Combination of tumor organoids with advanced technologies: A powerful platform for tumor evolution and treatment response (Review)

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Abstract. Malignant tumors notably decrease life expectancy. Despite advances in cancer diagnosis and treatment, the mechanisms underlying tumorigenesis, progression and drug resistance have not been fully elucidated. An emerging method to study tumors is tumor organoids, which are a three-dimensional miniature structure. These retain the patient-specific tumor heterogeneity while demonstrating the histological, genetic and molecular features of original tumors. Compared with conventional cancer cell lines and animal models, patient-derived tumor organoids are more advanced at physiological and clinical levels. Their synergistic combination with other technologies, such as organ-on-a-chip, 3D-bioprinting, tissue-engineered cell scaffolds and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9, may overcome limitations of the conventional 3D organoid culture and result in the development of more appropriate model systems that preserve the complex tumor stroma, inter-organ and intra-organ communications. The present review summarizes the evolution of tumor organoids and their combination with advanced technologies, as well as the application of tumor organoids in basic and clinical research.

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Abbreviations: CRC, colorectal cancer; 2D, two-dimensional; PDX, patient-derived xenograft; CRISPR-Cas9, clustered regularly interspaced short palindromic repeats-associated protein; iPSC, induced pluripotent stem cell; CTC, circulating tumor cell; TME, tumor microenvironment; ECM, extracellular matrix; PDO, patient-derived organoid; ccRCC, clear cell renal cell carcinoma; PEG, polyethylene glycol; MCHO, multicellular hepatocellular carcinoma organoid; HCC, hepatocellular carcinoma; EC, epithelial cell; PBMC, peripheral blood mononuclear cell; RBMS3, RNA binding motif, single stranded interacting protein 3; SCLC, small cell lung cancer; YAP, Yes-associated protein; TAZ, transcriptional coactivator with PDZ binding motif

Key words: tumor organoid, organ-on-a-chip, 3D-bioprinting, tissue-engineered scaffold, CRISPR-Cas9

Contents

1. Introduction

Cancer, a leading cause of mortality, notably decreases life expectancy. In 2022, there were ~20.0 million new cases and ~9.7 million cancer-associated mortalities worldwide (1). Despite advances in cancer diagnosis and treatment, mechanisms underlying tumorigenesis, progression and drug resistance have not been fully elucidated. Due to tumor

heterogeneity, different individuals with the same type of cancer may exhibit different responses to the same therapy; thus models need to be established that can recapitulate the tumors to study the mechanisms of tumorigenesis, progression and drug resistance.

Preclinical models include two-dimensional (2D) cell lines, patient-derived xenografts (PDXs) and organoids. Despite simple operation and culture, 2D cell lines cannot definitively predict the drug response of patients due to accumulation of gene mutations during passaging (2). Additionally, 2D cell lines are unreliable compared with *in vivo* models because of variations in cell phenotypical behaviors (3). PDXs, which are created by engraftment of patient tumor tissue into immunocompetent mice, recapitulate the tumor heterogeneity while preserving the biological and molecular features of original tumors (4,5), but they are time-consuming, expensive and may undergo mouse-specific tumor evolution rendering them unable to reflect the pathogenic process of patients (6,7). Therefore, application of PDXs is limited by the complex operation, duration, high cost and low success rate. Organoids, a novel type of three-dimensional (3D) miniature structure derived from adult or embryonic stem cells (SCs), not only retains *in vivo* tumor characteristics and heterogeneity, but also can predict the sensitivity of multiple drugs simultaneously, with the advantages of high success rate of generation, short time-frame and low cost (8) (Table I). Currently, organoids from multiple types of cancer have been established, including colorectal cancer (CRC), breast, pancreatic and lung cancer (9-12). These tumor organoids not only preserve the features of original tumors at genomic, molecular and epigenetic levels, but also contribute to predicting patient responses to therapies, thus offering potential for unveiling the biology of tumorigenesis, promoting drug discovery and personalized treatment in cancer.

The present review aimed to summarize evolution of tumor organoids and their combination with advanced technologies, such as organ-on-a-chip, 3D-bioprinting, tissue-engineered cell scaffolds and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (CRISPR-Cas9), as well as the application of tumor organoids in basic and clinical research.

2. Definition and evolution of organoids

Organoids, a type of 3D microstructure cultured *in vivo* under similar conditions to the human microenvironment, are primarily established based on the self-organization and differentiation of cells with stem cell characteristics (13). Organoids exhibit a natural self-organizing structure similar to native organs. In 1907, separated sponge cells were shown to self-organize into a complete organism (14). Subsequently, limbal SCs from 3T3 trophoblast cells were transplanted into damaged eyes following culture, laying the basis for the development of 3D organoid technology (15,16). Induced pluripotent SCs (iPSCs) are widely used in *in vitro* and *in vivo* preclinical studies (17-19). Since the first human iPSC-derived motor neurons from patients were cultured in a petri dish, several techniques, such as somatic cell nuclear transfer approach and transcription factor-based somatic cell reprogramming, have been developed to generate iPSC lines (20). Since 2009, when the intestinal organoids were first cultured successfully, organoids have gained much attention in cancer research (21,22).

In 2011, gut and retinal organoids from SCs were generated successfully (23,24). Long-term culture protocols for primary colon adenoma/adenocarcinoma have been established (25). In 2014, prostate cancer organoids from metastatic biopsy and circulating tumor cells (CTCs) were first constructed (26) and breast cancer organoids derived from CTCs resemble the immunohistochemical features of breast tumors (27). In 2015, Boj *et al* (28) first generated pancreatic organoids from human neoplastic tissue; orthotopically transplanted organoids can be used to study the pathogenesis of pancreatic ductal adenocarcinoma. In the same year, Bartfeld *et al* (29) demonstrated the feasibility of establishing human gastric cancer organoids. Subsequently, other types of tumor organoid have been established, including glioblastoma and liver, endometrial, bladder and esophageal cancer (30-39) (Fig. 1). Therefore, tumor organoids have value in cancer research. With the rapid development of biotechnology, they can not only supplement to the current evidence-based medicine, but also have advantages in clinical and translational research (40,41). Tumor organoids combined with advanced technologies, such as organ-on-a chip and 3D bio-printing, are also emerging, which not only promote the drug-testing process, but also reveal the synergistic effect in complex treatment regimens.

3. Combination of tumor organoids with advanced technologies

Tumor organoids reflect diverse key characteristics of tumor progression, but they lack characteristics such as vasculature, stomal components and tissue-resident immune cells (42). Moreover, multiple biophysical and biochemical factors from the tumor microenvironment (TME) are difficult to replicate accurately using conventional 3D organoid culture. Therefore, technologies, including organ-on-a-chip, 3D bio-printing, tissue-engineered cell scaffolds and CRISPR-Cas9 (Table II), are combined with tumor organoids as more precise models to study the mechanisms of tumorigenesis, progression and drug resistance (Fig. 2).

Organ-on-a-chip. As a microfabricated device, the organ-on-chip is designated to integrate the culture of extracellular matrix (ECM), living cells and microstructures imitating organs or tissue (43,44). Integrating living human cells into a synthetically produced microenvironment models physiological homeostasis and the process of complex diseases (Fig. 3A) (43). Demers *et al* (45) developed a versatile microfluidic platform that mimics *in vivo* spatial and temporal chemical environments during neural tube development. Using similar techniques, Wang *et al* (46) developed a brain organoid-on-a-chip system from human iPSCs, promoting 3D culture, *in situ* neural differentiation and self-organization of brain organoids under continuous perfusion of neural differentiation medium in a controlled manner.

The organ-on-a-chip has the advantages of specific stroma, requiring a small amount of tissue for analysis, high-resolution optical measurement, real-time tracking of organoid morphogenesis and inexpensive manufacture. Although tumor microsystems are used to explore the cancer-specific

Table I. Advantages and limitations of cancer cell lines, PDXs and tumor organoids in cancer research.

Category	Cancer cell lines	PDXs	Tumor organoids
Advantages	Simple operation and culture; high throughput	Replicate tumor heterogeneity while preserving the biological features of the tumor and predict the drug sensitivity	High throughput; able to recapitulate the tumor heterogeneity while preserving the biological features of the tumor and predict the drug sensitivity; high success rate of generation, short time; low cost
Limitations	Unable to reflect the physiological status of tumors and predict drug sensitivity; difficulty in replicating tumor microenvironment	Complex operation; time-consuming; high cost; low success rate; can possibly undergo mouse-specific tumor evolution	Lack of immune system; unable to model tumor-stroma interactions

PDX, patient-derived xenograft.

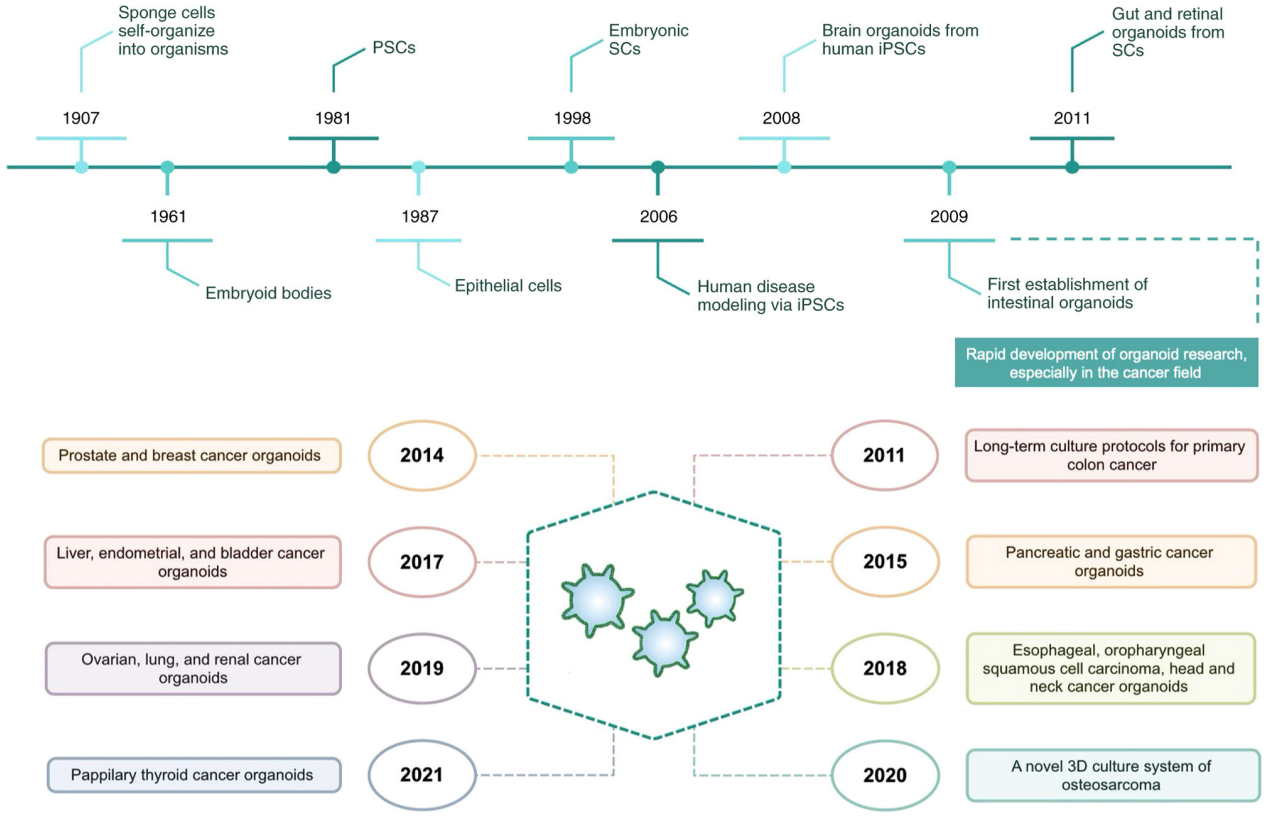

Figure 1. Evolution of tumor organoid research. iPSC, induced pluripotent stem cell.

hallmarks, the TME complexity cannot be recapitulated due to simultaneous or successive occurrence of cancer-specific hallmarks. Shirure *et al* (47) designed a tumor-on-a-chip microfluidic platform and that this could simultaneously model the hallmark characteristics of tumor progression in both cell lines and patient-derived organoids (PDOs), such as proliferation, migration, angiogenesis and intravasation.

At present, the culture environment of organoids lacks vascularization, leading to decreased organoid lifespan and changeability in tissue-specific functionality and architecture (48). In a previous study, 3D vascularized liver organoids comprising induced hepatic cells and decellularized liver ECM were developed based on the microfluidic system, exhibiting improved liver functionality, biosynthetic and metabolic activity, as well as drug response; this study also confirmed the feasibility of vascularized liver organ-on-a-chip systems as a high-throughput drug screening platform (49). To study tumor angiogenesis, the human primary clear cell renal cell carcinoma (ccRCC) cells are combined with endothelial cells in a vascularized, flow-directed, 3D culture system. Under continuous flow, cRCC clusters preserve the key angiogenic signaling axis between ccRCC and endothelial cells by

Table II. Advantages and limitations of advanced technologies.

Category	Organ-on-a-chip	3D bio-printing	Tissue-engineered cell scaffolds	CRISPR-Cas9
Advantages	Specific stroma; a small amount of tissue required for analysis; high-resolution optical measurement; real-time tracking of organoid morphogenesis; inexpensive to manufacture	Precisely controls the spatial arrangement of cells, biomaterials and soluble factors; can scale up the organoid systems with hierarchical architecture	Supports cell proliferation and attachment; simulates extracellular matrix function *in vivo*; can enlarge the organoid systems with vascular network	Identifies novel targets for cancer therapy; produces genetically inhibited models for drug development
Limitations	High variability and lack of standardization in methods; small size of the organoid system	Unable to replicate tumor progression when monodispersed tumor cells are used as bio-printing building blocks	Difficult to adjust for the unique tissue microenvironment	High off-target effects; difficult to validate the mutations in target genes

CRISPR-Cas9, clustered regularly interspaced short palindromic repeats-associated protein 9.

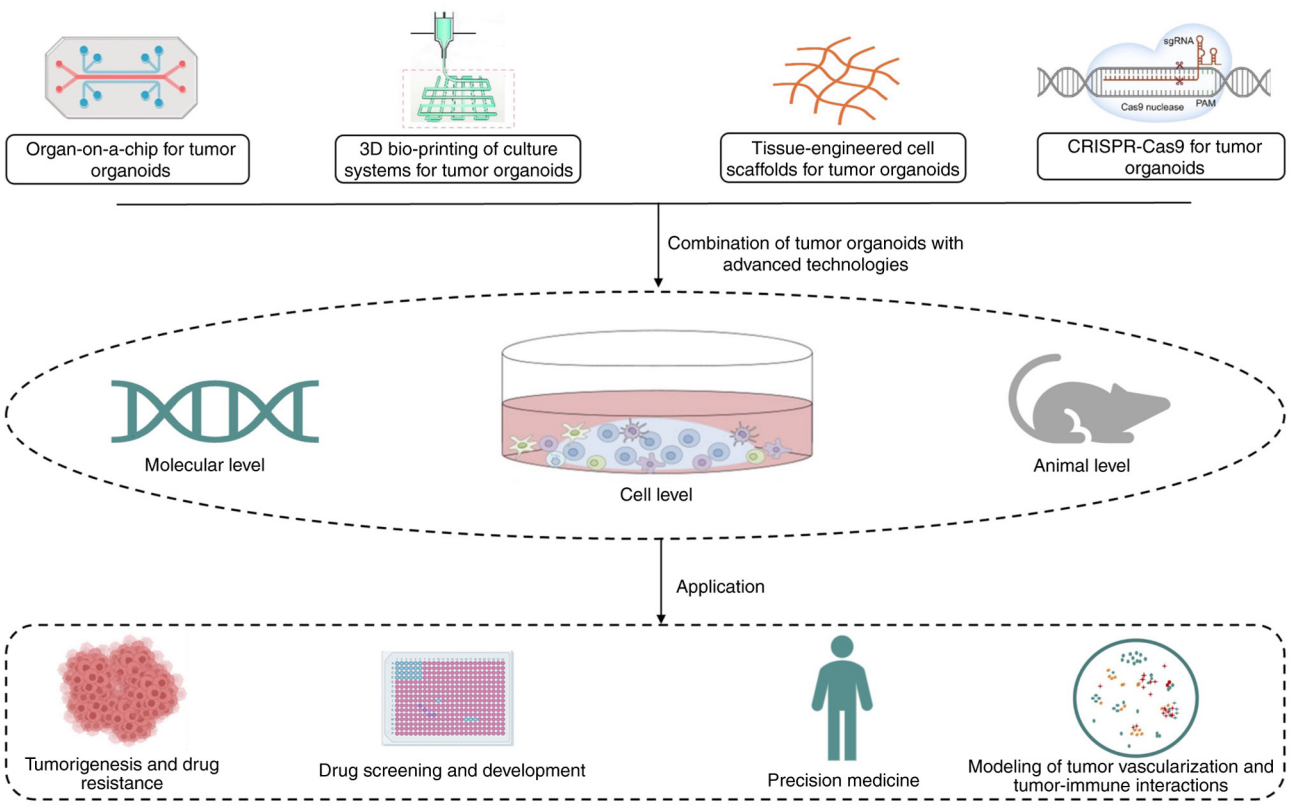

Figure 2. Synergistic combination and application of tumor organoids and advanced technologies, including organ-on-a-chip, 3D bio-printing, tissue-engineered cell scaffolds and CRISPR-Cas9, for studying the mechanisms of tumorigenesis and drug resistance, drug screening and development, personalized treatment and modeling vascularization and tumor-immune interactions. CRISPR-Cas9, clustered regularly interspaced short palindromic repeats-associated protein 9; sgRNA, single guide RNA; PAM, protospacer adjacent motif.

promoting endothelial cell sprouting. This system signifies a vascularized tumor model with adjustable perfusate, input cells and matrices (50). The PDOs established in a multicellular microfluidic chip may prolong cellular function and longevity and construct an intricate organotypic TME (Fig. 3B). Targeting stroma in a tumor-chip model notably increases response to chemotherapy in cancer cells, further verifying the application of the tumor-chip device in drug testing (51). Additionally, multiorgan models of coculture with SC-derived stomach, intestinal and liver organoids have

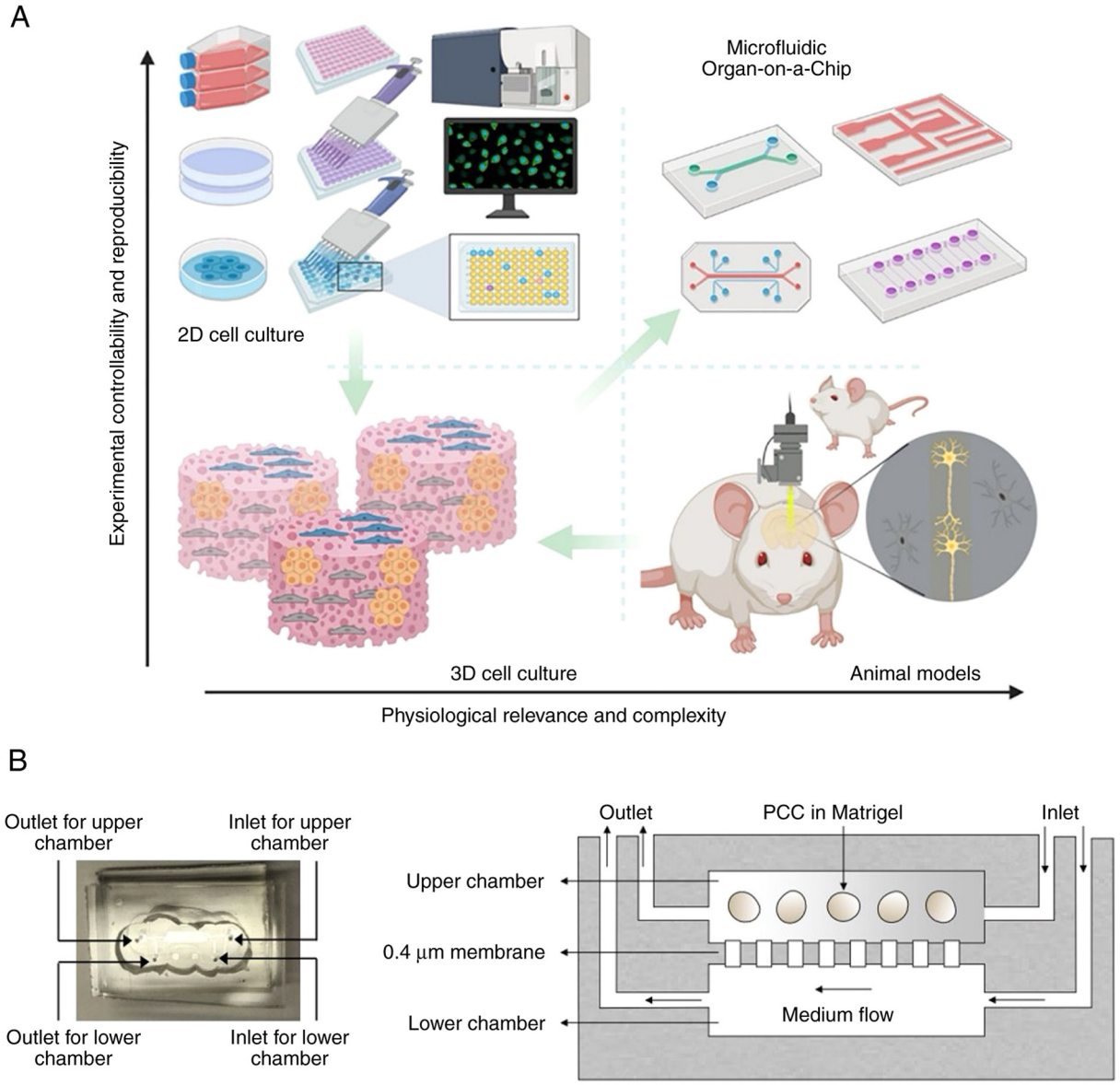

Figure 3. Microfluidic organ-on-a-chip platform. (A) Microfluidic organ-on-chip platform enables controllable cell culture within an organotypic microarchitectural environment (reproduced with permission. Copyright 2021, Elsevier Inc.). (B) Schematic diagram of the multi-chamber microfluidic device. The chip consists of two chambers separated by a 0.4-μm porous membrane. Cells are installed in the upper chamber through the inlet and culture medium is perfused through the lower chamber to maintain the cell viability (reproduced with permission. Copyright 2022, Springer Nature).

also been established, promoting the discovery of interorgan crosstalk characteristics (52). The aforementioned findings indicate how the organoids combined with organ-on-a-chip technique replicate cell maturity.

3D bio-printing. 3D bio-printing accurately controls the spatial arrangement of cells, biomaterials and soluble factors, forming intricate multicellular structures (53,54). By offering tumor-specific ECM, accurate geometric architecture and biophysical properties, bio-printing can replicate the TME, thus promoting the establishment of complicated and controllable 3D tissue models. In most studies, however, monodispersed tumor cells used as bio-printing building blocks do not effectively replicate the tumor progression due to the rare presence of volumetric tumor cells in isolation (55,56). The combination of 3D bio-printing and tumor organoids allows for the introduction of miniaturized tumor aggregations into a heterogeneous 3D niche containing stromal cells and hydrogels, which are more cell-specific for simulation of TME features and high-throughput drug screening (Fig. 4A) (57,58).

Mollica *et al* (59) revealed that 3D bio-printed organoids and tumoroid formation are preserved effectively using novel 3D culture substrates. Reid *et al* (60) applied bio-printing to analyze tumorigenesis and microenvironmental redirection in breast cancer cells and demonstrated that bio-printing could promote the formation of tumor organoids in 3D collagen gels and tumor organoid arrays. Moreover, *in vivo* findings are accurately simulated through bio-printed organoids, highlighting the feasibility of the 3D bio-printing technique in understanding tumorigenesis and TME control. To increase the throughput of 3D drug screening, an immersion bio-printing technique has been developed to bio-print tumor organoids in

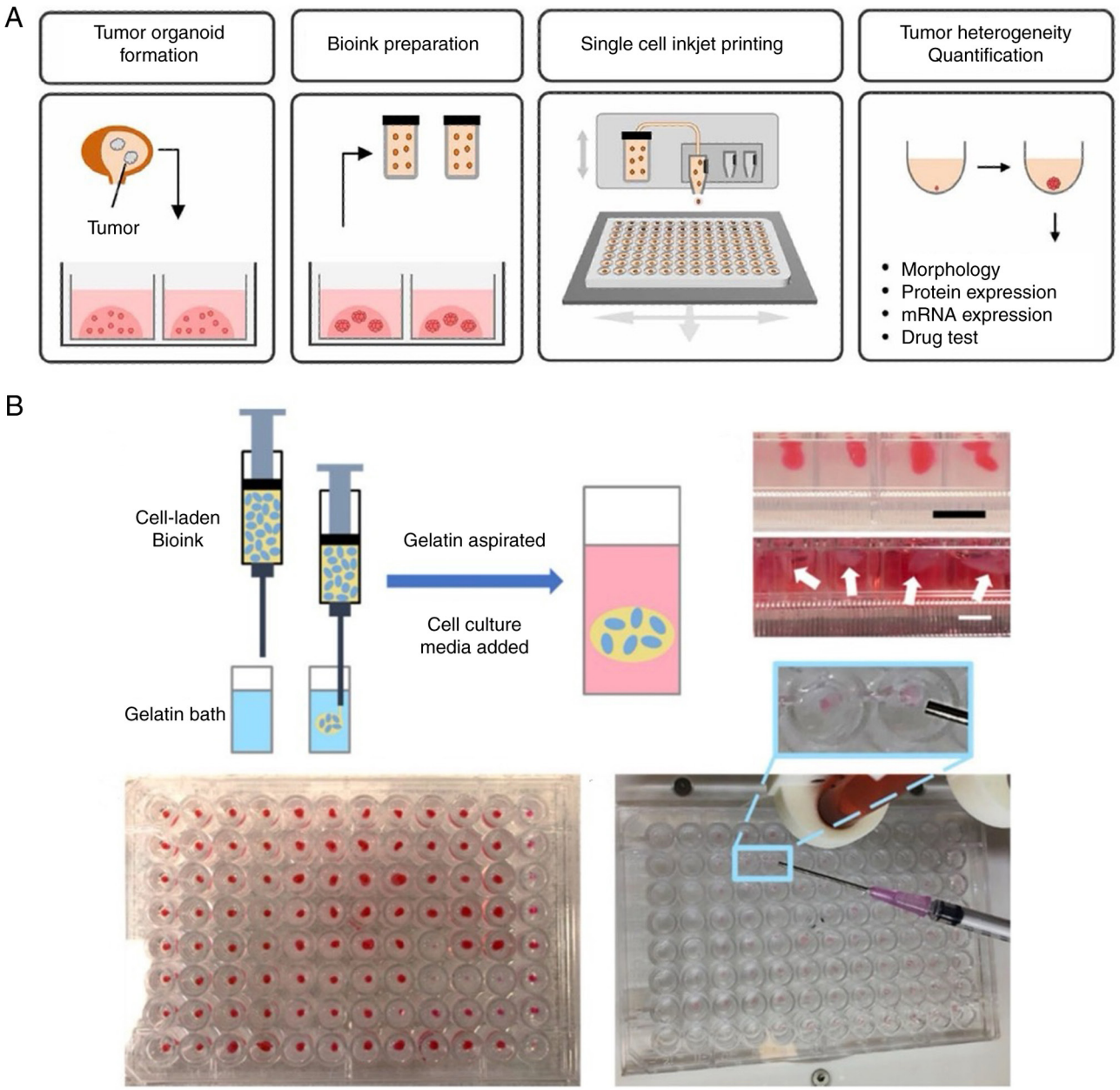

Figure 4. Tumor organoid culture based on 3D bio-printing. (A) Inkjet printing to quantify tumor heterogeneity (reproduced with permission. Copyright 2020, IOP Publishing). (B) Schematic diagram of immersion printing into a support gelatin bath (reproduced with permission. Copyright 2020, MDPI).

multi-well plates (Fig. 4B) (61). Additionally, a 3D-bioprinted construct is used to test the resistance of anti-cancer drugs (sorafenib, cisplatin and 5-fluorouracil) in patient-derived cholangiocarcinoma cells. Compared with 2D cultures, bio-printed cholangiocarcinoma cells exhibit stem-like properties and high resistance to the aforementioned anti-cancer drugs, indicating the potential of 3D bio-printed tumor models in the discovery of targeted drugs (62). Additionally, bio-printed organoids undergo hepatocytic differentiation, including liver-specific enzyme activity and albumin synthesis (63), creating novel possibilities for regenerative medicine and individualized drug screening.

Tissue-engineered cell scaffolds. Matrigel is key for the culture of most organoids, but it may elevate the risk of animal-derived microbial infection and batch-to-batch variability in organoids, leading to unreproducible experimental results (64,65). Tissue-engineered cell scaffolds support cell proliferation and attachment and simulate ECM function *in vivo* (66). For cell- and tissue-derived matrices, synthetic scaffolds, such as polyethylene glycol (PEG)-based hydrogel scaffolds, allow control over the growth conditions.

For conventional organoid cultures, generic matrices are usually applied, but they are difficult to adjust to replicate the unique TME. Ng *et al* (67) used gelatin-based hydrogels to demonstrate CRC organoid sensitivity to multiple drugs *in vivo*, and found that these hydrogels may be a promising platform for biochemically and mechanically defined matrices used in multiple types of tumor organoid. In a previous study, a fully synthetic hydrogel scaffold was constructed based

on the 8-arm PEG and pancreatic cancer organoids were generated successfully (68). Through regulation of hydrogel properties, the proliferation of pancreatic cancer organoids is controlled, and the phenotypic traits of the TME *in vivo* are effectively replicated when stromal cells are incorporated into the hydrogels (68). These findings suggest that synthetic scaffolds replicate a pathologically remodeled TME for studying normal and pancreatic cancer cells *in vitro*. Another study showed that ECM hydrogels generated organoids appropriate for gastrointestinal disease modeling, tissue regeneration and drug development (Fig. 5A), which may serve as effective alternatives to Matrigel (69). Accordingly, tissue-engineered cell scaffolds are promising next-generation materials for organoid technology to understand organ-based developmental biology and predict drug response in tumor organoids (67-69).

CRISPR-Cas9. CRISPR-Cas9 enables more efficient gene knockout and knock-in than other types of genome editor through introduction of DNA double-strand breaks at specific genomic loci (70). In addition to identification of novel targets for cancer therapy, CRISPR-Cas9 is also used to produce genetically inhibited animal models for drug development (71,72). CRISPR-Cas9 combined with 3D organoid systems facilitates development of precise cancer models for studying diverse mechanisms of tumor progression, metastasis, interactions and drug resistance. Organoid systems not only mimic the human disease and tailor therapeutic strategies, but also serve as an experimental platform for mechanistically studying the gene function in humans (73).

Matano *et al* (74) introduced mutations into organoids from normal human intestinal epithelium using CRISPR-Cas9 and demonstrated that the isogenic organoids with mutations show tumorigenicity in mice. Murine gallbladder organoids with *KRAS* mutations or overexpression of Erb-B2 receptor tyrosine kinase 2 cause gallbladder cancer in transplanted immunocompromised mice when CRISPR-Cas9 is used for *p53* or *PTEN* deletion (75). In another study, specific subtypes of breast cancer organoids were established following targeted knockdown of four breast cancer-associated suppressor genes in mammary progenitor cells through CRISPR-Cas9; these organoids respond to endocrine therapy or chemotherapy (Fig. 5B and C) (76,77). Vaishnavi *et al* (78) used CRISPR/Cas9 technology to silence RNA binding motif, single stranded interacting protein 3 (RBMS3); this facilitated the growth of BRAFV600E lung organoids and malignant progression of lung cancer. Additionally, CRISPR-Cas9 was also used to validate CRC driver genes in mouse intestinal tumor organoids and human CRC-derived organoids (79). Notably, CRISPR-Cas9-mediated homology-independent organoid transgenesis, a genetic tool for labeling specific genes in human organoids, effectively generates genetically engineered human liver duct and fetal hepatocyte organoids within 2-3 months (80,81). This genetic tool can be used to study cell fates and differentiation and identify targets for drug development, showing promise for cancer research.

4. Application of tumor organoids

Mechanisms of tumorigenesis and drug resistance. Tumorigenesis and progression primarily depend on the accumulation of genetic alterations. Understanding the mutational process is key to analyzing the mechanism of tumorigenesis. Multiple studies have demonstrated the feasibility of introducing pathological mutations into normal organoids using genetic modification to simulate tumorigenesis (74,76,82,83) (Fig. 6). As reported by Matano *et al* (74), isogenic organoids with mutations show tumorigenicity in mice when gene mutations from driver pathways are introduced into organoids derived from normal human intestinal epithelium (84). By establishing AT-rich binding domain protein 1A-deficient human gastric cancer organoids using CRISPR/Cas9 technology, modes of oncogenic transformation are revealed, including essential transcriptional forkhead box protein M1/baculoviral IAP repeat-containing 5-stimulated proliferation and non-essential Wnt-inhibited mucinous differentiation (82). As a key cause of mortality in patients with cancer, the migration and invasion of tumor cells also serve important roles in tumor progression. Through coculture with mammary tumor organoids, a tissue-engineered model with physiologically realistic microvessels was created, which allows quantitative and real-time evaluation of tumor-vessel interactions under the conditions that retain various *in vivo* characteristics to identify targetable mechanisms of vascular recruitment and intravasation (84). In the patient-derived breast cancer organoids, CD homophilic interactions and subsequent CD44-p21-activated kinase 2 interactions mediate tumor cluster migration and aggregation (85). Moreover, invasion could also be triggered in breast cancer subtypes by basal epithelial gene expression (86).

To investigate the role of tumor organoids in treatment resistance, Boos *et al* (87) modeled the acquisition of chemotherapy tolerance in metastatic CRC organoids during first-line combined chemotherapy, highlighting the potential of CRC organoids in modeling chemotherapy tolerance *in vivo*. Similarly, in intestinal cancer organoids, atypical expression of cyclin P facilitates stemness-like phenotypes (88), which usually results in tumor metastasis, relapse and treatment resistance. Through establishment of oxaliplatin-resistant organoids and assessment of their gene expression, myoferlin was shown to be involved in oxaliplatin resistance and tumor progression in gastric cancer (89). As commonly used anti-tumor drugs in prostate cancer, resistance to androgen receptor pathway inhibitors may develop due to epigenetic reprogramming turning castration-resistant adenocarcinoma to neuroendocrine prostate cancer. Bioengineered ECM regulates the response of prostate cancer organoids to small-molecule inhibitors of epigenetic targets and dopamine receptor D2, suggesting that synthetic organoids exert a regulatory effect on ECM in response to targeted therapy in prostate cancer and serve as a novel treatment strategy to overcome resistance (90). Overall, tumor organoids serve as a powerful platform for studying drug resistance mechanisms.

Precision medicine. Tumor organoids not only contribute to understanding the mechanism of tumorigenesis, but also predict the response to therapies, including chemotherapy, radiotherapy and targeted therapy (91-93). Vlachogiannis *et al* (92) established organoids from metastatic gastrointestinal cancer to predict the response to targeted drugs or chemotherapy, showing sensitivity of 100 and specificity of 93%. Gastric

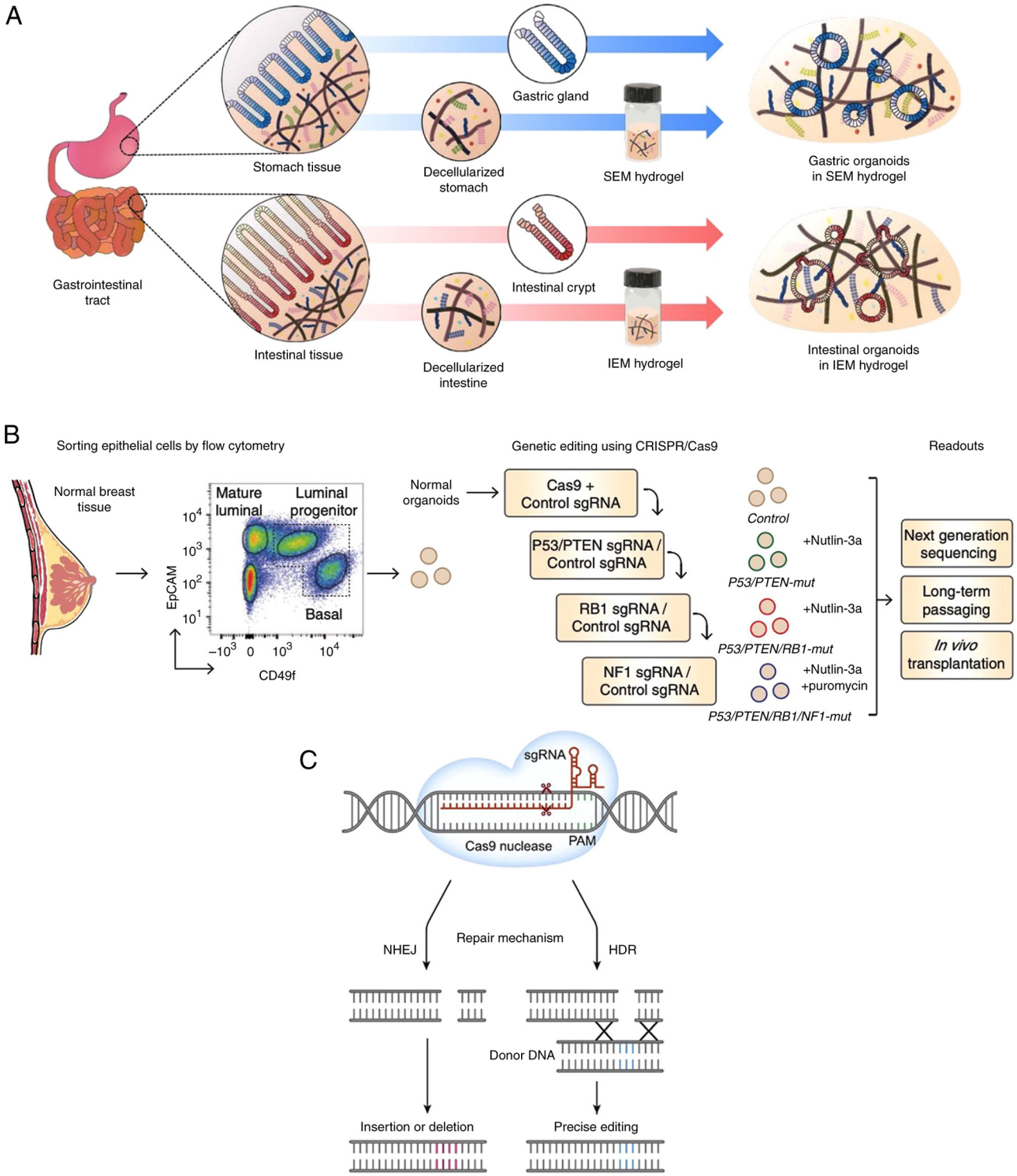

Figure 5. Schematic diagrams of organoid generation based on ECM hydrogels and CRISPR-Cas9. (A) Generation of gastrointestinal organoids using ECM hydrogels (reproduced with permission Copyright 2022, Springer Nature). (B) Generation of organoids from human normal breast basal and luminal progenitor cells and sequential CRISPR-Cas9-mediated gene editing in organoids of four tumor suppressor genes in breast cancer (reproduced with permission. Copyright 2020, Oxford University Press). (C) Mechanism of CRISPR/Cas9 (reproduced with permission. Copyright 2019, Elsevier Inc.). SEM, stomach extracellular matrix; IEM, intestinal extracellular matrix; sgRNA, single guide RNA; PAM, protospacer adjacent motif; HDR, homology-directed repair; NHEJ, non-homologous DNA end joining.

cancer organoids faithfully reflect responses to commonly used chemotherapy drugs, such as irinotecan, oxaliplatin, docetaxel, epirubicin and 5-fluerouracil (91). Tiriac *et al* (94) generated a pancreatic cancer organoid library and found that PDO profiling based on the next-generation sequencing of DNA and RNA and pharmacotyping may predict responses to chemotherapy in pancreatic cancer. Ji *et al* (95) identified potential drug combination therapies based on pharmaco-proteogenomic profiling of liver cancer organoids, offering guidance for clinical patient selection and drug combination therapies.

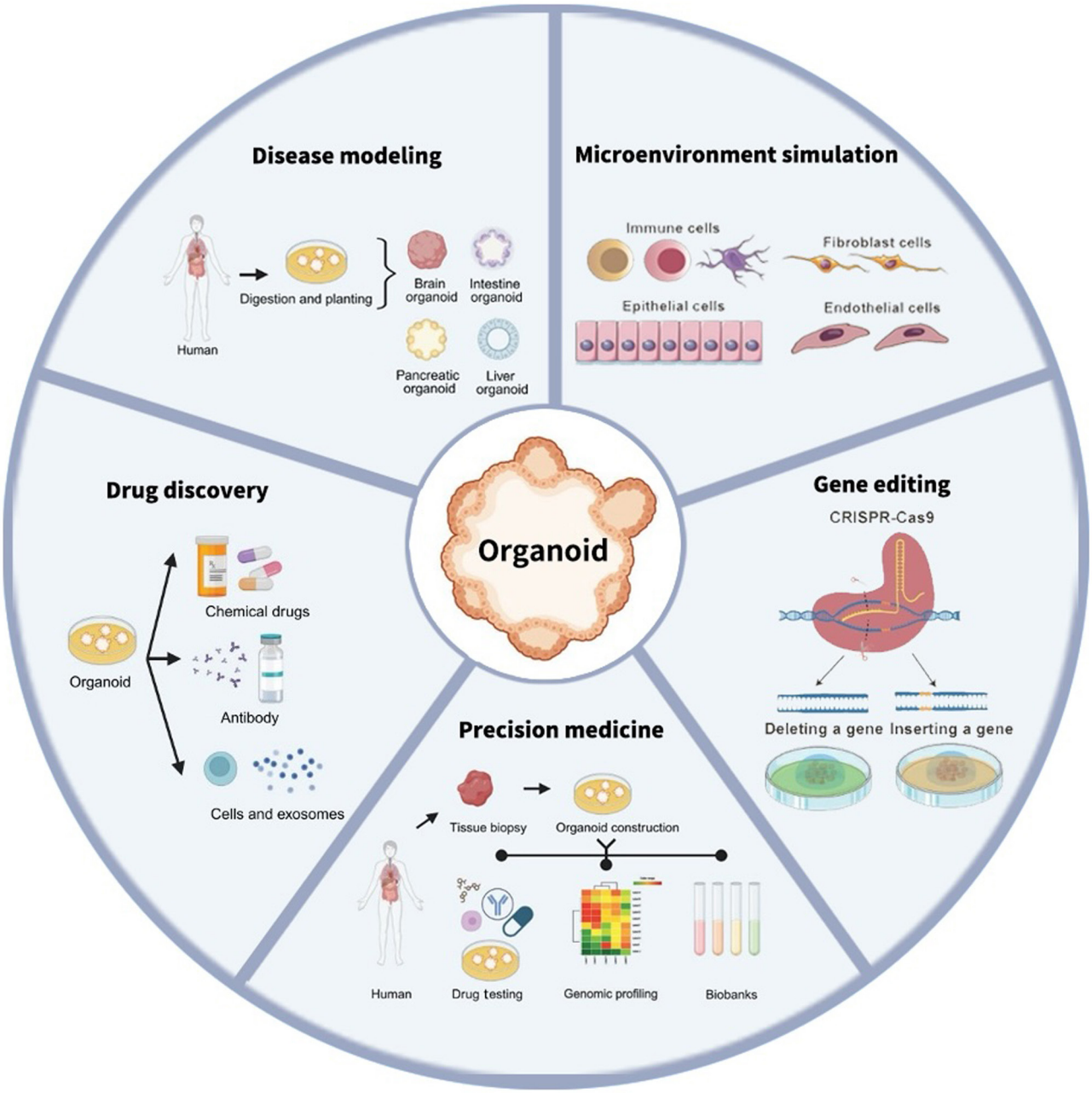

Figure 6. Applications of organoids, including disease modeling, drug discovery, precision medicine, microenvironment stimulation and gene editing (reproduced with permission. Copyright 2024, Wiley). CRISPR-Cas9, clustered regularly interspaced short palindromic repeats-associated protein 9.

Ganesh *et al* (93) revealed the heterogeneity of rectal cancer organoids in chemoradiation and e*x vivo* responses to clinically relevant chemoradiation associated with clinical responses. Moreover, *KRAS*-wild-type rectal cancer organoids are sensitive to cetuximab, while *KRAS*-mutant organoids are resistant, which is consistent with the results of a clinical trial that *KRAS* mutations are associated with resistance to EGFR-targeted therapy (93). Importantly, in a prospective, interventional clinical trial of the last-line systemic therapy based on PDOs, improved clinical outcomes were observed in patients with CRC compared with those receiving the best supportive care alone (96). In addition, the association between tumor organoids and clinical response has been identified in other types of cancer. By establishing PDOs from different stages of bladder cancer, Minoli *et al* (97) demonstrated that the PDOs exhibit heterogenous drug responses to standard-of-care treatment and drug screening showed sensitivity to targeted therapy. In a real-world study, lung cancer organoids were used to validate the response to osimertinib, chemotherapy and dual-targeted therapy and a high concordance was identified between lung cancer organoids and clinical response (12). PDO pharmaco-phenotyping not only reflects previous treatment responses of patients with advanced breast cancer but also serves as a potential platform to guide personalized treatment (9). Breast cancer organoids may also be used to predict patient-specific response to drug treatment (98).

Coculture of tumor organoids with immune components may generate tumor-reactive T cells, which may promote the

prediction and evaluation of tumor responses at an individual level by blocking PD-1/PD-L1 (98,99). Cattaneo *et al* (100) described the generation and function of tumor-reactive T cells based on the coculture of tumor organoids with peripheral blood mononuclear cells (PBMCs), demonstrating the feasibility of establishing *ex vivo* models of T cell immunotherapy at an individual level. Meng *et al* (101) developed a platform for the expansion of tumor-targeted T cells from peripheral blood and revealed that the coculture of tumor organoids with PBMCs generates tumor-reactive T cells, thereby promoting personalized immunotherapy. Moreover, tumor organoids cocultured with PBMCs are also used to enrich tumor-reactive T cells from peripheral blood of patients with mismatch repair-deficient CRC and non-small-cell lung cancer (SCLC); these T cells are useful for assessing the killing efficiency of matched tumor organoids (102). In certain organoids established from immunotherapy-responsive tumors, activation of T cells and tumor killing activity have been identified using PD-1/PD-L1 blockade (98). Meanwhile, Votanopoulos *et al* (103) developed an immune-enhanced tumor organoid model with a high clinical association between these organoids and response to checkpoint inhibitors. Collectively, tumor organoids can predict the response to immunotherapy in cancer.

Identification of novel treatment targets. In a longitudinal, observational co-clinical study, second mitochondria-derived activator of caspase mimetic LCL161 was shown to serve as a treatment target in the organoids derived from recurrent, *KRAS*-mutated liver metastases from rectal cancer (104). Compared with THZ1, YPN-005, a potent inhibitor of CDK7, shows potent antitumor effects in SCLC organoids, suggesting its treatment value in SCLC (105). Based on the interaction of breast cancer organoids and tumor-specific cytotoxic T cells, epigenetic inhibitors GSK-LSD1, CUDC-101 and BML-210 identified via high-throughput screening show antitumor activities (106). Furthermore, BML-210 promotes the efficacy of PD-1-based immune checkpoint blockage.

The tumor-suppressing function and efficient delivery of drugs have key roles in cancer treatment. In multicellular hepatocellular carcinoma organoids (MCHOs) with activated Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ) signaling, there is stromal activation and damaged penetration of verteporfin; inhibiting YAP/TAZ transcriptional activity in hepatocellular carcinoma (HCC) cells may facilitate the penetration of drugs into MCHOs. These findings suggest that the treatment targeting activated tumor stroma may promote drug delivery into HCC cells with increased YAP/TAZ activity (107).

Modeling tumor vascularization. To improve understanding of angiogenic signaling pathways and investigate effective treatment strategies, tumor vasculature must be preserved in organoid cultures. For organoid vascularization, implantation of organoids into highly vascularized tissue is a frequently used method (108,109). Another method is coculture with cells including vascular smooth muscle and epithelial cells (ECs) based on gene editing or microfluidic platforms (110). By integrating mesodermal progenitor cells into organoids, Wörsdörfer *et al* (111) found that the vascularized organoids formed following coculture with tumor cells or neural spheroids and the vessels in tumor organoids are associated with the host vessels after transplantation. Breast cancer organoids with ECs and immune cells show an obvious angiogenic response when cultured with the vascular network (112). Similarly, in the collagen- and hyaluronic acid-enriched ECM with human fibroblasts and MCF-7 cells, vascularized breast cancer organoids have been established successfully (113). Additionally, in the coculture system of organoids and ECs, vascularization is triggered by vascular endothelial growth factors and hypoxia gradients based on compartmentalized microfluidic chips (109,114). The aforementioned findings underscore the importance of coculture models in organoid vascularization.

Modeling tumor-immune interactions. Coculture of tumor organoids with immune components, such as fibroblasts, stroma, ECs and immune cells, models the tumor-immune interactions, which provides insights into cancer immunotherapy (115). Using the tumor organoid culture for expansion and characterization of tumor-reactive T cells, Dijkstra *et al* (102) developed a multifunctional platform to study tumor-immune interactions and concluded that $CD8^+$ T cells in PBMCs of the same patient are activated in half of the CRC organoids, with similar results in non-SCLC organoids. Meanwhile, a platform for expanding tumor-targeted T cells has been reported in patients with pancreatic cancer (116). By coculturing PBMCs and autologous tumor organoids, this platform enables recognition and expansion of tumor-targeted cytotoxic T cells (99). Based on the coculture of tumor organoids with PBMCs, the establishment and functional assessment of tumor-reactive T cells has also been described (98).

By generating organoids from surgically resected types of cancer based on the air-liquid interface, Neal *et al* (98) demonstrated that these organoid cultures retain various endogenous immune cell types and non-immune matrix components; immune checkpoint blockade with anti-PD-1 and/or anti-PD-L1 kills the tumor cells through induction of the expansion and activation of tumor antigen-specific T cells in organoid cultures. Moreover, a previous study suggested the potential of the organoid culture system in predicting adoptive immunotherapy responses following incorporation of patient-specific mature lymph node antigen-presenting cells into organoids (103).

5. Challenges

There are limitations to the application of tumor organoids that need to be addressed. First, there are no standardized evaluation criteria and culture protocols. Currently, the culture conditions of tumor organoids are diverse, leading to large differences in results between laboratories and teams. These differences may arise from inconsistent tissue dissociation, undefined formulation of culture medium and different matrices. To promote the standardization and reproducibility of tumor organoid cultures, culture conditions and laboratory operations should be unified as much as possible. Organoid culture is also affected by tumor cell composition, cell activity and tumor heterogeneity. For certain types of cancer, such as prostate cancer (26), the low success rate hinders repeatability and reproducibility, thereby affecting high-throughput screening. Hence, development of standard culture and

evaluation protocols and application of well-defined materials are required for improving the success rate of organoid generation. Second, due to potential inclusion of normal cells, TME reconstruction is challenging. Tumor organoid models lack certain *in vivo* components, such as endothelial and immune cells and fibroblasts. Although it is challenging to establish the organoids comprising immune and vascular cells, this limitation may be resolved in the future with the development of organoid technology. Third, the currently established tumor organoids are primarily from ECs. In the future, studies should establish organoids from non-ECs, which may further optimize the treatment of tumors such as CRC and lung cancer (117,118). Additionally, during the long-term culture and passage of tumor organoids epigenetic drift may occur (43). To avoid normal cells being contaminated and make organoids more mature, investigating the mechanisms underlying epigenetic drift is needed. Shi *et al* assessed the tumor purity in long-term cultures and found that none were contaminated with normal or non-human cells (119). In addition to recapitulating the biology that drives histologic appearance of original tumors, their organoid models had not drifted at the molecular level. More importantly, tumor organoids should be improved to model the interactions between cells, tissue and organs. Although TME can be replicated through coculture with stromal cells and ECM, the role of peripheral immune systems is not evaluated (77). Combination of tumor organoids with advanced technologies allows modeling of a more complex and realistic state, which may overcome the aforementioned challenges and create more appropriate model systems for cancer treatment.

6. Conclusion

Patient-derived tumor organoids are more advanced at physiological and clinical levels compared with conventional cancer cell lines and PDXs. Despite challenges, tumor organoids show potential in the treatment of cancer. Tumor organoids may be combined with advanced technologies, such as organ-on-a-chip, 3D-bioprinting, tissue-engineered cell scaffolds and CRISPR-Cas9, which may not only overcome defects of conventional culture methods, but also expand the application range, offering insights into the treatment strategies in cancer. Combined application of tumor organoids and advanced technologies allows accurate simulation of tumor heterogeneity, vascularization and tumor-immune interactions, facilitating comprehensive high-throughput drug screening to predict drug responses and optimize treatment options to promote personalized treatment in cancer.

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Authors' contributions

YW, FZ, JH and SW conceived and designed the study and wrote the manuscript. FD acquired data and revised the manuscript critially. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

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