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3D tumor cultures for drug resistance and screening development in clinical applications

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Abstract

Tumor drug resistance presents a growing challenge in medical practice, particularly during anti-cancer therapies, where the emergence of drug-resistant cancer cells significantly complicates clinical treatment. In recent years, three-dimensional (3D) tumor culture technology, which more effectively simulates the in vivo physiological environment, has gained increasing attention in tumor drug resistance research and clinical applications. By mimicking the in vivo cellular microenvironment, 3D tumor culture technology not only recapitulates cell-cell interactions but also more faithfully reproduces the biological effects of therapeutic agents. Consequently, 3D tumor culture technology is emerging as a crucial tool in biomedical and clinical research. We summarize the benefits of 3D culture models and organoid technology, explore their application in the realm of drug resistance, drug screening, and personalized therapy, and discuss their potential application prospects and challenges in clinical transformation, with the aim of providing insights for optimizing cancer treatment strategies and advancing precision therapy.

Keywords Cancer resistance, 3D tumor culture model, Organoids, Tumor microenvironment, Drug screening

Introduction

Cancer is one of the leading causes of premature death worldwide [1, 2]. The health burden associated with cancer is projected to rise substantially in the coming decades [2]. A challenge in preclinical cancer research will be the development of model systems capable of reliably mimicking the patient's condition. An effective model should ideally replicate tumor physiology in vivo. However, the heterogeneity of tumors and the complexity of the tumor microenvironment (TME) pose significant challenges to clinical treatment. For patients, timely and optimal treatment is often critical. While two-dimensional (2D) tumor cell culture models, commonly used for high-throughput drug screening and analysis, are cost- and time-efficient, they generally fail to accurately replicate the complex tumor physiology [3]. Mouse models, including genetically engineered mouse models and

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patient-derived tumor xenografts(PDX), are both expensive and time-consuming, limiting their utility in studying complex tumor physiology. Additionally, these models raise ethical concerns regarding animal welfare, and their predictive value for human disease is often limited [4]. In contrast, tumor organoids and three-dimensional (3D) cell culture systems offer promising alternatives. These in vitro models offer 3D structural and functional characteristics, resulting in significant cost and time savings. Additionally, they are believed to better replicate tumor physiology, potentially bridging the gap between simple 2D cell cultures and complex mouse models [3].

Establishing accurate preclinical drug screening models is essential prior to administering antitumor therapies. Traditional 2D cell culture models fail to accurately replicate the phenotype and genetic characteristics of tumor cells in vivo, and they do not capture the complexity or dynamic progression of cancer. However, 3D culture system offers a more accurate simulation of physiological and disease states in the humans, eliminating the disparities between animal models and human conditions. Compared to animal experiments, 3D culture systems offer significant cost reductions in drug screening. This is particularly important as approximately most of in-vivo results from drug screening do not align with clinical trial outcomes [5]. 3D culture systems can also considerably shorten the screening timeline, providing a more diverse array of cell types and greater physiological complexity, thereby improving the relevance and efficiency of the drug screening process. Animal and patient-derived organoids (PDOs) are miniature 3D tumor cell models cultured in the laboratory from primary tumor samples collected from patients [6, 7]. This allows the PDOs model to achieve more accurate phenotypic replication and efficient growth of tumor organoids [8]. Because PDOs closely resemble the histological features of the corresponding parental tumor and can reproduce the physiological functions of this organ, they have become widely used in various fields. These include tumor etiology, drug resistance studies, and personalized medicine research(Fig. 1). This review summarizes the advantages of 3D culture models and organoid technology, highlighting their significant contributions to drug resistance, drug screening, and personalized therapies. Furthermore, it discusses the potential clinical applications of these technologies, along with their prospects and challenges in clinical translation. The paper aims to provide a foundation for the development of optimized tumor treatment strategies and precision therapies, offering insights into how these advanced models could enhance the accuracy and efficacy of cancer treatment.

3D cell culture

Cell growth, differentiation, and organization in 3D cell culture technology closely resemble the behavior of tissues in vivo. This technology allows cells to grow and interact within a 3D space, forming physiologically relevant structures. Its primary advantage lies in overcoming the limitations of 2D culture by enabling the simulation of the 3D structure of cells to the greatest extent, fully utilizing the function of tumor cells, thereby fully harnessing the functional capabilities of tumor cells. This system supports cell adhesion, extension, and differentiation [3]. 3D culture systems include suspension drop culture, rotating cell culture, 3D scaffold-based culture, and 3D bioprinting technology (Fig. 2).

Suspension drop culture method

The hanging drop culture method involves placing droplets of cell suspension on the underside of a culture plate, utilizing surface tension. The culture is then rotated, allowing the cells within the droplets to aggregate into a 3D structure, driven by gravity and intercellular adhesion. This method is straightforward and does not require special instruments or equipment [9]. However, the volume of the cell suspension in each hanging drop is typically limited by surface tension, restricting the number of cells that can be cultured. Additionally, the hanging drop method can be cumbersome and prone to errors in drug handling and morphological observation [10]. These limitations make it unsuitable for large-scale culture applications, as maintaining consistency and control over the process becomes challenging.

Rotating cell culture

Cell culture vessels and coaxial rotating oxidizers make up rotating cell culture. As the system rotates, the culture container, filled with media, rotates around a horizontal axis. This rotation facilitates the uniform distribution of nutrients and oxygen while preventing cell sedimentation, allowing cells to grow in a more 3D environment [11]. During rotation, the medium rotates at the same angular speed, and the cells remain suspended due to the combined effects of gravity, centrifugal force, and Coriolis force, leading to their aggregation into tissue-like 3D structures. In the rotating cell culture system (RCCS) [12], cells are exposed to minimal mechanical external forces while receiving adequate nutrients, oxygen, and other essential substances. This environment effectively promotes cell proliferation, induces differentiation, and facilitates cell signaling. The RCCS relies on the use of propellers and stirrers, generating very low shear force and causing minimal damage to cells, making it especially suitable for the growth of nerve cells. Additionally, the volume of the rotary fermenter can be scaled up, making it ideal for large-scale cell culture.

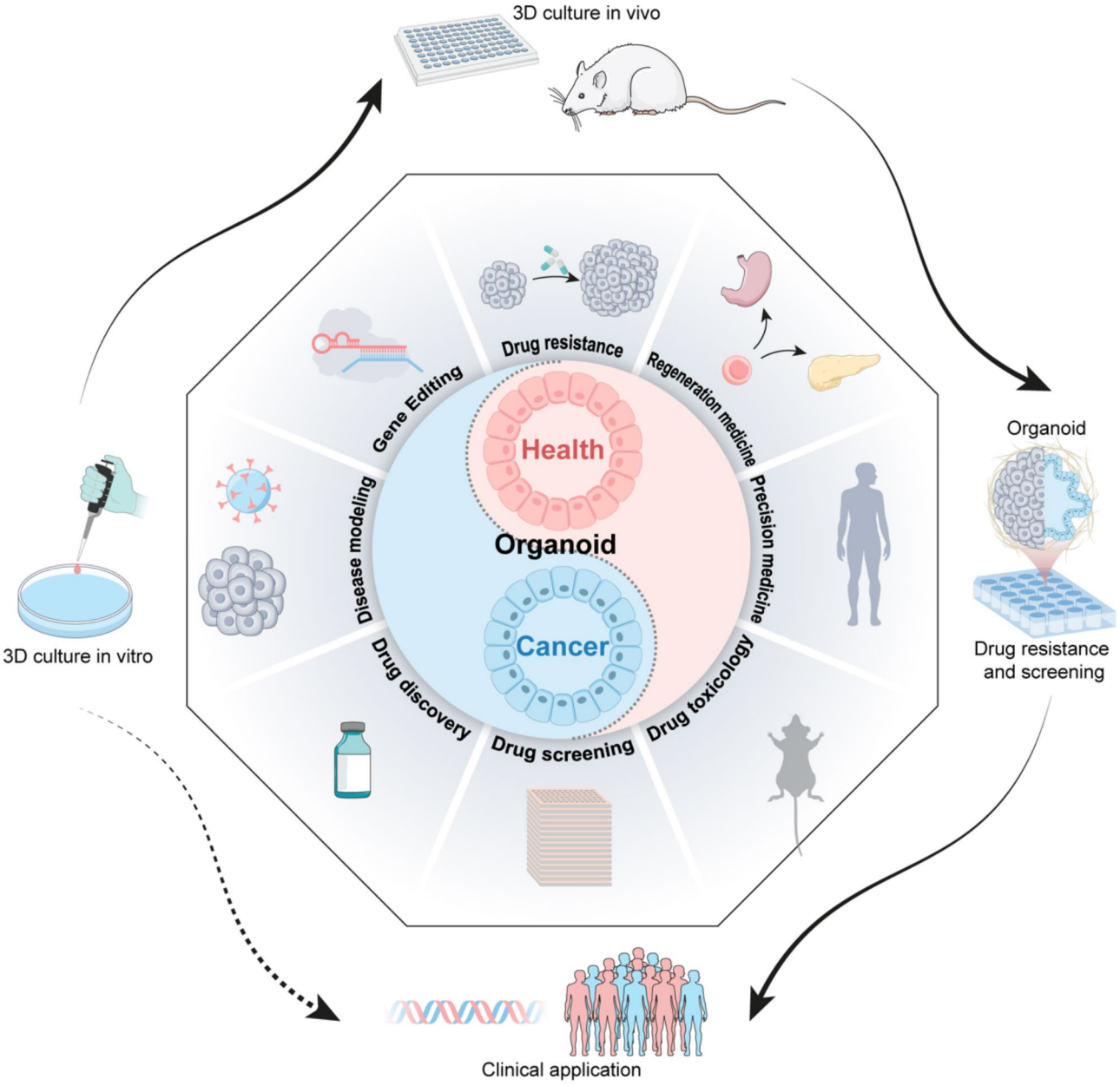

Fig. 1 A schematic depicting the balance of Yin and Yang through the Chinese Eight Trigrams, applied to health and cancer in 3D cultures, to support the development of drug resistance mechanisms and screening strategies for clinical applications

3D scaffold support culture

In 3D scaffold-based cell culture involves inoculating or dispersing cells within a loose, porous scaffold structure to form 3D cell structures. The ideal scaffold material should be non-toxic, biocompatible, possess certain porosity, and have good surface activity. It should also be biodegradable, malleable, and possess suitable mechanical strength to promote cell-cell adhesion and proliferation. Depending on the different scaffold materials and preparation methods, 3D scaffolds are categorized into hydrogel scaffolds [13], microcarrier scaffolds [14], etc. The hydrogel scaffold consists of hydrophilic polymer chains forming a 3D network structure in a water-rich environment [15]. Currently, synthetic hydrogels, including widely used gels like Matrigel, have been commercialized for 3D cell culture. By adjusting the molecular weight and cross-linking density of the synthetic materials, the properties of the gel, such as pore size and biodegradation rate, can be tailored. This customization makes synthetic hydrogels suitable for the 3D culture of various tumor cells, providing an environment that mimics in vivo conditions and supports tumor cell growth and differentiation. The primary function of the soluble microcarrier scaffold is to provide initial support for cells

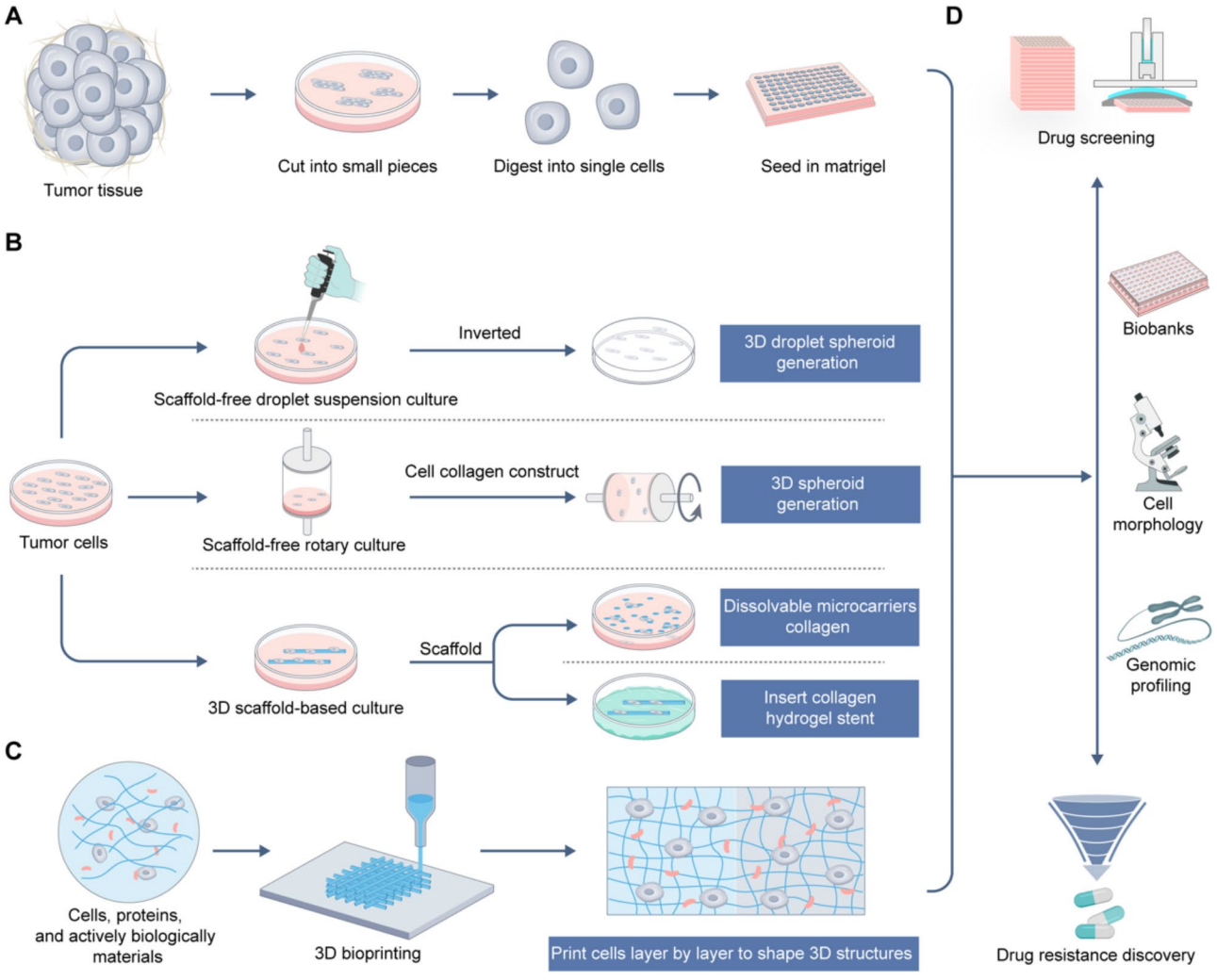

Fig. 2 The typical 3D tumor cell culture model (**A**)tumor tissues are cut into small pieces, followed by digestion to isolate single cells, which are then seeded into Matrigel for culture; (**B**) includes both scaffold-based and scaffold-free culture methods. Suspension droplet culture and rotary-type culture are used to aggregate into 3D scaffold-free spheroid culture. Dissolvable microcarriers collagen scaffolds, and insert collagen scaffold hydrogel stents constitute 3D scaffold-based culture. (**C**) 3D bioprinting. Cells are combined with proteins and other biologically active materials to produce bioink, which is then printed layer by layer to shape 3D structures; (**D**) Drug resistance and drug screening discoveries are explored through the 3D tumor cell culture model

while serving as a medium for the diffusion of soluble factors [14]. This facilitates better adhesion, migration, proliferation, differentiation, and long-term cell growth. The scaffold enhances the interaction between cells and the materials, promoting cellular processes that are crucial for tissue development and regeneration.

3D Bioprinting technology

Cells, proteins, and other biologically active materials, like DNA and growth factors, serve as the fundamental units for 3D printing in bioprinting technology [16, 17]. 3D bioprinting directly constructs in vitro biological structures, tissues, or organ models by precisely arranging cells, proteins, and other bioactive materials. A key focus of 3D bioprinting is the creation of biomimetic objects, often replicating the extracellular matrix (ECM) [18]. The ECM, rich in collagen, proteoglycans, and glycoproteins, provides both structural and biochemical support to cells in living organisms. 3D bioprinting allows for the preparation of complete ECM scaffolds, enabling the analysis of ECM composition, spatial distribution, and biological functions [19]. Through 3D bioprinting, specific ECM can be precisely replicated in vitro by controlling the presentation of functional materials [16, 17, 20]. Additionally, 3D bioprinting can produce and deposit ECM by directly printing cells or cell aggregates, which spontaneously form a microenvironment conducive to cell growth and function. Shabani et al. [21]. investigated the structure and function of ECM components and found that various ECM mixtures regulate a

wide range of biological functions. The 3D bioprinting of tumor cells has rapidly advanced and is increasingly applied in clinical medicine and other fields, owing to its robust cell control capabilities. Advanced tumor research and drug development still rely on organoid and animal models for support.

Organoids

Organoids known as microorgans or mini-organs, are self-assembled 3D cell clusters that develop through in vitro culture. Derived from stem cells, these clusters consist of multiple cell types characteristic of the corresponding organs and possess a spatial structure similar to that of real organs, allowing them to simulate certain organ functions [22, 23]. Organoids can be categorized based on their origin into two main types: those derived from pluripotent stem cells (PSCs) and those derived from neonatal or adult stem cells (ASCs). PSC-derived organoids include those from embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). The cellular source of iPSCs and ASCs-derived organoids can also be from the patient, known as PDOs [24]. Tumor organoids, a subset of PDOs, are widely used in research. As a new generation of preclinical drug evaluation models, organoids offer an advancement over traditional 2D cell models and animal models. They are cultured using various methods, including the embedding method, suspension method, and air-liquid interface method [25]. In drug development, iPSC-derived organoids are especially valuable for studying genetic diseases and drug toxicity, while ASC-derived organoids, especially those from tumor patients, have demonstrated clinical predictive advantages in drug target validation and drug sensitivity prediction [26]. This section will summarize the progress in the development of 3D tumor sections culture, tumor organoids, and organoid tissue chips according to model classification.

3D tumor section culture model (3D-TSC)

The 3D-TSC is established by sectioning fresh tumor tissue and embedding it in a gel matrix for tissue culture, resulting in a 3D-TSC model. Drug candidates are screened by treating the 3D-TSC model with these candidates in 3D cultures, followed by evaluation of apoptotic cell reporter signals [27]. Xing et al. [28] utilized a 3D-TSC model culture method, integrating label-free technology and/or time-lapse imaging of apoptosis reporter substances, to achieve high-throughput drug screening. This approach facilitated the identification of high-efficiency drugs for specific cancer samples within a week. The culture of the 3D-TSC model preserves the immune components of the original tumor, enabling successful immune checkpoint blockade assays using immune checkpoint inhibitors. This technology provides a cost-effective, rapid, and straightforward platform for the discovery of anticancer drugs, significantly accelerating the development of precision anticancer therapies. We monitored drug pharmacodynamics via time-course visualization and quantitatively assessed the efficacy of anticancer drugs using a 3D-TSC model that simulates the TME [27]. This model enables the evaluation of individualized drug responses and targeted immunotherapy within 4–7 days post-surgical tumor resection, incorporating chimeric antigen receptor natural killer (CAR-NK) cells. This platform technology has significantly accelerated the development of rapid and personalized treatment options for targeted immune agents, contributing to advancements in preclinical research and enhancing the potential for tailored cancer therapies.

Tumor organoids

Tumor Organoids (TOs) often referred to PDOs, possess significant commercial potential [29]. Compared to traditional 2D cell lines, TOs demonstrate a higher success rate, greater heterogeneity, and more effectively replicate the processes of tumor initiation and progression. PDOs are constructed more rapidly and cost-effectively than PDX, making them an attractive option for cancer research and drug screening [4]. Currently, non-metastatic colorectal cancer and hormone-sensitive breast cancer PDOs can be successfully gathered. Importantly, PDOs avoid the risk of genetic drift often associated with transplantation in PDX models, preserving the original genetic characteristics of the patient's tumor [4, 30]. However, PDOs face some limitations, such as slower growth rates compared to normal tissue organoids, potentially due to limited mitosis activity. The construction process also requires careful consideration of the TME, and PDOs have stringent requirements for material purity and should be cultured in specific media, such as matrix gel and basement membrane extracts [31].

Although conventional culture techniques can generate TOs containing one or more cell types, these organoids generally exhibit limited tissue-level characteristics. By utilizing an engineering culture methods with natural scaffolds or synthetic scaffolds, the biological, physical, and biochemical properties of TOs can be modulated to better simulate tumor tissue. Additionally, synthetic biology techniques can be employed to program cells at the genomic level to further improve the simulation effect of TOs [32]. TOs are widely used in tumor growth, angiogenesis, and drug resistance. They are also employed in high-throughput drug screening, drug sensitivity prediction, and biomarker analysis. Additionally, TOs can be combined with genomics to identify drug resistance genes or with proteomics to discover new antigens, which can then be applied to precision medicine and vaccine development. Moreover, co-culturing TOs with

immune cells or pathogenic microorganisms, provides a valuable approach to study the immune microenvironment and elucidate the relationship between pathogenic microorganisms and the onset and progression of malignant tumors [29].

Organ tissue chip

The organ tissue chip is akin to the microphysiological systems (MPSs) [33], but it is classified by the US FDA as a subclass of MPSs. It comprises a miniaturized physiological environment that generates and analyzes functional tissue units, mimicking specific or target organ-level responses. Organoids can be cultivated using engineered organoid chip technology [34]. The characteristics and applications of organoids from various sources and their corresponding chips are illustrated in Fig. 3. Jin et al. [35] established 3D vascularized liver organoids constructed from induced hepatocytes and a microfluidic system, successfully establishing a drug concentration gradient for efficient drug screening. Furthermore, the microfluidic multi-organ platform, which integrates microfluidic equipment with organoids, can simulate the metabolic process of drugs in the body and conduct studies on drug efficacy, safety, and pharmacokinetics [35], thus enhancing drug screening efficiency. Organ-on-chip technology demonstrates a unique application value in drug evaluation, including pharmacokinetics and toxicology [36]. However, organoid technology is not yet a complete replacement for traditional animal testing. In drug evaluation, data reliability is crucial, placing high demands on the reliability, standardization, and normalization of the evaluation model. For instance, organoid vascularization techniques primarily rely on transplantation, and replicating the peripheral nervous system in organoids remains a significant challenge. The relatively low degree of systematization weakens the near-physiological nature

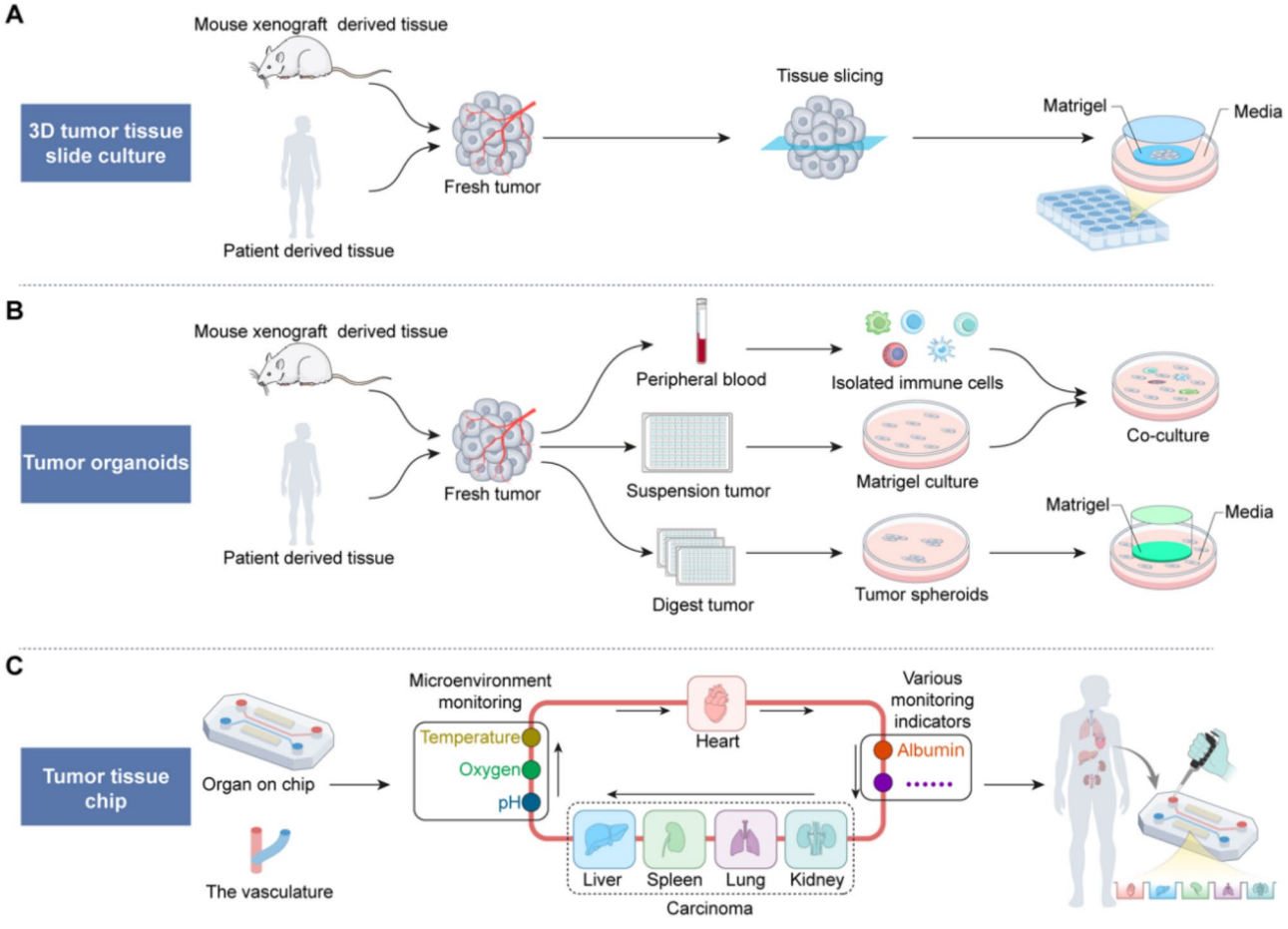

Fig. 3 Types of 3D tumor tissue culture processes include: (**A**) 3D tumor tissue slide culture utilized fresh tumor tissue, including xenograft and human models, to cut into tissue slicing and then cultured in matrigel; (**B**) Tumor organoids involve the co-culture of isolated immune cells and suspension tumor cells to generate organoids. Additionally, tumor tissue is digested to produce spheroids, which are then cultured in Matrigel media to maintain their three-dimensional structure and mimic the in vivo tumor microenvironment; (**C**) Tumor tissue chips integrate vasculature and organ-on-chip technology to construct a microenvironment, enabling the creation of various carcinoma models, such as those for the liver, spleen, lung, and kidney. This microenvironment is closely monitored, and various indicators in the tumor models are measured to assess and explore drug responses

of organoids, restricting their ability to fully replicate the in vivo environment. This presents challenges in studying complex systemic tumor diseases and assessing drug systemic toxicity, as PDOs may not fully replicate the intricate interactions and responses seen in living organisms. Therefore, the advancement of organoid technology requires not only technological breakthroughs but also the control of uncertain factors during organoid construction through standardized methods [37, 38]. Additionally, improving the reliability of data generated by PDOs depends on establishing normative requirements that ensure consistency and reproducibility across studies, facilitating their more effective application in research and clinical settings.The development and application of organoid technology must adhere to establish norms and standards. It is essential to evaluate the reliability and maturity of organoid technology protocols based on specific contexts. At the same time, efforts should focus on developing standardized and reliable models and evaluation methods that ensure the effectiveness and safety of drugs (Fig. 3).

3D cultures used for drug screening

Before a drug enters clinical trials, it must undergo rigorous screening and evaluation procedures to fully verify its indications, efficacy, and safety. The preclinical development process of new drugs is often slow, costly, and inefficient due to the limitations of current in vitro and in vivo drug screening models. The introduction of 3D culture technology more accurately simulates the TME, allowing tumor cell growth to more closely resemble the conditions in the human body. This, in turn, significantly enhances the accuracy and efficiency of drug screening. In addition, 3D culture technology can evaluate the effects of different drugs on tumor cells, providing a scientific basis for personalized treatment strategies(Fig. 4). Wong et al. [39] analyzed 406,038 clinical trial data points and found that the overall success rate of Phase III drug clinical trials was just 13.8%, with the cancer drugs having success rate as low as 3.4%. The primary reason for this low success rate is the insufficient efficacy and safety of the drugs.

Compared to traditional 2D tumor cell lines and PDX models, tumor organoids show advantages such as lower

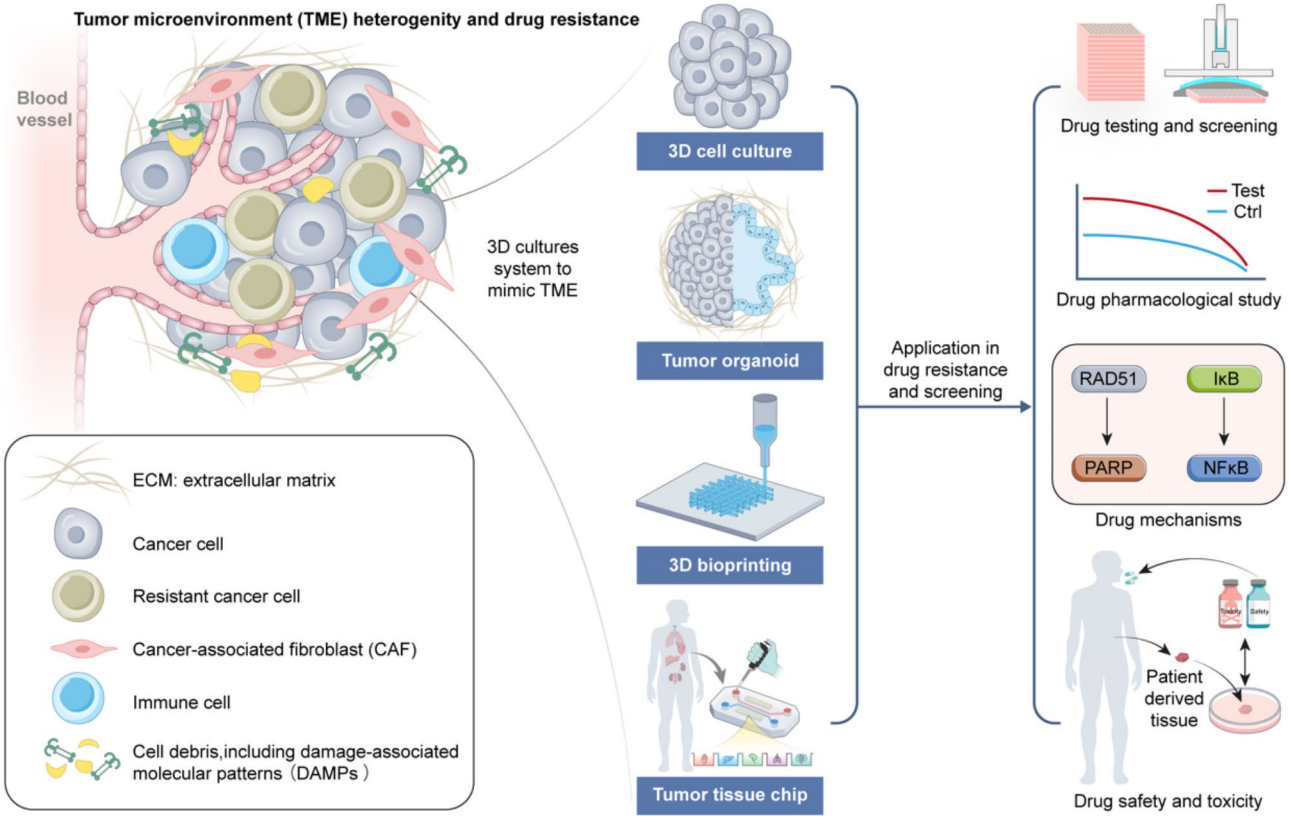

Fig. 4 .A proposed workflow for applying 3D culture technology and organoids in the study of drug resistance and screening. The TME encompasses the ECM, cancer cells, drug-resistant cancer cells, CAFs, immune cells, and cellular debris, including damage-associated molecular patterns (DAMPs). The 3D culture technology and organoids are employed to replicate the heterogeneity of the TME, enabling the exploration of drug pharmacology, mechanisms, safety, toxicity, as well as drug testing and screening. Patient-derived tissues are utilized to culture organoids for assessing drug safety and toxicity, providing valuable insights to guide clinical therapy

Table 1 Advantages and disadvantages of 2D and 3D culture applications in drug resistance and screening

Culture type	Advantage	Disadvantage
2D culture	• - Easy to operate, simple, and cost-effective [40, 41]. • - HTS and easy implementation of standardized assays [42, 43]. • - The simple structure of the cell monolayer facilitates microscopic observation and data analysis [40, 41]. • - Suitable for studying the biological behavior of a single cell type and the mechanism of drug action [41]. • - Uniform drug distribution and consistent efficacy assessment results [41, 44].	• -Inability to reflect tumor heterogeneity and spatial structure [41, 43]. • -Lack of ECM and intercellular interactions to mimic the TME [41, 42]. • -Inability to model key mechanisms of tumor resistance, such as barriers to drug penetration and hypoxic environments [45, 46]. • - Drug susceptibility results poorly correlated with clinical practice [41, 42]. • -High failure rate of drug screening results in clinical translation [41, 46].
3D culture	• - Simulating the 3D tumor structure, cell-to-cell interactions, and ECM dynamics provides a model that closely mimics the in vivo environment [42, 46]. • - Tumor heterogeneity is preserved, enabling the study of tumor resistance mechanisms [41]. • -The model simulates the penetration and distribution of drugs within tumor tissue, allowing for more accurate predictions of drug efficacy[40, 41]. • - It is suitable for studying the effect of the TME on drug response [42, 44].	• - Complex cultural conditions and high costs. [41, 43] • - Difficulty in meeting the needs of large-scale drug screening due to low throughput [42, 43]. • - Complex data acquisition and analysis [41, 46]. • - Inhomogeneous drug penetration within the 3D structure may lead to biases in efficacy assessment [40, 41, 43]. • - Challenges in standardizing 3D cultures [43, 46].

2D, two-dimensional;3D, three-dimensional; HTS, High-throughput screening; ECM, extracellular matrix; TME, tumor microenvironment

cost, shorter culture cycle, higher success and conversion rates, making them a promising new platform for drug screening and research. Tumor organoids are also effective in predicting patient responses to drugs(Table 1). For instance, Lui et al. [47] tested the antitumor activity of five natural compounds—cypermethrin, sparfloxacin, allicin, berberine, and betaine—using PDOs. The results indicated that PDOs were sensitive to berberine, which exhibited resistance in cell lines; betaine causes acquired resistance in non-small cell lung cancer (NSCLC); and cypermethrin, sparfloxacin, and begonia inhibited the proliferation of NSCLC in both PDOs and cell lines [48, 49]. Furthermore, PDOs can be used to monitor the progression of drug resistance during cancer treatment. For example, halofuginone (HF) has been shown to inhibit tumor proliferation, induce tumor cell arrest in G0/G1 phase, and promote lung cancer cell apoptosis in a dose-dependent manner when tested in PDOs models [24, 50].

In the era of precision oncology, oncologists are increasingly focused on developing more effective drug screening models to assess patient responses to therapeutic agents [51]. PDOs derived from prostate cancer, bladder cancer, endometrial cancer, liver cancer, lung cancer, and other organs, to a large extent, the histological, genetics, and pathological characteristics of parent tissue. These models are better equipped to simulate drug penetration and are widely used in drug testing, patient-specific drug testing, targeted therapies, and proof-of-concept studies on drug resistance mechanism. Organoids thus serve not only as reliable systems for studying tumor evolution and therapeutic response, but also as novel tool for testing cancer drugs and individualized cancer therapies [51, 52]. In the case of a genetically engineered rat PDOs cultured by Duarte et al. [53]., these organoids are designed to be compatible with in vivo screening methods, allowing them to be used for studying responses to various cytotoxic agents. More importantly, PDOs can be effectively modified by the CRISPR/Cas9 system to target the gene needed for research [54]. This genetic manipulation enhances the flexibility and adaptability of PDOs, facilitating precise alterations and replacing generalized models.By enabling targeted gene modifications, CRISPR/Cas9 improves the study of drug resistance, as well as the mechanisms underlying tumor initiation, progression, and metastasis. In addition to gene editing, tools such as single-cell analysis, optogenetics, chemical genetics, super-resolution microscopy, and other advanced imaging techniques, when combined with electronic tools, significantly contribute to the generation and refinement of organoids. By continuously optimizing the functional diversity of organoids. Continuous optimization of the functional diversity of organoids is driving the advancement of drug evaluation and screening systems in a more comprehensive and precise direction [54].

Application of 3D culture techniques in Pharmacological studies

The organoid model (PDO) offers a distinct advantage in simulating drug response, as it more effectively mimics tumor heterogeneity compared to conventional PDC and PDX models. Kim et al. [55]. developed a novel PDO with a specific mutations that respond to targeted drugs. For instance, organoids with mutations in the breast cancer susceptibility gene 2 (BRCA2) are sensitive to olaparib, those with specific mutations in the epidermal growth

factor receptor (EGFR) are sensitive to erlotinib, and organoids with EGFR mutations and hepatocyte growth factor tyrosine kinase receptor (MET) mutations are sensitive to crizotinib and other [33–35]. This targeted drug response emphasizes that the gene expression profile of the organoid transcriptome can serve as a valuable guide for drug sensitivity screening.

Kim S et al. [46]evaluated the efficacy of the darafenib/trametinib combination using PDO with both an exon 19 deletion mutation in the EGFR gene and mutations in the serine/threonine kinase (BRAF) gene, including the BRAF (G464A) mutation. Their findings confirmed the potential of this combination therapy [56]. Chen et al. [57] selected 26 anticancer drugs based on the NSCLC treatment guidelines and tested drug responsiveness in 50 PDO models. Their study demonstrated that PDO models could serve as predictors for targeted therapies. For example, PDO with the EGFR exon 20 insertion mutation showed a positive response to osimertinib and chemotherapy but were resistant to gefitinib, aligning with clinical outcomes. PDO overexpressing c-MET was significantly responsive to osimertinib but resistant to gefitinib, reflecting the mechanism of gefitinib resistance. Additionally, ERBB inhibitor tyrosine kinase inhibitors in PDOs carrying the KRAS (G12C) mutation were also evaluated.

PDOs in investigating mechanisms of drug action

PDOs are valuable tools for investigating drug response mechanisms, enhancing tumor -specific cytotoxicity while minimizing toxic side effects on normal tissues. In a study [58], a two-organ model involving a nuclear factor E2-erythroid 2-related factor 2 (Nrf2) inhibitor and knockdown of the Nrf2 gene was constructed. Their findings revealed that Nrf2 promotes the growth of lung squamous cell carcinoma by activating the PI3K-Akt-mTOR signaling pathway. This underscores the potential of PDOs in understanding molecular mechanisms and guiding therapeutic strategies. Inhibition of Nrf2 was shown to decrease PI3K-Akt-mTOR signaling, and Nrf2 inhibitors were found to suppress the growth of lung squamous cell carcinoma. Taverna et al. [59] developed short-term lung adenocarcinoma organoids from patients with high expression of tyrosine protein kinase receptors, specifically Anexelekto (AXL) and Janus kinase (JAK). They tested the sensitivity of these organoids to JAK inhibitors, providing further insights into potential therapeutic strategies targeting tyrosine kinase pathways in lung adenocarcinoma. The results confirmed that AXL inhibitors compensated for AXL loss by inhibiting SMAD4/TGFβ signaling and inducing JAK1-STAT3 signaling.

PDO has the potential to explore new tumor targets

PDO can be employed to identify new tumor targets and offer novel approaches for intervening in tumor progression. Pyroptosis, a form of programmed cell necrosis driven by gasdermin, is induced by chemotherapy involving 0–5 integrins. High levels of 0–5 integrin are associated with tumor progression [60]. Su et al. [61] demonstrated that by using Src or ceramidase inhibitors in chemoresistant patient-derived tumor organoids (PDTO), the reactivation of pyroptosis restores drug resistance, proving the feasibility of pyroptosis-based cancer therapies. Pan et al. [62], during the transformation of Kmt2d gene-deficient lung basal cell organoids into lung squamous cell carcinoma tumor organoids, found that Kmt2d loss increased the activation of tyrosine kinase receptors EGFR and ERBB2, resulting in enhanced oncogenic RTK-RAS signaling. This suggests that Kmt2d is a key regulator in the carcinogenesis of lung squamous cell carcinoma. The combination of the tyrosine kinase inhibitor SHP099 and the ERBB inhibitor afatinib in PDX models demonstrated that lung squamous cell carcinoma with Kmt2d loss is therapeutically sensitive to RTK-RAS pathway inhibitors.

Several research teams have utilized tumor organoids for drug-targeting effectiveness screening. Vlachogiannis et al. [63] screened 55 drugs in phase I clinical trials or clinical practice and found that patients with BRAF mutations had a suboptimal response to MEK/ERK inhibitors. Additionally, the efficacy of GDC-0980 (a mammalian target of rapamycin (mTOR)/PI3K inhibitor) in PIK3CA mutant organoids varied, highlighting the importance of genetic context in determining drug responses. Yan et al. [64] established nine organoid lines and tested 37 drugs, revealing that ARID1A mutant organoids responded well to the ATR inhibitor VE-822. Additionally, the study showed that organoids can not only maintain patient-specific genetic mutations but also acquire rare genetic mutations, providing a stable genetic background for studying drug targets and facilitating personalized medicine approaches.

Safety testing of organoids in a drug preclinical setting

Normal tissue organoids can be employed to assess toxicity to healthy tissues through cytotoxic tests, such as the lactate dehydrogenase release test, combined with live cell counting methods, such as CellTiter. Vrious healthy tissue organoids have been successfully developed, including liver organoids [65, 66], cardiac organoids [67, 68], and kidney organoids [69, 70], have been successfully developed and utilized in the drugs toxicological evaluation. Researchers have utilized liver organoids to examine dose-dependent hepatotoxicity and have delved into the molecular mechanisms of hepatotoxicity for certain drugs [71]; Mun et al. [65] assessed the safety of various

drugs that were recalled due to adverse reactions, such as trevafloxacin, which was associated with a higher risk of liver failure. The team employed both 2D models and PDOs for drug testing, and under the same drug concentration, the 2D model indicated that trevafloxacin had little to no liver toxicity effect. However, 3D liver organoids exhibited cell mitochondrial dysfunction and evident cell death, suggesting that 3D healthy organ models may be more sensitive to drug toxicity than 2D models. Incorporating organoid systems as a complement to existing drug screening and development testing methodologies can enhance the assessment of drug safety and efficacy. This approach holds the potential to provide more accurate toxicity profiles and improve the success rate of drug development by offering a more physiologically relevant model for evaluating drug effects on healthy tissue.

3D culture model for drug resistance

Tumor cell drug resistance is a major factor influencing the effectiveness of tumor treatment and patient survival [72]. The mechanisms underlying this resistance are complex and multifaceted, involving drug inactivation, qualitative and quantitative alterations in drug targets, drug efflux, enhanced DNA damage repair capacity, inhibition of cell death pathways, and drug sequestration in lysosomes or vesicles [73]. These diverse mechanisms pose substantial challenges in overcoming resistance and improving therapeutic outcomes for cancer patients [74]. 3D culture technology can mimic the in vivo growth environment of tumor cells, including angiogenesis and oxygen supply, allowing for a deeper study of tumor cell drug resistance mechanisms. Additionally, 3D culture can be used to assess the impact of different drugs on tumor cell drug resistance, offering valuable insights for the research and development of antitumor therapies. In contrast, traditional 2D tumor cell culture significantly differs from the microenvironment and cell behavior of tumor cells in vivo, limiting the study of tumor cell drug resistance(Table 1). The 3D culture model, which more closely resembles the in vivo environment, provides a microenvironment that closely mimics the actual conditions. It better simulates the material basis and spatial structure of the cell microenvironment, offering intuitive and controllable cell culture and drug screening, with cell gene expression patterns and life activities that are more reflective of a real organism [75].

3D tumor cell culture model for drug resistance and its mechanism

Utilizing a 3D tumor cell culture model to study drug resistance and the mechanism of tumor cells is highly significant for clinical anti-tumor research. A study on MCF-7 breast cancer cells revealed a significant improvement in cell resistance in the 3D collagen culture model. The study further explored the impact of the 3D collagen microenvironment on tumor cell stemness, the cell cycle, and the drug-related ABC transporters involved in drug resistance. It also examined the connection between drug resistance, stemness, and the cell cycle. This research provides a foundation for studying drug resistance and its mechanisms in breast cancer cells [76]. Cancer stem cells (CSCs) play a crucial role in acquired drug resistance due to their robust DNA repair capabilities, high expression of ATP-binding cassette transporters, and anti-apoptotic characteristics [77]. CSCs engage multiple signaling pathways, including Wnt, Notch, and Hedgehog, to maintain their self-renewal, survival, and drug resistance characteristics [78, 79]. Usui et al. [80]. employed air-liquid interface (ALI) technology to successfully build a 3D organoid culture model containing a significant number of CSCs derived from human colorectal cancer tissue. Their study demonstrated that these organoids exhibited resistance to 5-fluorouracil (5-FU) and irinotecan. Ukai et al. [81]. also established an anti-5-FU PDO and conducted a comprehensive gene expression analysis using microarray technology. They found that KHDRBS3 was specifically upregulated in 5-FU resistant GCO and played an important role in multidrug resistance by acquiring GC GC stem cell characteristics by regulating the expression of CD44v. Furthermore, the knockdown of KHDRBS3 inhibited resistance and suppressed organoid formation in GCOs and gastric cancer cell lines.

Furthermore, in intestinal cancer cells, both normal and transformed enterocytes are believed to be Wnt-dependent, meaning that activation of the Wnt signaling pathway is crucial for tumor maintenance [82]. Some scientists designed a series of PDOs and found that alterations in oncogenes play a key role in the WNT signaling pathway. In KRAS or BRAF mutant intestinal epithelial cells, accompanied by p53 and SMAD4 knockdown, activation of TGF-β leads to YAP/TAZ-dependent transcriptional reprogramming and lineage reversal [24, 82, 83]. This process results in the acquisition of an embryonic intestinal identity, which is characterized by the permanent loss of the adult intestinal lineage. Additionally, this reprogramming enables long-term Wnt-independent growth, highlighting the significant role of TGF-β signaling in driving lineage plasticity and tumorigenic properties in the context of these genetic alterations [82].

Screening study of drug resistance against various cancer

3D culture model can simulate the growth microenvironment of tumor cells, which can be used for the study of tumor drug resistance screening. Considering that patients with invasive breast cancer frequently develop resistance to standard chemotherapeutic agents, Borges et al. [84]. proposed a novel therapeutic strategy. This strategy involves combining the DNA methyltransferase

inhibitor decitabine with suramin as well as the combination of both drugs, on the toxic response and cell invasiveness of breast cancer cells in a 3D cell culture model. The findings indicated that neither suramin nor decitabine alone significantly inhibited tumor cell growth, toxicity response, or cell invasiveness in 3D culture conditions. However, the combination of the two drugs effectively prevented cancer cells from invading the surrounding ECM, suggesting that while the combination of decitabine and suramin does not inhibit the growth of cancer cells, it can significantly reduce the invasion potential of breast cancer cells. This implies that the combination of suramin and decitabine could serve as a treatment regimen for aggressive, multidrug-resistant breast cancer.

In addressing the issue of PARP inhibitors (PARPi) targeting homologous recombination deficiency (HRD) in tumors lacking BRCA 1 or BRCA 2, Duarte et al. [85]. developed a panel of KB 1 P (M) and KB 2 P breast tumor-derived organoids and tested organoids with pairs of PARPi-sensitive and PARP-inhibited BRCA 1- and BRCA 2-deficient tumors. Results of in vitro and in vivo PARPi response analysis showed that in vitro organoids are more sensitive to PARPi. However, some mechanisms of PARPi resistance in KB 1 P (M) and KB 2 P tumors were not effectively recapitulated, as analyzed by in vitro viability assays of tumor-driven organoids. These findings suggest that while PDOs are useful for testing PARPi sensitivity, they may not fully mimic all aspects of resistance mechanisms present in tumors. The studies highlight the importance of 3D culture techniques in screening novel chemotherapeutic agents or combination chemotherapy regimens for the treatment of breast cancer, offering a more accurate representation of tumor behavior and response to therapy [23].

Considering that the traditional 2D cell culture method fails to accurately mimic the natural progression of malignancy in primary drug screening, Cha et al. [86] investigated the antitumor effect of BGJ398, a selective inhibitor of the fibroblast growth factor receptor (FGFR), on ovarian cancer SKOV3ip1 cells under both monolayer and spheroid culture conditions. Cell viability was assessed before and after treatment with the inhibitor. While no significant decrease was observed after 48 and 72 h of BGJ398 treatment in monolayer cultures, a notable reduction in viability was noted in spheroid ovarian cancer cells, with this decrease being time-dependent. This indicates that the 3D culture model of tumor cells is crucial for screening anti-ovarian cancer drugs. Hirst et al. [87]cultured various epithelial ovarian cancer (EOC) cell lines in a 3D cell culture environment, forming multicellular tumor spheroids, and compared them with 2D cell cultures. These findings further emphasize the importance of the 3D spheroid drug screening model in identifying effective treatments for ovarian cancer. Patients with advanced endometrial cancer often exhibit resistance to chemotherapeutic agents. Chitcholtan et al. [88], cultured the 3D multicellular structure of endometrial cancer cells using suspension culture and compared the antitumor activity of doxorubicin and cisplatin in both 3D multicellular structures and 2D monolayer cultures. Their results indicated that doxorubicin and cisplatin-induced less apoptosis in 3D multicellular structures and had higher viability compared to 2D monolayer cultures. This suggests that 3D multicellular structures provide a more accurate model for detecting sensitivity to chemotherapeutic drugs than 2D cultures, as they better mimic the in vivo tumor environment and resistance mechanisms.

In conclusion, compared to 2D monolayer cell culture, the 3D stereoscopic culture model of malignant tumor cells more closely resembles the morphological and cell biological characteristics of in vivo tumors, allowing for a more accurate simulation of the actual tumor growth situation in vivo. 3D culture technology is essential for screening anti-tumor drug resistance in malignant tumors.

The application prospect and challenges of 3D culture technology in clinical transformation

Despite significant progress in 3D culture techniques for resistance research and clinical applications, several challenges persist. These include the need for standardization of 3D culture methods, the lack of clinical approval for tumor organoids, the complexity of cell models, and the high cost associated with their culture. Addressing these issues is crucial for advancing the field. Consequently, the use of tumor organoids in drug screening remains challenging and requires further advancements in both the models and their validation processes. Moreover, the comprehensive application of 3D culture in drug response and drug resistance mechanisms is still in the exploratory stage. In the future, 3D culture will likely be combined with emerging technologies such as genomics and single-cell analysis, offering more accurate methods for drug screening and drug resistance prediction. However, the complexity of 3D culture technology and the high cost of scaffold materials hinder its ability to meet the demands of large-scale experiments. Additionally, most 3D culture techniques fail to replicate the vascular structure, significantly limiting the growth and development of tumor cells. As a result, these in vitro culture techniques are primarily used for early tumor manifestation, drug screening, and mechanism research.

The development of tumor organoids offers a novel approach to personalized treatment by enabling researchers to create in vitro tumor PDOs that reflect the specific characteristics of a patient's tumor. These models can

predict the efficacy and prognosis of drug regimens, facilitating a more individualized approach to treatment. This personalized method has the potential to improve therapeutic outcomes and minimize adverse effects. Vlachogiannis et al. [63] established PDOs before, during, and after treatment, as well as following metastasis or progression. They found that, aside from histopathological characteristics, the molecular profiles between organoids and the original tumor tissue largely overlapped, with a 96% overlap in the gene mutation spectrum. This suggests that PDOs can closely mimic the genetic features of the patient's tumor, making them valuable for personalized treatment strategies and drug testing. They tested the drug response of patients and attempted to identify drugs that might be effective after disease progression using PDTO. The results showed that organoids predicted drug response with 100% sensitivity, 93% specificity, 88% positive predictive value, and 100% negative predictive value. These findings highlight the potential of PDTO models in guiding personalized treatment decisions and improving the precision of drug selection, particularly after tumor progression. Yao [89] conducted a drug sensitivity test on a library of 96 organoids derived from locally advanced colon cancer. Initially, 18 organoids were established, and whole exome sequencing (WES) was performed on corresponding tumor biopsy samples. They found that the overlap rate of PDTO and the corresponding tumor gene mutation spectrum was 94.4%. Additionally, they assessed the organoids' sensitivity to radiation and chemotherapy drugs, including fluorouracil and irinotecan. By comparing these drug responses with the clinical efficacy observed in patients, they achieved an accuracy of 84.43%, a sensitivity of 78.01%, and a specificity of 91.97%.

Despite the promising potential of tumor organoids in predicting patient sensitivity to drugs, variability in drug response remains a significant challenge. Even organoids derived from the same tumor can exhibit considerable differences in their responses to treatment. For instance, Schumacher et al. [90]. observed up to a 30-fold difference in drug response among organoid cultures obtained from the same colon cancer through simultaneous multiregion sampling. This suggests that intra-tumor heterogeneity can significantly influence the establishment of organoids and the accuracy of their predicted drug sensitivity outcomes in vitro. Kim et al. [91]. addressed this challenge by establishing tumor organoids and corresponding cell lines from different regions of colon cancer patient tissue. This could serve as a new approach for drug selection, testing, and assessing patient prognosis. However, given the heterogeneity within tumor tissue, PDTO, established from a single biopsy, may have limitations in fully predicting patient response.

Although tumor organoids can recapitulate the molecular and pathological features of the parental tumor, they are composed solely of tumor epithelial cells, excluding stromal cells. As a result, they lack tumor-matrix interactions and the TME [92]. Consequently, tumor organoids have limitations for clinical application, such as the inability to predict responses [63]to drugs like antiangiogenesis and immunotherapy. To bridge the gap between tumor organoids and the in vivo TME, researchers have attempted to co-culture organoids with tumor stromal cells and immune lymphocytes, among others [29].Cancer-associated fibroblasts (CAFs) are a crucial cellular component of the TME, playing a critical role in tumor initiation, progression, metastasis, and drug resistance through various mechanisms [93]. Ohlund et al. [94]. co-cultured pancreatic ductal adenocarcinoma organoids with mouse pancreatic stellate cells and observed that HSCs could differentiate into various subtypes of CAFs under different conditions, indicating the intratumoral heterogeneity of CAFs. Seino et al. [23, 95] co-cultured pancreatic cancer organoids with CAFs and found that CAFs provided a microenvironment essential for the growth of tumor organoids. Similarly, Marusyk et al. [96], who co-cultured CAF with breast cancer organoids, discovered that CAFs could protect cancer cells from lapatinib toxicity, thereby mediating drug resistance. Dijkstra et al. [97] expanded tumor-reactive T lymphocytes by combining PDTO and patient-derived T-lymphocytes. The co-culture model has also been utilized to amplify tumor-specific reactive T lymphocytes by adding cytokines such as interferon γ, interleukin-2 (IL-2), and fixed concentrations of PD-1 antibodies to the T-cell medium. Thus, co-culture represents a key direction for future organoid-based research.

In addition, artificial intelligence (AI) technology has promoted the accuracy, objectivity and processing ability of complex data in organoid research, especially AI-based 3D organoid image analysis. For example, organoids experiment need fast acquisition and low illumination to avoid phototoxicity, which often cannot obtain high-quality images. McAleer et al. [98] proposed two deep learning models to obtain high-resolution organoid images using reduced light exposure. Super-resolution image reconstruction can be achieved by training on a training set consisting of low-resolution images from human retina and skin organoids collected under two-photon excitation fluorescence and corresponding high-resolution images [98]. In future, AI will play an important role in the research of organoids, accelerating the transformation of organoids into clinical practice and precision treatment.

Conclusions and perspective

While 3D culture systems and tumor organoids are anticipated to capture the heterogeneity of tumors and assess alterations in drug responses for clinical applications, the correlation between in vitro sensitivity and the ultimate patient outcome has yet to be established. In conclusion, the development of 3D culture systems and the refinement of their cultivation and analytical methods presents substantial challenges. Nevertheless, 3D culture technology, with its adaptability and strong potential for in vivo simulation, has forged a link between fundamental cancer research and clinical cancer therapy, hoping to provide a basis for the exploration of optimized tumor treatment strategies and precision therapy.

Personalized cancer medicine focuses on tailoring the most appropriate therapy

Personalized treatment options for each patient are challenging due to the absence of representative patient tumor models that accurately reflect the key characteristics of individual tumors, complicating the precise evaluation of treatment predictions. Tumor organoids provide a promising platform for studying human development and drug screening, offering a system free from ethical constraints. PDTO, in particular, holds significant promise for predicting clinical drug responses and guiding treatment selection [99]. To enable high-throughput analysis, enhanced culture methods are required to reduce the time needed for organoid generation, lower costs, and ensure the availability of essential input materials. Several studies have developed automated microfluidic platforms that integrate organoids with microfluidic technologies, facilitating high-throughput culture, modulation, and analysis of organoids under dynamic conditions [100, 101]. Additionally, the integration of CRISPR/Cas9 technology allows for genome editing in tumor organoids. Greater emphasis should be placed on controlling organoid-specific phenotypes, such as the regulation of stem cell-related Wnt and Notch pathways, to better align with the physiological and biochemical conditions required for organoid growth and the demand-driven transformation of organoids [102, 103].

Established the link between ex vivo sensitivity and final patient outcomes via 3D organoid cultures

The establishment of live biobanks of cancer organoids presents new opportunities for expanded testing and development of anticancer drugs, as well as improved stratification of cancer patient cohorts. However, in 3D organoid cultures, reconstructing the TME with patient-derived CAFs, immune cells, capillary systems, and biomechanical stimulation to simulate disease progression and evolution remains a significant challenge. This microenvironment is crucial for the various features and therapeutic responses that define cancer. To address these limitations, future co-culture systems should integrate these elements—such as CAFs, immune cells, capillary systems, and biomechanical factors—into organoid cultures. Furthermore, although tumor organoids are expected to reflect inter-tumor heterogeneity and assess changes in drug response in clinical settings, the link between ex vivo sensitivity and final patient outcomes remains to be established. Different parts of the same tumor may also contain cancer cell populations with varying drug sensitivities, necessitating multiple sampling to capture the full diversity of the tumor's response to treatment [104]. In conclusion, while organoid development is in its early stages and faces challenges in maturation and analytical methods, their versatility, strong in vivo simulation capabilities, and rapidly evolving applications suggest that organoid technology will continue to have a significant impact on both basic cancer research and clinical cancer treatment in the future.

Abbreviations

3D	Three-dimensional
2D	Two-dimensional
CAF	Cancer-associated fibroblasts
PDX	Patient-derived tumor xenografts
PDC	Patient-derived cell
PDO	Patient-derived organoids
RCCS	Rotating cell culture system
ECM	Extracellular matrix
PSC	Pluripotent stem cells
ASCs	Adult stem cells
ESCs	Embryonic stem cells
3D-TSC	The 3D tumor section culture
HF	Halofuginone
BRCA2	Breast cancer susceptibility gene 2
EGFR	Epidermal growth factor receptor
PI3K	Phosphatidylinositol 3-kinase
FGFR	Fibroblast growth factor receptor
EOC	Epithelial ovarian cancer
CSCs	Cancer stem cells
HRD	Homologous recombination deficiency
MMP	Matrix metalloproteinase
PDTO	Patient-derived tumor organoids
TME	Tumor microenvironment
DAMPs	Damage associated molecular patterns

Author contributions

Conceptualization, S.G.H. L.N. Z and.Z P.; writing, review and editing, S.G.H.,Z.P, XL. L, H.S and L.N.Z; supervision, S.G.H.and L.N.Z. All authors have read and approved the final version of the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

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