorporated into human tumors and neural organoids, thereby forming vascularized organoids *in vitro*.[153] Vessels may add layer-by-layer deposition to *in vitro* co-cultures or form tubular voids by selective removal of material that is seeded with endothelial cells and connected to the perfusion network.[154] Alternatively, vascular endothelial growth factor (VEGF) and hypoxia gradients within a compartmentalized microfluidic chip have been used to induce vascularization in organoid-endothelial cell co-culture.[155]

Tumor immunobiology

Bone marrow and thymus are the sites of development, differentiation, and maturation of immune cells and aid the development of peripheral immune organs. Stromal cells form a scaffold for thymic tissue that promotes differentiation and proliferation of thymic epithelial cells (TECs) to support thymic function and T lymphocyte maturation.[156,157] 3D thymic organoids may be generated by thymocytes isolated from NOD SCID mice or postpartum tissue which are dissociated with deoxyguanosine and cultured on gel foam sponges during the process of reaggregation fetal thymic organ culture (FTOC). Thymocyte aggregates are cultured with hematopoietic stem cells (HSCs) to support the development of T cells after transplantation into athymic mice.[158—160] However, to avoid T cell dysplasia due to different sizes of tissue samples, isolated tissues have been cultured in a single suspension and on gelatin foam at the air-liquid interface, in the process of reaggregation thymic organ culture (RTOC) which maintains the 3D conformation necessary for T cell function.[161] An artificial thymic organoid system (ATO) has also been constructed by adding aggregates co-cultured from HSCs and MS5-hDLL1 cells (genetically modified allantois cell line expressing human DLL1 or DLL4) to cell culture inserts at the air-liquid interface in serum-free medium.[162] The ATO system has been used to assist ESC and iPSC in the production of conventional T cells, providing a controllable, efficient, and stable tool for studying human thymus function.[163,164]

The lymph node peripheral cortex is characterized by a spatially organized extracellular matrix (ECM) with lymphoid follicles, rich in B cells and follicular dendritic cells (FDCs).[165] Suematsu et al implanted thymus-derived stromal cell lines together with dendritic cells into biocompatible scaffolds, after which they were transplanted into the subcapsular space of mouse kidneys and generated a lymphoid tissue organoid which retains a secondary lymphoid organ structure and contains a network of compartmentalized B and T cell clusters, high endothelial venule-like vessels, germinal centers, and follicular dendritic cells. This simplified lymphoid tissue organoid assists with studies of secondary lymphoid organ development and induction of adaptive immune responses and may be used to interrogate tumor immune escape mechanisms.[166] Votanopoulos et al generated melanoma/lymph node organoids by introducing patient tissue into an ECM-based hydrogel system to create three-dimensional (3D) mixed immune-enhanced patient-derived tumor organoid (iPDO) after washing them with saline, antibiotics, and erythrolysis buffer. This mixed organoid preserves both tumor heterogeneity and stromal and immune cell components. The iPDO reflects immunotherapy responses in 85% of patients and activates patient-matched peripheral blood T cells for killing tumor cells in the naïve PDO.[167] Immune organoids allow the immune system and tumor cells of individual patients to remain viable and permit the mechanistic study of hematological malignancies. Using these organoids, mutations in mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) protease and caspase recruitment domain-containing protein 11 (CARD11) mutations were found to be involved in activated B-cell like diffuse large B-cell lymphoma, the molecular subtypes of which are activated through different genetic pathways.[168,169]

Tumor immunotherapy

Immune checkpoint inhibitors (ICIs)

Targeting of PD1/PD-L1 and CTLA-4 by ICIs produces a clinical response among only some patients with melanoma,[170] skin squamous cell carcinoma,[171] non-small cell lung cancer,[172] renal cell carcinoma,[173] head and neck cancer,[174] and tumors with mismatch repair defects.[175] *In vitro* systems that preserve the tumor microenvironment (TME) may facilitate the development of more precise immuno-oncology. Kong et al co-cultured CRC organoids with autologous TILs to demonstrate the migratory and tumor cytotoxic activities of TILs, indicating the

maintenance of the immune checkpoint, the PD-1/PD-L1 pathway.[176] Courau and colleagues demonstrated that CRC organoids respond to immune infiltration by up-regulating HLA-E, a ligand for the CD8 and NK cell receptor, NKG2A, and that anti-MICA/B increases NKG2A while stimulating anti-tumor responses. Synergistic actions of anti-MICA/B and anti-NKG2A may be a potential immunomodulator in CRC patients.[177] Chakrabarti et al investigated the potential link between the human epidermal growth factor receptor 2 (HER2) and PD-1/PD-L1 in co-cultures of human gastric cancer organoids, cytotoxic T lymphocytes (CTLs), and myeloid-derived suppressor cells (MDSCs). Knockdown of HER2 decreased PD-L1 expression and increased CTL infiltration and an antitumor effect. Co-expression of HER2 and PD-L1 may contribute to tumor cell immune escape, indicating a potential treatment for patients with anti-PD-1/anti-PD-L1 resistance.[72] However, it must be acknowledged that the addition of a single immune cell type may not completely reproduce the complex interactions between different immune cell populations.

Holistic culture systems, including microfluidic and ALI methods, can be used for the functional modeling of ICIs. 3D microfluidic culture can reproduce *in vivo* therapeutic sensitivity when organoids were generated from MC38 and GL261 tumors (sensitive to PD1), CT26 (moderately sensitive to PD1) or B16F10, human melanoma and Merkel cell carcinoma (resistant to PD-1). Moreover, a combination of anti-PD-1 and TBK1/IKKϵ inhibitors helped to overcome immunosuppressive TME.[150] Deng et al identified the CDK4/6 inhibitor which increased T cell infiltration and had a synergistic action with that of anti-PD-1, thereby improving anti-tumor activity.[178] ALI culture of organoids with a blockade by anti-PD-1 or/and anti-PD-L1 resulted in expansion and activation of tumor antigen-specific T cells.[179] These examples show the potential of immune-tumor organoids in detecting antibody-mediated therapy, identifying novel therapeutic targets, and visualizing and enhancing immune cell migration and tumor infiltration.

Adoptive cell transfer-therapy

Adoptive cell transfer therapy (ACT) includes TILs, chimeric antigen receptor T cell therapy (CAR-T), and T cell receptor therapy (TCR).[180] CAR-T and TCR cell therapies involve extracting T cells from the patient's peripheral blood and genetically engineering them to express tumor-specific ligands. Cancer organoid models, particularly those containing TME components, are capable of monitoring and enhancing CAR cell recruitment and penetration in tumor tissue and subsequent killing. CAR NK-92 cells expressing EGFRvIII[181] and CAR-T cells targeting bladder cancer-specific antigens[52] have been analyzed using organoids. Glioblastoma organoids co-cultured with CAR-T cells have been a tool in immunotherapy research.[115] These organoid immune cell co-culture systems can be used to assess car-mediated tumor-specific cytotoxicity in normal and tumor organoids. In addition, PDOs can be used as a source of tumor-reactive T cells, a culture platform to enrich them, and can induce and assess the killing efficiency of tumor-specific T cells. CAR-T cells expressing ligands for CD39 hepatitis B virus (HBV) surface protein and personalized tumor-reactive CD8 T had activity against hepatocellular carcinoma organoids.[182] Although this study is currently limited to low patient numbers it proves the concept of organoid models being suitable for immunotherapy development.

Oncolytic virus-therapy

Oncolytic viruses are replication-competent and tumor-targeted to infect and kill tumor cells while stimulating the host to produce an anti-tumor immune response.[183] Organoid models of pancreatic cancer,[102] bladder cancer,[184] glioblastomas,[184] and breast cancer[48] have been used as a screening platform for oncolytic viral therapy. A renal cell carcinoma PDO (RCC PDO) was tested with an oncolytic adenovirus into which a cross-hybridized Fc-fusion peptide, consisting of Fc with IgA1 and IgG1 constant domains, attached to the PD-1 outer domain had been cloned. The fusion peptide bound to PD-L1 and activated IgA1 neutrophils and IgG1 natural killer and complement activation functions enhancing tumor killing relative to anti-PD-L1 (atezolizumab), IgG1-PDL1, and IgA-PDL1.[185] Although these studies suggest promise for PDOs in studying oncolytic virus infectivity and cytotoxicity, to our knowledge, immune responses triggered by oncolytic viruses have not been investigated in complex immune-organoids.

Immunotherapy research has driven the development of novel cancer therapies, either alone or in combination with other therapies, to target immunomodulatory pathways and advance the control of cancer cells. PDOs combine TME characteristics and mimic immunotherapy responses, while co-culture with immune cells mimics the mechanism of immunotherapy efficiency and drug resistance. Overall, cancer organoids can not only elucidate potential resistance pathways but may also contribute to therapeutic efforts, such as *in vitro* screening and optimization of drugs and cellular immunotherapies; if short-term responses in culture do accurately reflect clinical responses and long-term outcomes, they can also determine patient sensitivity to single or combination therapies in real time. In the future, organoid approaches will greatly facilitate the basic science and translation of immuno-oncology and accelerate the development of personalized immunotherapy for human tumors.

Current limitations and future opportunities

Many features of human cancer development and progression are reproduced by cancer organoids but the pathophysiology and clinical relevance could be improved. Firstly, although three-dimensional organoids constructed *in vitro* already have structures of some organs compared with intact organs *in vivo*, the structures are still relatively simple and can only partially reflect tissue characteristics. Additional supplements, such as IL-2, anti-CD3, and anti-CD28 antibodies, are often required to maintain immune cells in co-culture with organoids. The animal origins of the 3-D collagen-rich lamin, Matrigel, which is essential for organoid culture may also influence treatment outcomes. There is also variation in PDO generation due to a lack of standardized methodology plus ethical issues around the transplantation of organoids into humans, all of which must be addressed. Secondly, organoid culture varies according to the tumor type and starting material with treatment-directed tumor shrinkage affecting the success of the

culture. Similarly, metastasis-derived organoids are difficult to generate, especially with inter-organ spread, and must be validated using mouse xenograft models. Thirdly, tumor-derived organoids usually grow more slowly than healthy tissue-derived organoids which leads to the contamination of tumor organoids with healthy tissue.[85,186] Non-solid tumors cannot be reproduced as organoids and more complex brain organoids are still a major challenge. Fourthly, PDOs establishment takes 4—6 weeks plus approximately 2—4 weeks to screen for anticancer drugs, reducing the chances of coinciding with the optimal therapeutic window for patients. Moreover, the production of PDOs is currently very expensive, and the technology is immature and difficult to incorporate into existing healthcare systems. The ethical significance of tumor organoid biobanking also requires further consideration. Despite these limitations, organoid cultures are a physiologically relevant model for translational applications and personalized cancer drug development.

Yin et al[187] established an *in vitro* tumor model of a patient-derived tumor cell cluster by simultaneously optimizing the medium in Matrigel-free conditions. This model allows short-term cell expansions from primary tumors, including immune and fibroblast populations, allowing the investigation of hundreds of therapeutic options per patient in a manner that correlated with clinical performance. This model excludes the influence of extrinsic factors such as Matrigel on subsequent studies while allowing drug prediction within the patient's optimal therapeutic time window. However, the chronic culture of patient-derived tumor cell clusters leads to the loss of stromal cells and their drug response patterns may be altered.

At present, the "Organ-on-a-chip" technology formed by the integration of organ chip and organoid can address some problems very well. A typical example is the application of microfluidic devices in an *in vitro* tumor organoid model. The interaction between tumor and stroma was investigated mechanically by simulating the spatial organization of the tumor microenvironment on a chip. It has been observed that tumor-associated fibroblasts (CAF) enhance the expression of glycoprotein non-metastatic B in breast cancer cells, thereby promoting invasion.[188] The organoid-on-a-chip allows simulation of the microenvironment and establishment of interrelationships between tissues and multiple organs.[189] Lung cancer organoids combined with microfluidic chip technology have produced drug responses within a week.[190] In addition, Shirure et al[191] used this technique and organoid integration to mimic perfused vessels for investigating the progression and response of cell lines and PDOs to chemotherapy and anti-angiogenic therapy. Many aspects remain to be improved in order to obtain more accurate and reliable results. Skilled and experienced researchers are required for the fabrication of microfluidic devices and sophisticated tissue engineering techniques. Another potential limitation is that biomaterials used to fabricate many organ chips cannot be effectively used for drug research due to drug absorption.

Optical metabolic imaging (OMI) measures drug-induced changes in cellular metabolism and may shorten drug turnover times and be used to test drug responses in individualized cancer treatment.[192,193] The susceptibility of gastroenteropancreatic neuroendocrine tumor (GEP-NET) organoids to navitoclaxa (Bcl-2 inhibitor) and everolimus (mTOR inhibitor GEP-NET treatment) has been evaluated by OMI.[194] 3D bioprinting allows precise control of spatial heterogeneity in the tumor microenvironment through spatially deterministic deposition of predefined biobanks that may contain multiple cell types, biochemical factors, and ECM. Reid et al generated chimeric organoids by co-printing cancer cells and normal breast epithelial cells and showed that cancer cells within the chimeric construct had significantly increased levels of 5-hydroxymethylcytosine compared to bio-printed tumor-like cells.[195] In addition, organoid technology, microfluidic technology, and 3D printing technology have been combined into an automated organoid platform for one-week high-throughput drug screening and personalized medicines.[196] When limitations are eventually overcome, organoids may give new hope to cancer patients and greatly promote human health.

Conclusions

Patient-derived tumor organoids are physiologically and clinically more advanced and maintain patient-specific tumor heterogeneity, constituting a good platform for different tumors. This model allows the simulation of tumor development to provide new targets for cancer therapy and facilitate appropriate treatment selection. Some complex cancer organoids may become tools for personalized immunotherapy validation, response and toxicity testing, and therapeutic development in early clinical trials. Therefore, tumor-derived organoids are an important tool through which we can improve the understanding of cancer and promote the treatment of cancer patients in the future.

Author contributions

XM and QW collected the related paper and drafted the manuscript. GL and HL revised the manuscript and prepared the figures. DP and SX participated in the design of the review and helped draft and modify the manuscript. All authors read and approved the final manuscript.

Conflict of interests

The authors declared that there is no conflict of interests.

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