



Review Article

Tumor organoids: A review of culture methods, applications in cancer research, precision medicine, and drug development

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ABSTRACT

Three-dimensional (3D) culture systems known as organoids have emerged as powerful preclinical models for human tumors, supporting the advancement of cancer research from the laboratory to clinical application. They have transformed preclinical cancer research by preserving the complexity and biological characteristics of human cancers. Organoids more accurately replicate the intricate architecture and microenvironment of clinical tumors compared to conventional *in vitro* cell cultures and *in vivo* animal models. Nonetheless, a thorough overview of the potential applications of cancer organoids is still lacking. In this review, we outline the current knowledge of cancer organoid culture. In addition, organoids and 3D cultures can accurately simulate the *in vivo* tumor environment. We explore the key features and underlying processes of tumor development and spread and examine recent progress in using patient-derived tumor organoids for drug testing and immunological research. In additionally, we address the existing obstacles and limitations of organoid technology in clinical settings, along with its future potential. This review underscores the promise of organoids as an innovative approach in cancer therapy and research.

KEYWORDS: Cancer research, Organoids, Patient-derived tumor organoids, Three-dimensional culture systems, Tumor environment

INTRODUCTION

Cancer remains one of the top causes of death globally, posing serious challenges to public health, social stability, and economic growth. This underscores the critical need for advancements in both prevention and treatment. Projections indicate that by 2040, the global number of cancer cases could rise to approximately 28.4 million [1]. Consequently, developing accurate and reliable preclinical tumor models is essential for studying cancer biology and supporting the

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advancement of personalized cancer treatments in clinical practice.

Comparison of two-dimensional and three-dimensional culture models, as well as the patient-derived xenograft animal model (the advantage of three-dimensional-cell culture model)

One of the main challenges in developing effective preclinical models is preserving tumor heterogeneity and accurately replicating the tumor microenvironment (TME). Conventional two-dimensional (2D) cancer cell cultures fall short in areas like high-throughput drug screening, as they lose their native structure and fail to reflect the complex architecture and environmental cues of tumors, which are essential for proper cell-cell and cell-matrix signaling [2]. Recently, several tumor patient-derived xenograft (PDX) models have been established, including breast [3], colorectal [4], lung [5], and pancreatic [6]. PDX models are particularly advantageous due to their ability to maintain vascularized tumors and a functional circulation system, which connects the transplanted tumor to other organs *in vivo*. This feature allows PDX models to accurately mimic metastatic processes, tumor-vascular interactions, and drug pharmacokinetics, providing insights into systemic responses to therapy [7]. Moreover, PDX models retain the molecular and histological features of the original tumor, making them useful tools for investigating tumor diversity and disease progression. However, these models come with certain limitations, including low establishment success rates [8], extended development times, high costs, and concerns about their clinical relevance [9]. A comparison of the strengths and weaknesses of two-dimensional (2D), three-dimensional (3D), and PDX models is presented in Table 1. Furthermore, differences in human immune system can complicate the interpretation of immune-related responses and their clinical relevance [12]. Consequently, the PDX models have not yet been fully validated in human clinical trials [5]. Although patient-derived tumor organoids (PDOs), an advanced 3D culture system, have demonstrated high success rates, easy maintenance, and rapid expansion, they have been proposed as effective preclinical models. In addition, studies have suggested that integrating both PDX and PDO models could open new avenues for the application of precision medicine [13].

The characteristics and the advantages of the three-dimensional cell culture model

To address these issues, 3D cell culture techniques have been established for growing tissues *in vitro* over the last decade. The

development of “organoids” initially stemmed from two primary sources: Pluripotent embryonic stem cells (ESCs) and their engineered counterparts, induced pluripotent stem cells (iPSCs), as well as tissue-specific adult stem cells (aSCs) [14]. Organoids are multicellular structures generated from ESCs, iPSCs, aSCs, or differentiated cells obtained from healthy individuals or patients, including tumor tissues. They have the capacity for self-renewal and proliferation *in vitro* while preserving the structural and functional characteristics of their tissue of origin [15]. Compared to conventional 2D cell culture models, 3D culture systems more accurately replicate the biological functions of real tissues. These systems exhibit: (1) layered cell organization with varying proliferation rates; (2) gradients of nutrients, oxygen, and metabolic waste; (3) authentic interactions between cells and with the extracellular matrix (ECM); (4) gene expression profiles that closely resemble those *in vivo*; and (5) an increased capacity for developing chemoresistance [16]. Two main 3D culture systems are widely used: Multicellular tumor spheroids (MCTS) [17] and PDOs [18]. Spheroids (including MCTS), simple and cost-effective models, exhibit lower structural complexity and are simple, easy-to-use models for drug screening [19]. They effectively mimic *in vivo* tumor characteristics, such as the formation of hypoxic cores, nutrient gradients, and drug penetration barriers, which are essential for assessing therapeutic efficacy [20]. However, spheroids have limited structural complexity and genetic stability over time. In addition, cancer organoids offer unique advantages, including compatibility with emerging technologies and preservation of the architecture, histopathology, and genetic mutations during long-term culture compared to traditional 2D cell cultures, making them valuable *in vitro* models for studying tumorigenic progression and developing personalized drug therapies [14,21]. Accordingly, organoids are promising 3D cell culture models that represent near-physiological models of human cancers and serve as valuable tools for translational cancer research. The following sections provide an overview of different strategies for culturing cancer organoids, along with recent progress spanning from fundamental biological insights to potential clinical applications.

In addition, PDO production remains costly and time-consuming, and the technology remains immature and unstandardized, making its integration into current healthcare systems challenging. Overall, the establishment of 3D cell lines is more time- and labor-intensive than that of traditional 2D cell lines.

Table 1: Comparison of two-dimensional, three-dimensional, and patient-derived xenograft models in preclinical research

Feature	2D cell culture [9-11]	3D organoid culture [9,10]	PDX models [8-10]
Success rate	High	Very high	Low
Reproducibility	High	High	Moderate
Tumor microenvironment fidelity	Poor	Moderate to high	High
Cost	Low	Moderate	High
Time required	Short	Moderate	Long
Applications	Drug screening, basic research	Drug screening, personalized therapies	<i>In vivo</i> validation, translational research
Structural complexity	None	Mimics <i>in vivo</i> structure	Fully mimics tumor features
Maintenance	Easy	Easy to moderate	Difficult
Clinical relevance	Low	Moderate to high	Moderate

PDX: Patient-derived xenograft, 3D: Three-dimensional, 2D: Two-dimensional

ORGANOIDS ESTABLISHMENT

Organoids are self-organizing, 3D tissue models that replicate organ development *in vitro*. They are derived from stem cells, such as ESCs, iPSCs, or aSCs, as well as patient-derived tumor tissues, and are cultivated in a specialized 3D environment that mimics the *in vivo* TME. These organoids retain the characteristics of their original organs and can be stably expanded in 3D culture systems, cryopreserved in biobanks for further research, and easily manipulated using techniques similar to those used in traditional 2D cell culture system [22]. Organoid culture techniques differ based on the source tissue. Typically, tissue samples or biopsy specimens are processed through enzymatic and/or mechanical digestion. The resulting cell mixtures can then be cultured using various methods: Embedded in a basement membrane extract (BME) [23], maintained in a floating primary culture [24], or grown at the air–liquid interface (ALI) using Transwell inserts [25]. Organoids are usually passaged every 1–2 weeks, and the culture medium is specifically tailored to reflect the characteristics of the tissue from which the organoids were derived [21]. An essential aspect of organoid culture media is the inclusion of key growth factors. For instance, R-spondins serve as Wnt signaling agonists that support stem and progenitor cell function and guide cell fate decisions. Meanwhile, BMP pathway inhibitors like Noggin or Gremlin 1 help prevent unwanted differentiation [26]. As an example, small intestinal organoids are maintained using a combination of epidermal growth factor, R-spondin, and Noggin within a BME matrix [27]. In contrast, the growth and expansion of colonic organoids require additional components such as nicotinamide, prostaglandin E2 (to support human gastrointestinal cell growth), and inhibitors targeting p38 and TGF- β /Activin signaling pathways, such as A83-01 [28]. Furthermore, the culture medium composition critically influences organoid growth, as specific recipes may enrich particular cell subpopulations, potentially limiting the organoids' ability to fully reflect the original tumor's heterogeneity [29].

At present, both normal and cancerous organoids can be developed from a variety of sources, including iPSCs, ESCs, aSCs, differentiated iPSCs, and tissue-derived cancer cells obtained from patient samples or liquid biopsies collected via surgical procedures or needle aspiration [30]. Numerous cancer organoids have been successfully established from both primary and metastatic tumors across various cancer types, including breast [31] and liver [32].

Multiple factors can influence not only the growth rate and size of cancer organoids but also the overall stability and success of the culture. These include the chosen culture

system and conditions, the viability and level of necrosis in the original tumor tissue, whether the sample was collected before or after treatment, as well as the timing and techniques used during sample processing [21,33]. Furthermore, monitoring and characterizing the genetic and phenotypic stability of organoids over time is crucial to confirm their fidelity to the original tumor. Techniques such as transcriptome sequencing, whole exome sequencing, whole genome sequencing, and DNA methylation profiling can be employed to detect genetic mutations, assess gene expression patterns, and analyze key signaling pathways [34]. In addition, single-cell RNA sequencing (scRNA-seq) has become a valuable tool for exploring the transcriptomic profiles of individual cells within organoids. This technique has been applied to patient-derived pancreatic ductal adenocarcinoma organoids, uncovering cellular heterogeneity and a conserved developmental hierarchy [35]. Moreover, scRNA-seq has been used to compare the single-cell transcriptomes of primary tumors and their corresponding organoids in pancreatobiliary cancers, providing deeper insights into cellular composition and tumor fidelity [36]. Those provide insights into their cellular composition and potential applications in diagnostics and translational studies.

APPLICATIONS OF ORGANOIDS THROUGH TECHNOLOGICAL AND METHODOLOGICAL REVOLUTION

Several organoid technologies and methods have been established to fulfill the various requirements of cancer research. First, various cancer organoid biobanks, such as colorectal cancer (CRC) [37] and breast cancer [31], have been developed for drug screening and the study of tumor molecular characteristics, promoting the development of therapeutic approaches for precision and personalized medicine. Second, organoid–immune cell coculture models have been primarily developed using two main approaches: (1) direct coculture, where exogenous immune cells, either autologous or allogeneic, or endogenous immune cells are introduced and cultured alongside tumor organoids [38]; and (2) organoid-on-a-chip systems, which involve coculturing tumor organoids with immune cells within a microfluidic, continuously perfused chamber designed to replicate key features of the TME [39,40]. And overview of the diverse applications of organoid models and their significance in advancing cancer research is summarized in Table 2.

Table 2: Summary of tumor organoid models and their applications in cancer research

Tumor organoid model	Applications	References
Colorectal cancer organoids	Drug screening, tumorigenesis studies, immune cell coculture, precision medicine	[33-37]
Breast cancer organoids	Drug screening, gene editing, immunotherapy, precision medicine	[41]
Gastric cancer organoids	Tumorigenesis studies, drug screening, immune cell coculture, precision therapy	[40]
Pancreatic cancer organoids	Tumorigenesis studies, drug screen, immune checkpoint inhibitor testing	[35,36]
Lung cancer organoids	Drug screening, personalized therapy, tumorigenesis, immune cell coculture	[42,43]
Prostate cancer organoids	Screening of FDA-approved drugs, immune therapy evaluation, personalized medicine	[41-52]
Liver cancer organoids	Tumorigenesis studies, drug screening, immune therapy evaluation	[53]

FDA: Food and Drug Administration

APPLICATION OF VARIOUS CANCER ORGANOID IN CANCER RESEARCH

Organoids are valuable preclinical models due to their capacity to closely replicate the genetic, phenotypic, and cellular characteristics of the original tissue. Tumor-derived organoids function as personalized tumor models, reflecting the unique features of individual cancers. Notably, a 2018 study by Vlachogiannis *et al.* demonstrated that tumor organoids showed excellent predictive performance for patient drug responses, reporting a sensitivity of 100%, specificity of 93%, positive predictive value of 88%, and negative predictive value of 100% [54]. In addition, organoid models are suitable for gene editing using genome editing technologies to investigate the effects of specific oncogenic mutations [34]. Organoids have been utilized as *in vitro* tumor models to investigate tumor biology, perform drug screening, evaluate the responsiveness of PDOs to immunotherapy, and support the development of personalized treatment strategies.

ORGANOIDS IN TUMORIGENESIS MECHANISM STUDY

Cancer is caused by the accumulation of somatic mutations in genes that primarily control cell cycle and cell proliferation, and tumorigenesis is characterized by a series of multistep genetic alterations [55]. Organoids preserve the genetic heterogeneity of primary tumor tissue while maintaining genomic stability during long-term culture [55]. For example, pancreatic and gastric organoids harboring the *Kras* (G12D) mutation and *p53* loss contribute to dysplasia, while the combination of *Apc*, *p53*, *Kras* (G12D), and *Smad4* mutations is required for primary colon organoid formation, with this study also validating *miR-483* as a dominant driver oncogene in the *IGF2* region at chromosome 11p15.5 [25]. Tumor organoids have also been used to investigate tumorigenic signaling pathways. Using colon cancer organoids, it was found that lnc-RP11-536K7.3 plays an important role in resistance to oxaliplatin, primarily through the SOX2/USP7/HIF-1 α signaling axis [56]. Several cancer organoids have been established using CRISPR/Cas9-mediated genome engineering [57]. Furthermore, using CRISPR/Cas9, a gene editing technology, to introduce oncogenic mutations, tumor organoids that mimic the process of tumorigenesis, allowing researchers to investigate the mechanisms underlying cancer development [58]. Using CRISPR/Cas9 genome editing, the introduction of *ARID1A* mutations, which are common in human cancers, causes morphological dysplasia, tumorigenicity, and mucinous differentiation in gastric cancer organoids with *FOXMI1/BIRC5*-simulated tumorigenesis [57]. In intestinal tumor and CRC organoids, CRISPR/Cas9 validated some CRC driver genes, showing that mutations in activating and TGF- β receptors synergistically promote tumorigenesis, with *Acvr1b*, *Acvr2a*, and *Arid2* acting as tumor suppressors [59]. Besides CRISPR/Cas9, CRISPR-Cas9-mediated homology-independent organoid transgenesis (CRISPR-HOT) has been developed to precisely integrate transgenes at specific sites in organoids, representing various tissues; also, combining CRISPR-HOT with fluorescent labeling enables the visualization of subcellular structural molecules [60]. The combination of CRISPR/Cas9

and CRISPR-HOT methods successfully generated long-term expansion of human fetal hepatocyte organoids, enabling the investigation of disease-related gene functions as well as cellular differentiation and division processes [60]. In addition, artificial intelligence (AI) provides robust tools for analyzing complex organoid datasets, ranging from scRNA-seq to high-content imaging, enabling deeper insights into disease mechanisms and therapeutic responses [61]. The integration of organoid and gene-editing technologies with AI-driven data analysis represents a powerful approach for investigating the roles of tumor driver genes in tumorigenesis and the related signaling pathways.

ORGANOIDS AS PLATFORM FOR SCREENING OF ANTITUMOR DRUG

Drug screening is crucial for disease treatment but is often time-consuming and costly, with low success rates [62]. Organoids mimic the structure and complex tumor tissue environment while retaining the characteristics of the original organs *in vivo*. Tumor organoids offer a promising solution by mimicking tumor growth more effectively than traditional models. For example, lung cancer organoids retain tumorigenic properties and genetic mutations of the original tumors, and their response to targeted therapeutics closely mirrors that of the parental tumor, demonstrating their reliability for antitumor drug screening [63]. Consequently, organoids offer a powerful platform for drug screening, enabling the identification of genetic alterations associated with drug responsiveness. This approach holds great promise for driving progress in personalized precision medicine.

Organoids in tumor precision therapy

Precision therapy focuses on customizing treatment plans for individual patients by considering their unique tumor profiles and specific pharmacodynamic responses, which are still not fully understood. PDOs serve as optimal models for this approach, as they preserve the genetic and structural characteristics of the original tumors. This enables high-throughput screening of various anticancer drugs to determine the most effective therapeutic options for each patient.

The establishment of tumor organoid biobanks has enabled the simultaneous screening of numerous anticancer agents across various cancer organoids, such as those from colorectal [27,64] and breast cancer [65], to assess their antitumor potential and confirm their efficacy in clinical trials. Studies have shown that prostate cancer organoids can be used to screen Food and Drug Administration (FDA)-approved drugs such as multikinase inhibitors (ponatinib, sunitinib, and sorafenib) to identify the most suitable therapeutic agents [44]. Furthermore, organoids have also been instrumental in screening drugs with fewer side effects, as observed in studies on colorectal diseases [64] and breast cancer [65], etc.

Organoids represent a transformative advancement in cancer research, offering unparalleled insights into tumorigenic mechanisms, drug screening, and precision therapies. Organoids offer a crucial link between conventional experimental models and real-world clinical applications, providing a more accurate representation of human tumors. This advancement has the potential to greatly enhance cancer

treatment outcomes and deepen our understanding of the disease's complexity [Figure 1].

ORGANOIDS MODEL OF TUMOR MICROENVIRONMENT AND IMMUNOTHERAPY STUDIES

In TME, tumors evade attacks from immune cells and constrain cancer outgrowth [45]. One of the key limitations in using organoids to evaluate immunotherapeutic responses is the lack of immune cell components. Recently, there has been growing interest in incorporating immune elements into organoid models to better replicate the TME. This enables the study of cancer immunobiology and helps identify patients likely to respond to immunotherapy [Figure 2]. Two primary coculture approaches have emerged: (i) maintaining and expanding endogenous immune cells within tumor organoids or introducing exogenous immune cells – either autologous or allogeneic [38]; and (ii) utilizing organoid-on-a-chip technology, which enables dynamic coculture of tumor organoids with immune cells in a perfused microfluidic chamber that mimics the architecture and conditions of the TME [39,40]. Organoid-on-chip has been established to mimic the TME, preserving tumor heterogeneity [46]. Innovative platforms like the integrated superhydrophobic microwell array chip (InSMAR-Chip) have revolutionized lung cancer organoid generation by enabling rapid, high-throughput production. This technology allows for the

assessment of clinically relevant drug responses within just 1 week, significantly accelerating the pace of personalized treatment evaluation [47]. Furthermore, the integration of organoid-on-chip technologies with sensors for real-time monitoring offers exciting possibilities for drug screening and optimizing personalized treatment strategies [48]. In 2022, the U.S. FDA approved organoid-on-a-chip research, with preclinical data progressing to clinical trials (NCT04658472). This milestone highlights the increasing importance of organoid-on-a-chip platforms in *in vitro* drug testing and their emerging role in the development of new therapeutics [49]. The shortcomings of the organoid-on-chip include the absence of immune cells and vascular structures [46]. To address this, organoid-on-chip integrated microfluidic technology has been used to create orthogonal gradients, which help regulate the development of the central nervous system [50]. In addition, another organoid chip has been developed that integrated immune cells into organoid models in a continuously perfused chamber to mimic the TME, as described in the following sections. The TME can be recapitulated by coculturing tumoroids and either tumor-infiltrating lymphocytes (TILs), stromal cells, or CAFs [51].

Immune checkpoint inhibitors and adoptive cell transfer therapy

Immune checkpoint inhibitors (ICIs) are a prominent class of immuno-oncology therapies that target key immune regulatory molecules, including programmed cell death-1 (PD-1), its ligand PD-L1, and cytotoxic T-lymphocyte-associated protein

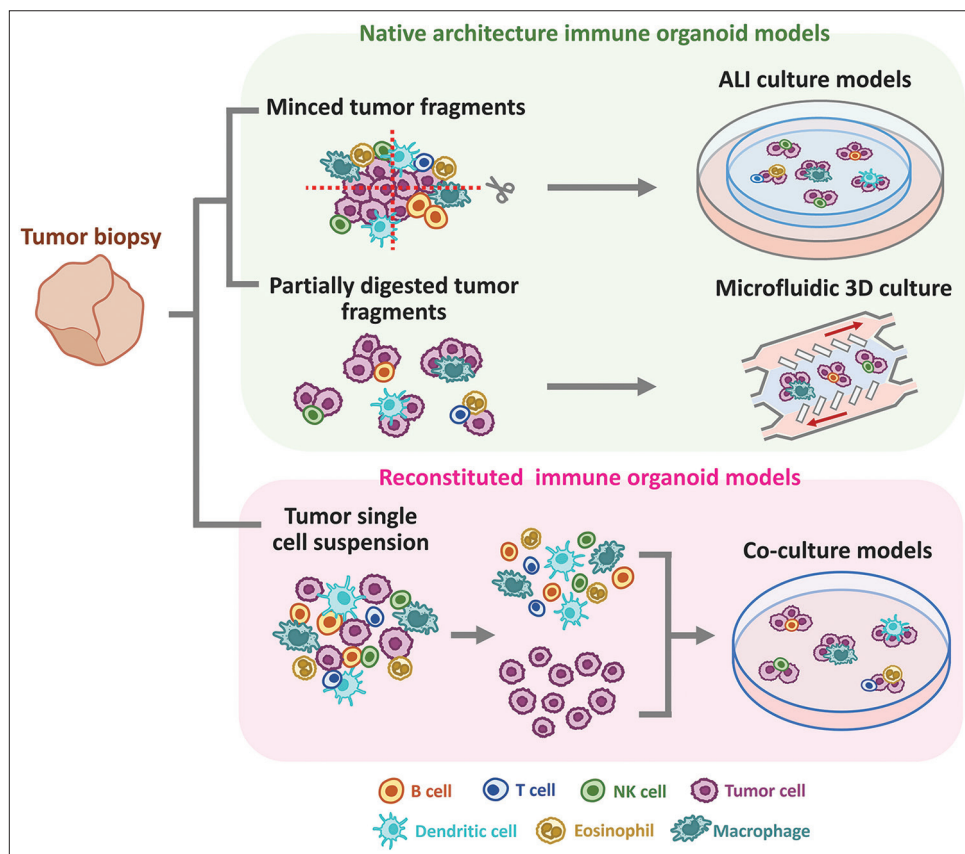


Figure 1: Various cancer organoids and their application in cancer research

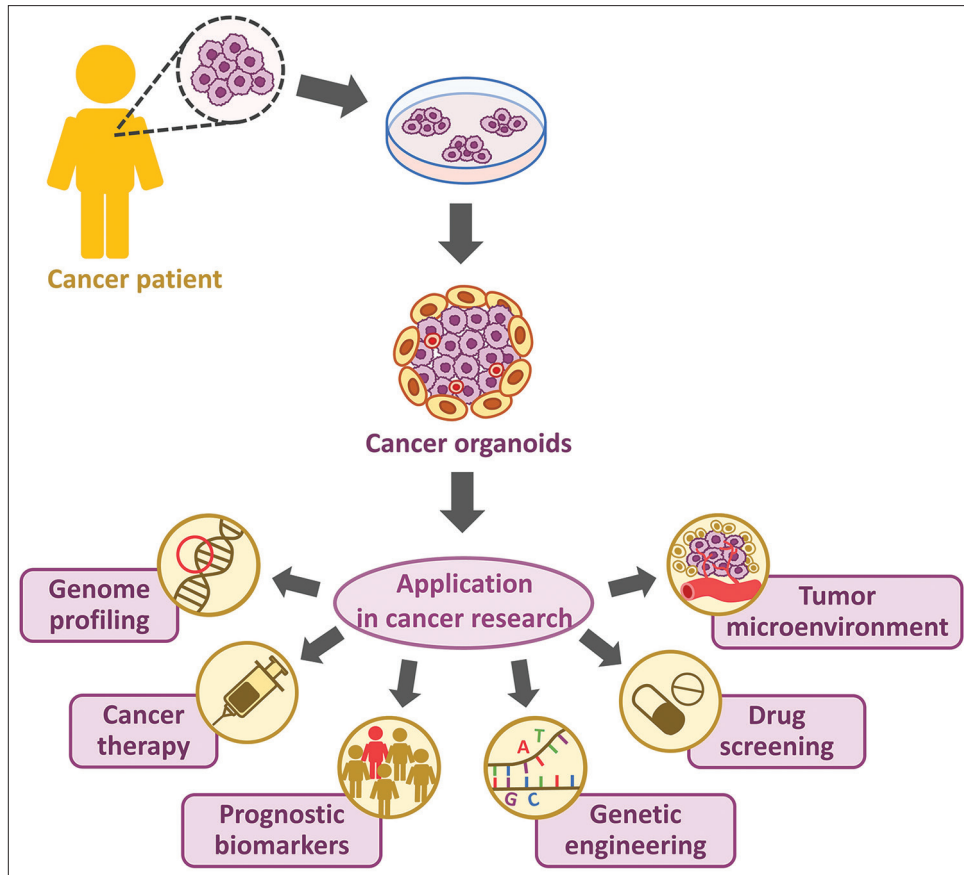


Figure 2: The coculture system of tumor organoids serves as an *in vitro* model of the tumor immune microenvironment

4. These therapies have notably enhanced clinical outcomes and survival rates in several cancer types, such as lung cancer [66] and head-and-neck cancer [67].

Adoptive cell transfer therapy represents another significant advancement in the field of immunotherapy. In a study by Kong *et al.*, CRC organoids were cocultured with autologous TILs to evaluate the effects of anti-PD-L1 antibody treatment. The results showed that this approach successfully restored the cytotoxic activity of TILs against the tumor cells [68]. Courau *et al.* demonstrated that CRC organoids cocultured with autologous TILs such as T and NK cells showed enhanced immune cell cytotoxic activity when an anti-NKG2A antibody was added to block the inhibitory signaling caused by the interaction between NKG2A and HLA-E [69]. Furthermore, comprehensive culture systems such as microfluidic platforms and ALI methods provide advanced tools for functionally modeling ICIs. These approaches allow for more physiologically relevant interactions between tumor cells and immune components, enhancing the evaluation of ICI responses *in vitro*. The ALI culture system effectively replicates the oxygenation and microenvironment dynamics of epithelial tissues exposed to air, making it a physiologically relevant model for respiratory, gastrointestinal, tumor-specific, and immune-related research [70]. Compared to conventional submerged cultures, ALI cultures provide accurate oxygen gradients, gas exchange, hypoxia maintenance, and functional epithelial differentiation, facilitating studies on

host-pathogen and immune-tumor interactions. However, limitations include restricted applicability to certain tissues and solid tumors (e.g., liver, pancreas, and brain), challenges in controlling oxygen levels, longer differentiation periods, complexity, and higher costs. ALI cultures do influence epithelial tissue differentiation and immune modulation [71]. They enhance cell proliferation, epithelial layer thickness, and the apical localization of microvilli-associated villin, closely resembling intestinal epithelial morphology *in vivo* [72]. In addition, human tracheobronchial epithelial cells cultured at the ALI produce apical secretions with a protein composition similar to induced sputum. Mass spectrometry identified 186 proteins, with mucins as major components, demonstrating the model's relevance for studying airway mucus-related innate defense mechanisms [71]. 3D microfluidic cultures replicate *in vivo* therapeutic sensitivity and can be used to study the underlying mechanisms of response and resistance to ICIs, develop novel combination therapies, and facilitate precise tumor immunotherapy [73]. In an ALI culture system using PDOs from clear-cell renal cell carcinoma, blocking PD-1 and/or PD-L1 was found to promote the expansion of antigen-specific T cells. This intervention also enhanced the cytotoxic activity of these T cells against the PDOs, demonstrating the potential of ALI systems for evaluating immunotherapeutic responses [74]. Dijkstra *et al.* cocultured CRC and NSCLC organoids with autologous circulating CD8⁺ T cells in combination with anti-PD-1, anti-CD28, and

interleukin-2 (IL-2) to induce T cell-mediated killing and MHC-dependent cytotoxicity against tumor organoids [75]. However, coculture systems can be challenging due to factors such as difficulty in maintaining long-term culture stability, variability in immune cell interactions, and the complexity of mimicking the entire TME, which may be less pronounced in conventional models. Despite these challenges, coculture systems allow for more personalized and realistic evaluations of immune responses in cancer treatment [76]. In addition, a coculture model of PDOs with chimeric antigen receptor (CAR)-engineered lymphocytes was used to monitor the cytotoxicity of CAR lymphocytes against PDOs, exploring CAR efficacy and tumor specificity in a personalized manner [77]. The personalized cytotoxic effects of CAR-T cells have been investigated using coculture models involving PDOs and immune cells, specifically in glioblastoma [78] and hepatocellular carcinoma [79]. These studies highlight the value of PDOs as *in vitro* platforms that maintain key features of the TME, supporting the advancement of more targeted and effective immuno-oncology therapies.

Oncolytic virotherapy

Recently, oncolytic adenoviruses have emerged as highly promising candidates owing to their ability to effectively transduce both proliferating and quiescent cells while carrying large transgenes. This capability contributes to their direct oncolytic effects, as the resulting lytic cell death induces antitumor immune responses, such as the release of cytokines and tumor antigens, which help modulate the TME and improve therapeutic responses [80]. A PDO model of renal cell carcinoma was utilized to evaluate the antitumor efficacy of a novel PD-L1 ICI, a cross-hybrid Fc fusion peptide combining IgA1 and IgG1 constant regions fused to the PD-1 ectodomain, delivered via an oncolytic adenovirus. This engineered construct demonstrated tumor-killing activity comparable to that of established agents such as atezolizumab, IgG1-PD-L1, and IgA-PD-L1. However, the full spectrum of immune responses induced by these oncolytic viral therapies remains to be thoroughly characterized [81].

Growing evidence suggests that PDOs can serve as a platform for evaluating the selectivity and efficacy of oncolytic virotherapy as a novel personalized medicine. Organoid-based models are poised to greatly enhance both fundamental research and translational efforts in immuno-oncology. By providing physiologically relevant platforms, these approaches have the potential to accelerate the development of personalized immunotherapies and improve treatment outcomes for cancer patients.

Current limitations and future opportunities

While cancer organoids successfully replicate numerous aspects of human tumor development and progression, there is still room to enhance their pathophysiological accuracy and clinical relevance. Although *in vitro* 3D organoids mimic certain structural features of organs, further refinement is needed to better represent the complexity of real tumor environments and improve their utility in clinical applications. In TME, cancer organoids interact with other cells, such as CAFs and immune cells; to better mimic these interactions, coculture models

that incorporate organoids with CAFs or immune cells have been developed. These models aim to provide a more accurate representation of the cellular dynamics and interactions within the TME [69].

These models include cocultures with engineered immune cells or tumor-infiltrating/autologous lymphocytes using microfluidic devices (microfluidic 3D culture) and ALI methods. However, there are several limitations to this approach, including challenges in maintaining long-term cultures and the presence of heterogeneous fragment [82]. In addition, maintaining immune cell viability and functionality in organoid cocultures often requires supplementation with factors such as IL-2, anti-CD3, and anti-CD28 antibodies [83]. Furthermore, while Matrigel – a non-hysiological ECM derived from animal sources and rich in components like laminin and collagen – is commonly used to support 3D organoid growth, its nonhuman origin and variable composition may impact experimental reproducibility and influence treatment responses [21,82]. Furthermore, as mentioned above “organoid-on-a-chip platform” represents a promising evolution in cancer research by combining the 3D complexity of organoid cultured with the dynamic microfluid environments of chips technology. There are some limitations of “organoid-on-a-chip platform” including these system components are often absent or only partially integrated, limiting the physiological fidelity of it [84], discrepancies between *in vitro* outputs from “organoid-on-a-chip platform” and clinical data underscore the need for further optimization to ensure that these systems adequately recapture the complexity and heterogeneity observed in human cancer [85]. Therefore, future advancements should prioritize integrating multi-organ systems, coculturing immune components with tumor organoids, and dynamically simulating systemic interactions to overcome current limitations and create more precise models for cancer research and therapeutic testing [85].

Despite their limitations, organoid cultures remain highly physiologically relevant and serve as powerful models for translational research. They hold significant promise in advancing personalized cancer drug development by closely mimicking patient-specific tumor biology. In addition, an *in vitro* tumor model optimized for Matrigel-free conditions was developed, allowing short-term cell expansion from primary tumors, which strongly correlates with clinical outcomes [86]. By eliminating extrinsic components like Matrigel, this model improves the accuracy of drug response predictions, enabling a more precise identification of the patient’s optimal therapeutic window. In 2022, the U.S. FDA Modernization Act 2.0 significantly impacted *in vitro* research, promoting alternatives to animal testing such as human-iPSCs, AI models, organoids, and organs-on-chips [87]. Following its enactment, government agencies, and private investors increased funding for organoid research, enhancing its appeal as a viable alternative. The removal of animal testing requirements has spurred innovation in organoid-based disease models, drug screening platforms, and personalized medicine, while also advancing high-throughput screening technologies for pharmaceutical development [87,88]. This legislative shift has accelerated organoid-based research, driving funding,

innovation, and industry adoption, further establishing it as a transformative approach in biomedical sciences.

Variability in the generation of PDOs arises from the absence of standardized protocols, as well as ethical concerns related to informed consent, data privacy, and the potential transplantation of organoids into humans. Addressing these challenges is essential for ensuring consistency, ethical integrity, and the safe clinical translation of organoid technologies [89]. Organoid culture methods differ based on the tumor type, the nature of the starting material (whether fresh or frozen), and prior treatment history. Tumor shrinkage resulting from therapy can significantly influence the success rate of organoid establishment and growth [90]. Similarly, organoids derived from metastatic tumors, particularly those involving interorgan spread, are challenging to establish and often require validation through mouse xenograft models [21]. In addition, tumor-derived organoids typically exhibit slower growth compared to those from healthy tissues. This may be attributed to increased mitotic failure and higher rates of cell death in cancer cells, which can result in the overgrowth or contamination of tumor organoids by healthy tissue-derived cells [91]. Non-solid tumors are currently difficult to model as organoids, with brain organoids posing additional complexity due to their intricate architecture and functional diversity. These challenges largely stem from limitations in existing culture systems, which are not yet fully equipped to replicate the structural and microenvironmental nuances of such tissues [92]. In addition, organoid research must navigate the stringent data privacy standards of the EU GDPR, emphasizing individual rights and robust enforcement, alongside the fragmented, sector-specific frameworks in the U. S., such as HIPAA, requiring adaptive compliance strategies to protect patient data and ensure ethical integrity [93].

Establishing PDOs and conducting anticancer drug screening can take several weeks, which may reduce the likelihood of alignment within a patient's optimal therapeutic window [21]. In addition, PDO production remains costly and time-consuming, and the technology remains immature and unstandardized, making its integration into current healthcare systems challenging. Overall, the establishment of 3D cell lines is more time- and labor-intensive than that of traditional 2D cell lines [94].

High-throughput screening using cancer organoids as model systems remains limited owing to nonstandardized analysis methods and the automation required to detect metabolic cell viability [94]. Therefore, readouts for high-throughput tumoroid screening have been developed [95]. The development of optical metabolic imaging enables the measurement of cellular metabolism in response to drug treatment, reduces drug turnover times, and facilitates the exploration of drug responses to individualized cancer treatment [96]. In addition, computational analysis methods such as multiparametric microscopy-based readout and a trained Stardist-3D convolutional neural network are still undergoing optimization for imaging 3D structures with defined parameters for their phenotypes [97]. The difficulty in calculating total cell numbers, combined with the complexities of

organoid culture methods, hinders the accuracy and efficiency of high-throughput screening, especially when culturing organoids with ECMs in multiwell plates, such as 384-well plates [98]. 3D bioprinting enables precise spatial control over the TME by allowing the deliberate placement of predefined bio-inks composed of various cell types, ECM components, and biochemical cues. For example, chimeric organoids created by coprinting cancer cells with normal breast epithelial cells have demonstrated that cancer cells within these constructs exhibit significantly elevated levels of 5-hydroxymethylcytosine compared to tumor-like cells printed alone. This highlights the potential of 3D bioprinting to model tumor heterogeneity and epigenetic regulation more accurately [99]. An integrated platform combining organoid technology, microfluidics, 3D bioprinting, and real-time monitoring has been developed to enable high-throughput drug screening within a 1-week timeframe. This automated system supports the rapid evaluation of therapeutic responses, advancing the potential for personalized medicine by tailoring treatments based on patient-specific organoid models [21]. In addition, organoid technology has been expanding its applications beyond cancer research. For instance, organoid platforms have demonstrated potential in modeling neurodegenerative diseases such as Alzheimer's disease and infectious diseases like SARS-CoV-2, showcasing their versatility in diverse biomedical fields [100]. Once current limitations are addressed, organoid technology holds the potential to transform cancer care and the treatment of other diseases, offering renewed hope to patients. This advancement could significantly contribute to improving human health and overall well-being by enabling more precise, personalized, and effective therapies.

CONCLUSION

Several tumor organoid models have been developed in recent years. Organoids were first developed from stem cells, and subsequently, culture methods for various tumor organoids derived from the tumor tissues of cancer patients have been established. These organoids retained the key characteristics of the original tissue *in vivo*, even after long-term culture, and successfully mimicked the complex architecture of tumor tissues and their microenvironment.

Genotyping and phenotyping profiles of organoids, as well as the underlying mechanisms, have been studied by integrating genome editing technologies (including CRISPR-Cas9 and CRISPR-HOT) with microfluidic devices and chips (such as microfluidic organoids-on-a-chip). Further applications of cancer organoids include drug screening for precise anticancer therapies, selection of cancer immunotherapies, and the identification of therapeutic responses to oncolytic virotherapy.

Organoid-based clinical decision-making faces many obstacles, such as organoids and *in vitro* models lack several *in vivo* mechanisms that are crucial for fully understanding tumor biology and treatment responses. In addition, as mentioned above, there are still some limitations associated with tumor organoids. However, ongoing research is expected to address these issues, making tumor organoids a powerful tool in cancer research and personalized medicine.

Data availability statement

The present study does not involve data sharing since no datasets were generated or analyzed.

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Conflicts of interest

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