Prospects and challenges of ovarian cancer organoids in chemotherapy research (Review)

WEIJIA ZHANG[1*], YUQING DING[1*], HUI HE[1], KEMING CHEN[2], QINGSONG ZENG[2], XIAOMING CAO[2], YING XIANG[3] and HAI ZENG[1]

[1]Department of Oncology, The First Affiliated Hospital of Yangtze University, Jingzhou, Hubei 434023, P.R. China; [2]Department of Gynecology and Obstetrics, First Affiliated Hospital of Yangtze University, Jingzhou, Hubei 434023, P.R. China; [3]Department of Cell Biology and Medical Genetics, School of Basic Medicine, Health Science Center, Yangtze University, Jingzhou, Hubei 434023, P.R. China

Received September 20, 2024; Accepted January 20, 2025

DOI: 10.3892/ol.2025.14944

Abstract. Ovarian cancer (OC), a prevalent and severe malignancy of the female reproductive system, often presents with mild early symptoms and is therefore diagnosed at advanced stages, leading to a poor prognosis. Current chemotherapeutic treatment relies on platinum-based combinational therapy and there have been no recent breakthroughs in the development of new drugs. Advances in organoid technology offer a novel approach to study OC by simulating tumors and their microenvironment, enhancing drug screening effectiveness and accuracy, and providing a foundation for personalized therapy. In recent years, researchers have made notable advancements, successfully developing a diverse array of OC organoid models, with biobanks serving a pivotal role in enhancing their success rates and overall efficiency. The present review summarizes the advantages of organoids over other models, such as two-dimensional cell models, three-dimensional spheres and patient-derived xenograft models, as well as the application of organoids. In particular, the current review emphasizes the application of organoids in chemotherapeutic drug screening, testing and personalized treatment. The limitations and prospects of organoid technology are also discussed. The present study aimed to reveal the unique advantages of OC organoids in chemotherapeutic applications, so as to provide insights into screening and testing new drugs for OC.

Correspondence to: Dr Hai Zeng, Department of Oncology, The First Affiliated Hospital of Yangtze University, 4 Aviation Road, Jingzhou, Hubei 434023, P.R. China
E-mail: 2023721157@yangtzeu.edu.cn

Professor Ying Xiang, Department of Cell Biology and Medical Genetics, School of Basic Medicine, Health Science Center, Yangtze University, 1 Nanhuan Road, Jingzhou, Hubei 434023, P.R. China
E-mail: xying316@yangtzeu.edu.cn

*Contributed equally

Abbreviations: OC, ovarian cancer; 2D, two-dimensional; 3D, three-dimensional; PDX, patient-derived xenograft; OS, overall survival; HGSOC, high-grade serous ovarian carcinoma; TME, tumor microenvironment; ECM, extracellular matrix; PDOs, patient-derived organoids; OCCC, ovarian clear cell carcinoma; ENOC, ovarian endometrioid carcinoma; MTSs, multicellular tumor spheroids; MCTSs, multicellular tumor spheres; EOC, epithelial ovarian carcinoma; IC_{50}, median inhibitory concentration; VEGF, vascular endothelial growth factor; PARPi, poly(ADP-ribose) polymerase inhibitor; AsPCs, ascites-derived primary cell cultures; HRec, homologous recombination; BBB, blood-brain barrier; CAFs, cancer-associated fibroblasts; HR, hazard ratio; HRD, homologous recombination deficiency

Key words: ovarian cancer, organoid, chemotherapy, precision medicine

Contents

1. Introduction

Ovarian cancer (OC) is a malignant tumor that poses a significant threat to women's health. Globally, OC is the third most prevalent gynecological malignancy, yet it is associated with the highest mortality rate among these types of cancer (1). In 2022, it was estimated that 324,398 new cases of OC would be diagnosed and 206,839 related deaths would occur worldwide (2). OC is often diagnosed in its advanced stages, with current screening techniques unable to facilitate an earlier diagnosis (3); thus, the overall 5-year relative survival rate for OC is 50% (4). Delayed clinical presentation and a late diagnosis also contribute to the high mortality rate associated with this disease, mainly due to a significant lack of sensitive and specific biomarkers for early-stage disease (5).

Currently, the standard chemotherapeutic regimen for OC commonly employs a combination of taxane-based (such as paclitaxel) and platinum-based drugs (6). Cisplatin was the first platinum compound used in the primary treatment of OC; however, it is associated with dose-limiting toxicity, including minor symptoms such as nausea, severe kidney impairment and peripheral neuropathy. To overcome the toxicity associated with cisplatin use, organic platinum analogs of cisplatin, such as carboplatin, have been developed and used instead of cisplatin in initial OC chemotherapy (7). In patients with advanced disease, carboplatin plus paclitaxel chemotherapy is considered an optimal choice (8). Over time, chemotherapy for OC has resulted in progressive enhancements in survival, and the use of platinum/paclitaxel combinations, particularly in combination with intraperitoneal delivery, has the potential to double or increase survival time (9). In addition, heat-intraperitoneal perfusion chemotherapy may improve the disease-free survival of patients with OC, especially when the residual tumor is ≤1 cm or invisible, but it may only improve overall survival (OS) in patients with recurrent disease with a residual tumor of ≤1 cm (10).

Several challenges remain in the treatment of OC, despite advances in chemotherapy. For example, all forms of maintenance therapy have thus far failed to prolong survival. Intraperitoneal therapy has been shown to improve survival in small-volume stage III disease but is associated with significant toxicity (11). Furthermore, a substantial proportion of patients diagnosed with advanced OC are at an increased risk of recurrence (12) and drug resistance (13), which presents a significant challenge for clinicians in terms of developing effective treatment strategies. It is estimated that 60-80% of patients with epithelial ovarian carcinoma (EOC) will achieve complete remission following a combination of surgical and postoperative chemotherapy treatments; however, ~80% of these patients face the risk of chemotherapeutic resistance and potential recurrence (14).

With advancements in technology and research, organoid models have been shown to demonstrate notable improvements over traditional models in multiple aspects, primarily benefiting from their ability to more accurately simulate the complexity and dynamics of biological systems. Traditional two-dimensional (2D) culture models often overlook the three-dimensional (3D) structure of cells and the interactions between cells (15), and xenograft models have drawbacks such as immune rejection and species differences (16). Although patient-derived xenograft (PDX) models retain the tumor characteristics of patients (17,18), they are costly and technically complex to establish and maintain (19). By contrast, organoids, which are 3D dynamic tumor models that can simulate tumor environments *in vitro*, can mimic the disease development process (20). Organoids can overcome the limitations of traditional models, and thus provide new perspectives for OC research and treatment. Thus, there is an urgent need to develop new *in vitro* cultivation techniques that can mimic the development of the disease and produce organoids, and can be successfully grown from ovarian tumors, ascites or pleural fluid (21). As a novel approach to cell culture, organoid technology offers significant potential for developing individualized treatments and screening anticancer drugs. This is due to the high degree of tumor replication, the preservation of tumor heterogeneity, the short growth cycle and the stable transmission observed in organoids, which collectively address the limitations of existing preclinical models, including cell lines and animal models (22). The efficacy and resistance of chemotherapeutic drugs can be more accurately predicted through organoid culture, providing a basis for individualized treatment; therefore, organoids may be considered a convenient and efficient platform for studying drug resistance mechanisms (23).

The objective of the present review was to present a thorough overview of the definition, formation and construction of OC organoids. Additionally, the current review considers the advancement of OC organoids in chemotherapy, and their applications, deficiencies and prospects.

2. Overview of OC organoids

Organoids represent a 3D *in vitro* cell culture system (23), which are derived from cellular or organ progenitors and, through *in vitro* differentiation, develop functional cell clusters that display the essential properties of their organ of origin (24). Furthermore, they can preserve the heterogeneity of the original tumor and mimic the tumor growth microenvironment (23); they are thus considered a powerful model for simulating human disease.

In a 2D cell model, cells are grown as a single cell layer in a petri dish. Notably, 2D cell culture models cannot accurately represent the heterogeneity of tumors since they lack the inherent complexity and the TME of the original tumor (25). In addition, researchers have transplanted patient-derived subcutaneous or *in situ* tumor cells into immunodeficient mice to simulate the heterogeneity of the original tumors; however, this PDX model is expensive, time-consuming, and has low transplantation rates (26,27) and ethical drawbacks (28). Patient-derived organoids (PDOs) have emerged as a pivotal preclinical model system in the field of cancer research and clinical studies (29,30). Organoids retain a high degree of genomic similarity to their corresponding tumors; moreover, they can exhibit the inheritance of certain diseases and the ability to epigenetically alter their phenotype, which may facilitate the development of specific interventions to promote personalized medicine (31,32). The primary advantages of *in vitro* organoids over other *in vitro* tumor models are: i) They retain a high degree of heterogeneity among tumor cells, ii) they can be cultivated in small samples, and iii) they maintain the ability for long-term expansion, cryopreservation and genetic modification (24). The advantages and disadvantages of *in vitro* models are shown in Table I.

In order to generate organoids, tumor cells isolated from primary tumor material, such as surgically excised tissues, biopsies, ascites, pleural fluid or circulating tumor cells, need to be capsulated in a 3D matrix and cultured in medium containing variable growth factors and hormones (33) (Fig. 1). The composition and concentration of certain substances in the medium vary depending on the specific experiment or study; however, advanced Dulbecco's Modified Eagle Medium (DMEM)/Nutrient Mixture F-12 (F-12) is widely used (34,35). DMEM and F-12 are often used in combination to obtain higher concentrations of components of DMEM and a wider variety of components in the F-12 nutrient mix. DMEM/F-12,

Table I. Advantages and disadvantages of *in vitro* models.

Model	Advantages	Disadvantages	(Refs.)
2D cell model	Straightforward and easy to use; low cost	Lack of tumor complexity; poor expression of tumor heterogeneity; lack of immune cells and tumor microenvironment	(19)
PDX model	Preservation of tumor heterogeneity; mimics original tumor characteristics	Time-consuming; low transplantation rate; ethical issues; cost issues	(20-22)
Organoid	Maintenance of high tumor heterogeneity; high genomic homogeneity; possible to culture small samples; retention of long-term amplification, cryopreservation and genetic modification	Technical limitations; individual differences; standardization issues; cost issues	(23-25,27, 28,88-97)

PDX, patient-derived xenograft; 2D, two-dimensional.

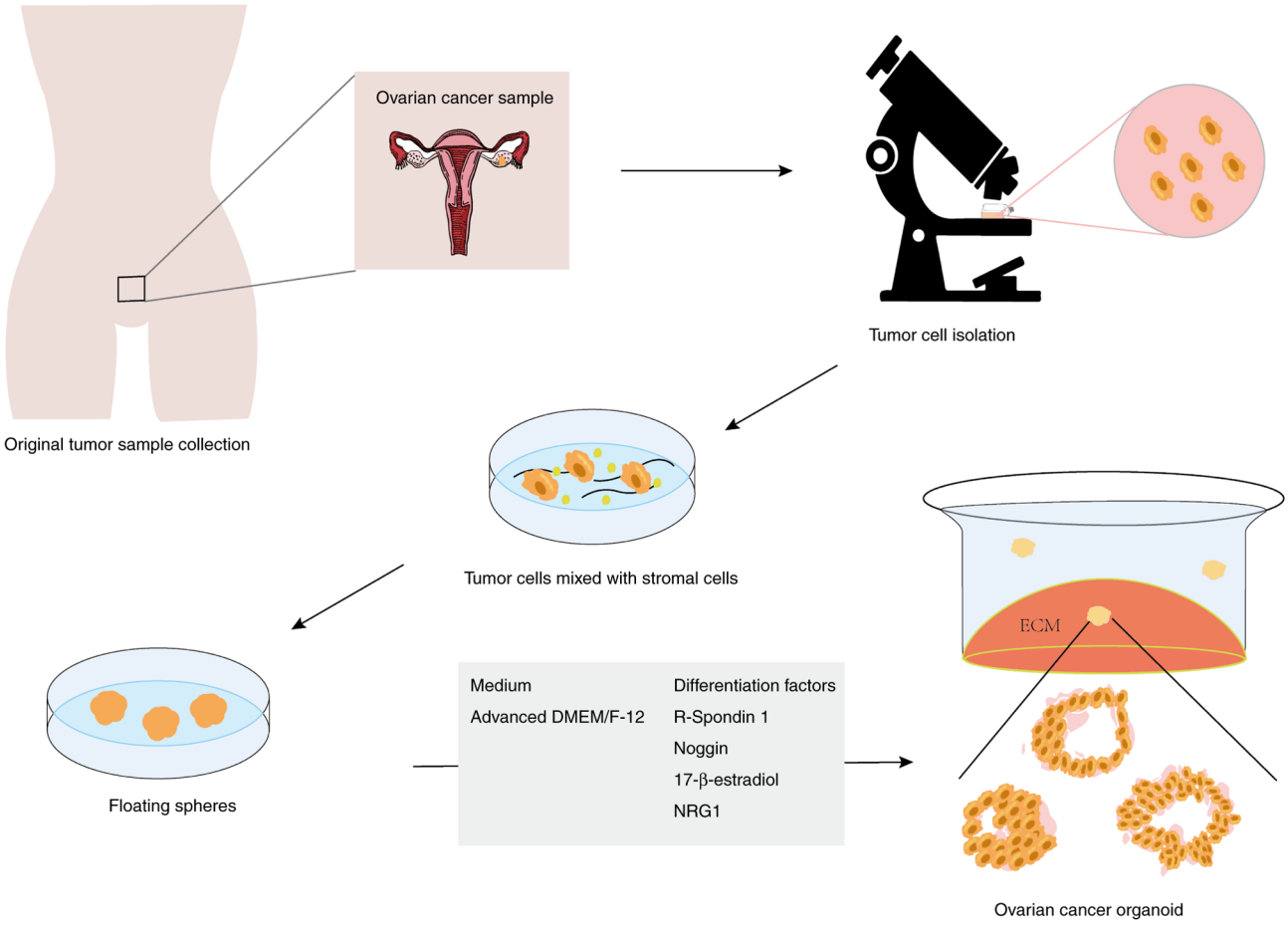

Figure 1. Formation of organoids from OC. Tumor cells from ovarian samples were mixed with ECM, aggregated into spheroids and placed in a medium with R-Spondin 1, Noggin, 17-β-estradiol, NRG1 and advanced DMEM/F-12, ultimately forming a fully developed OC organoid. ECM, extracellular matrix; NRG1, neuregulin 1; OC, ovarian cancer.

however, is free of growth factors, hormones and lipids; therefore, to achieve optimal cell proliferation when using DMEM/F-12, it is necessary to add an appropriate combination of growth factors, hormones, proteins or peptides, depending on the specific research needs and cell type. The mixture of growth factors utilized for OC organoid propagation is not standardized and may vary between studies. For OC organoids, it has been suggested that R-spondin 1 (an activator of the Wnt pathway), Noggin (an inhibitor of BMP-dependent differentiation) and 17β-estradiol are necessary for growth, while neuromodulin-1 (NRG1) may be needed for organoid expansion (36).

The development of OC organoids from multiple stages and subtypes (56 organoid lineages) has previously been successfully achieved (37). Organoid cultivation techniques can be applied to a wide range of patient samples, including tissues, biopsies, ascites and pleural effusions (38-40). Most OC organoids generated thus far have been high-grade serous ovarian carcinoma (HGSOC), but there are also other types of cancer, such as low-grade serous OC, mucinous OC, ovarian clear cell carcinoma (OCCC) and ovarian endometrioid carcinoma (ENOC) (41). Furthermore, the proliferation of primary ovarian organoids from different histological subtypes, including HGSOC, OCCC and ENOC, has been reported to have a success rate of 80% and to retain the original mutation pattern (42). Moreover, OC organoids are suitable for constructing biobanks to further improve organoid construction efficiency. For example, a study by Hoffmann *et al* (43) successfully generated 15 organoids (with an efficiency of ~30%) from 13-45 patients under conditions of low Wnt expression for long-term passaging culture. The OC organoids were cryopreserved for >5 months and passaged every 10-20 days. This previous study showed that there were no significant changes in the morphology or proliferation capacity of the organoids even after repeated thawing and freezing cycles.

3. OC organoid model applications

Application of OC organoid models for chemotherapeutic drug screening. Drug screening, which refers to testing the effects of a variety of drugs on several cell lines, is used to identify and develop medicines (44). In cancer therapy, drug screening aims to screen numerous compounds or new compounds for specific physiological activity against tumor cells. The present review aimed to summarize the differences in drug screening methods and their application in tumor therapy. For this purpose, models such as 2D cell cultures, multicellular tumor spheroids (MTSs) and organ tissues were assessed (Table II).

Notably, 2D cell cultures are the mainstay of fundamental cellular research, and are used to predict drug activity, metabolism and toxicity *in vitro* (45). In cancer screening, cytotoxicity testing is often performed on cultured tumor cell lines in monolayer culture due to the rapid and uncontrolled growth of these cell lines (46). By contrast, the main disadvantage of 2D systems is their inability to reproduce complex 3D structures or to communicate with the TME (47,48). Thus, 2D culture models may differ greatly from growing tumors with regard to cell morphology, proliferation, and gene and protein expression (49). Therefore, only 10% of the drugs that pass *in vitro* testing positively impact the clinic or lead to approval. The proportion of anticancer drugs that demonstrate clinical efficacy is even lower, at ~5% (50). Furthermore, a number of drugs reported to have potent anticancer effects in 2D cell culture models have failed clinical trials (51). In 2011, ~900 anticancer drugs passed cell-based tests, but only 12 were approved by the Food and Drug Administration after clinical testing (52).

The cells in MTS models more closely mimic the cell shape and environment *in vivo* than monolayer culture models (53,54); however, the size of spheroids has a substantial influence on their survival and reaction to drugs (55). Spheroids <100 μm do not reveal the complexity of the tumor or tissue (56). On the other hand, spheroids with diameters in the range of 400-500 μm can reflect the behavior of tumors *in vivo* due to the presence of different cellular layers: A necrotic core surrounded by a region of quiescent cells and an outer region composed of proliferating cells. The peripheral cells may reflect the *in vivo* tumor situation next to the capillaries, while the distant internal cells remain quiescent or die due to apoptosis or necrosis (57). This may also be why a number of reports (58-60) have shown that antitumor drugs are less effective against 3D cultured cancer cells than against 2D cultures.

It has also been shown that the MTS model has the potential to screen for antitumor drugs. Hirst *et al* (61) previously grew a series of EOC cell lines in 3D cell cultures to form multicellular tumor spheres (MCTSs). These MCTSs were characterized on the basis of the molecular and cellular properties of EOC, and were screened against cells grown in 2D cell culture to identify previously underappreciated anticancer agents. The MCTSs demonstrated enhanced resistance to chemotherapy, showed symptoms of senescence and hypoxia, and expressed numerous stem-cell-related transcripts, including ALDH1A and CD133 (PROM1). This previous study identified licofidone as a candidate with more activity in the MCTSs than in the 2D-cultured cells using a clinically repurposed drug library. Licofidone has been shown to be synergized with paclitaxel in an ovarian MCTS model, as well as in a patient-derived tumor xenograft model. The combination of licofidone and paclitaxel significantly increased the median survival time (>141 days) in mice compared with paclitaxel (115 days), licofidone (37 days) or vector (30 days). In addition, the Mantel-Haenszel hazard ratio (HR) was confirmed to be superior to the vector (HR=0.037) and paclitaxel (HR=0.017). The results of this previous study identified the potential use of an underappreciated anti-inflammatory agent in OC treatment.

Although the MTS model has demonstrated its unique advantages in antitumor drug screening, most identified drugs have failed in clinical applications due to the differences between MTSs and the real *in vivo* environment. Therefore, researchers have begun to explore OC organoid models. Cavarzerani *et al* (62) derived organoids from the ascites or tissues of patients with OC. In this previous study, the development of an organoid culture model with a higher growth rate, a faster drug response and better drug penetration into the extracellular matrix (ECM) was revealed to be possible in a passive microfluidic platform that maintained the vital signs of the sample and collected data for up to 16 medicines on a single plate. Achieving successful cultivation of organoids originating from patients with HGSOC was considered feasible in Mimetas Dual Lane OrganoPlates®. Thus, OC organoids may potentially be used to assess the antitumor activity of drugs.

OC organoids in chemosensitivity testing. Organoids can also be applied to chemosensitivity testing. *In vitro* susceptibility and resistance tests are used to determine whether a sample of a patient's tumor tissue exhibits a response (i.e., a reduction in tumor survival) when exposed to a selected chemotherapeutic agent under laboratory conditions (63). Drug response measurements aim to assess the therapeutic effectiveness of a

Table II. Multi-model perspective on ovarian cancer drug screening and sensitivity applications.

A, Drug screening

Model	Key findings	(Refs.)
2D cell cultures	Prediction of drug activity, metabolism and toxicity; only 10% of drugs tested *in vitro* are clinically effective and only 5% of anticancer drugs; only 12 of 900 drugs identified were approved by the Food and Drug Administration in 2011	(45,50,52)
MTSs	Potential of screening antitumor drugs; MCTSs demonstrate increased resistance to chemotherapeutic agents and the ability to screen for active drug candidates	(61)
Organoid	Improvement of antitumor activity effect; the potential for higher growth rates, faster drug response and better drug penetration; efficient screening in a passive microfluidic platform	(62)

B, Carboplatin

Model	Key findings	(Refs.)
2D monolayer, 3D spheres, 3D tumor *ex vivo*, mouse xenografts	3D sphere and mouse xenograft models best correlate with *in vivo* response; Variability in therapeutic response across models	(40)
2D monolayer, 3D spheres, mouse xenografts	3D sphere systems are in best agreement with *in vivo* models; 2D super-resistant cell lines are more sensitive in 3D and mouse models vice versa	(65)
Four cell lines, two PDOs, one PDX	PDX and PDO models reproduce patient tumor heterogeneity; Resistance to standard therapeutic drugs; Consistent with the clinic, PDO provides support for therapeutic decisions	(66)

C, Paclitaxel

Model	Key findings	(Refs.)
2D monolayer versus 3D hydrogel	3D sphere survival (40-60%) is better than 2D single layer survival (20%)	(67)
3D spheres (methylcellulose)	Spheres maintain cell density, and exhibit higher apoptotic behavior and higher paclitaxel resistance	(68)
PDO	PDO cell viability: 48.7%	(69)

D, Bevacizumab (Avastin®)

Model	Key findings	(Refs.)
Mouse xenograft model versus PDO	Mouse xenograft model may prolong survival and reduce ascites formation; models *in vivo* better reflect actual treatment effects, and PDO may be used for initial drug sensitivity screening; low effect on cell viability when used as a single agent; possibility of improvement of therapeutic effect when used in combination with chemotherapy	(69,73)

E, Olaparib

Model	Key findings	(Refs.)
Monolayer AsPCs versus 3D AsPCs	Monolayer AsPCs responded significantly more than 3D AsPCs to PARPis treatment	(41)
PDO	Only 2 of 33 organoid cultures tested (6%) were sensitive to olaparib	(41)

2D, two-dimensional; PDX, patient-derived xenograft; MTSs, multicellular tumor spheroids; MCTSs, multicellular tumor spheres; PDO, patient-derived organoid; AsPCs, ascites-derived primary cell cultures; PARPis, poly(ADP-ribose) polymerase inhibitors.

drug across a specified concentration range. Wherever possible, multi-drug response metrics should be used to account for possible experimental variations during the measurement, initial population and cell division count, including: Emax (maximal effect of the drug), EC_{50} (drug concentration that reaches Emax), IC_{50} (median inhibitory concentration), GI_{50} (concentration that decreases the overall cell growth by 50%), GR_{50} (50% inhibition of cell growth rate) and AUC (area under the dose-response curve, which represents the cumulative effect of the medicinal product) (64). Therefore, a multi-model perspective offers a comparative analysis of sensitivity testing in OC (Table II).

Carboplatin. Carboplatin is the most common first-line chemotherapy agent in the treatment of gynecological malignancies. The sensitivity of ovarian epithelial adenocarcinoma cell lines to carboplatin chemotherapy *in vitro* varies in 2D or 3D models (65). Compared with other *ex vivo* and *in vivo* models, the response of the 3D system to carboplatin has been revealed to be in best agreement with the *in vivo* model. To assess the current differences in treatment response in *in vitro* and *in vivo* models, and to deal with this problem, a comparative study was conducted by Maru *et al* (40) to distinguish the response of four different models to carboplatin chemotherapy, including 2D single layer, 3D sphere, 3D tumor *ex vivo* and mouse xenograft models. Six previously characterized EOC cell lines with different chemosensitivities were used and viability tests were carried out on each model. The *in vivo* results in the mouse model correlated with the 2D response in 3/6 cell lines, while they correlated with the 3D sphere response in 4/6 cell lines and the 3D *ex vivo* model in 5/5 cell lines. These results highlight the variability of treatment responses across models, and indicated that the response of carboplatin was best correlated with the response of EOC cell lines grown in a 3D *ex vivo* model.

Similarly, Brodeur *et al* (65) investigated the response of single-layer and spherical (3D) EOC cells to carboplatin, and then compared it with the *in vivo* response (xenografts). Mice were injected with six ovarian epithelial adenocarcinoma cell lines that received three different concentrations of carboplatin. Their responses were evaluated and classified based on tumor volume and immunofluorescence measurements. The same ovarian epithelial adenocarcinoma cell lines were seeded onto shallow adhesion plates to form spheroids and were processed. Flow cytometric analysis was performed to classify ovarian epithelial adenocarcinoma cell lines based on IC_{50}; this was compared to previously published 2D IC_{50} results. The results showed that the 3D system was in the best agreement with the *in vivo* model; in particular, the 2D super-resistant ovarian epithelial adenocarcinoma cell lines became more sensitive in either the mouse model or the 3D system. By contrast, the ultrasensitive ovarian epithelial adenocarcinoma cell line in the 2D system was more resistant in xenografts and spherical tissues.

Additionally, Thorel *et al* (66) established seven models (four cell lines, two PDOs and one PDX), all derived from the same OCCC. In order to establish their relevance, a comprehensive characterization was conducted using morphological, histological and transcriptomic analyses, as well as an evaluation of the patient's response to treatment, which was compared with the patient's chemotherapeutic response and showed resistance to drugs, carboplatin, gemcitabine and doxorubicin. Only PDX and PDO models derived from tumors could replicate the heterogeneity of patients. Patients were refractory to carboplatin, doxorubicin and gemcitabine, whereas cancer cells were susceptible to these agents. The PDX and PDO models, on the other hand, were resistant to these three drugs. Transcriptome analysis was consistent with these results, as models that faithfully reproduced the clinical response differed from the classical 2D cell culture models. Subsequently, the researchers examined the potential of previously unused drugs and determined that the histone deacetylase inhibitor belinostat was a potential therapeutic agent based on the response of the PDO. These findings indicated that PDX and PDO models may be the most relevant; however, only the PDO model offered all of the necessary preconditions for prediction and could be used in conventionally refractory types of cancer, especially aggressive ones.

Paclitaxel. Paclitaxel is a commonly used medicine for the treatment of OC. In a previous study, 3D patient-derived tumor spheroid structures were tested for paclitaxel susceptibility in comparison to 2D monolayer cultures. Loessner *et al* (67) performed an extensive study of EOC using hydrogel as a 3D model. The two cell lines (OV-MZ-6 and SKOV-3) were revealed to exhibit similar proliferation in 2D cultures, but different in 3D cultures. Spherical cell formation could only be observed in 3D cultures when the cells were embedded in hydrogels. The proliferation of OV-MZ-6 and SK-OV-3 cells in polyethylene glycol hydrogel possessing arginyl-glycyl-aspartic acid functionalized sites with glutamine, a general nutritional addition to the cell culture process, and matrix metalloproteinase sensitive sites for 14 days, followed by paclitaxel treatment, demonstrated a higher survival rate (40-60%) 7 days post-treatment compared with the single cells (20%) in 2D culture. Thus, 2D chemosensitivity evaluation does not necessarily reflect patient pathophysiology.

In order to determine the optimal conditions for analyzing the drug response of SKOV-3 and OVCAR-3 spheroids with the expected properties (roundness approaching 1.0; diameter in the range of 200-500 μm) to paclitaxel, two methods were used to evaluate the spheroids in two 3D cultures, after the addition of methylcellulose (0.25 and 0.5%, w/v) to the culture medium. Compared with 2D culture, SKOV-3 and OVCAR-3 spheroids retained cell density, increased apoptosis and increased resistance to paclitaxel treatment. The results indicated that 3D cultures may provide more reliable drug response results than 2D monolayer cultures (68). Meanwhile, Bi *et al* (69) assessed paired tumors and adjacent normal tissues from the same patient. At tumor-killing doses, they reported minimal cell destruction with variable chemotherapeutic agents. The results indicated that, in comparison with other drug treatments, the OC organoids of the patient were more sensitive to paclitaxel, and the survival rate was 48.7% after exposure to this single agent. The patient OC organoids were also more sensitive to gemcitabine (65% decrease in viability) and topotecan (56% decrease in viability) than the previously received chemotherapy regimen carboplatin + paclitaxel. Thus, organoids were considered more sensitive to paclitaxel compared to 2D monolayers.

Other drugs. Bevacizumab (Avastin®) is a recombinant humanized monoclonal antibody against vascular endothelial

growth factor (VEGF) (70), which binds to and neutralizes the biological properties of human VEGF by blocking VEGF interaction with its receptor (71). Bevacizumab is frequently used as an adjuvant treatment in gynecological tumors (72). In mice bearing HOC22 xenografts, OS and complete remission were markedly prolonged when bevacizumab was used in combination with chemotherapy and continued at the end of chemotherapy. Bevacizumab, on its own, inhibited the formation of ascites and had a limited impact on tumor load (73). Bi *et al* (69) also tested the sensitivity of PDOs to either bevacizumab alone or in combination with first-line standard chemotherapy. Overall, bevacizumab as a monotherapy had a minimal effect on cell viability and, when combined with chemotherapy, had little additive effect on cell killing

Olaparib was the first approved poly (ADP-ribose) polymerase inhibitor (PARPi) used to treat patients with advanced BRCA-mutated OC (70,72,74). PARPis inhibit PARP in cells with BRCA-mutated tumors; they can cause 'synthetic lethality' by targeting two DNA repair pathways at the same time and do not affect normal cells (73). Hill *et al* (41) formulated a 3D spherical functional evaluation methodology aimed at quantifying the responsiveness of two PARPi agents, specifically niraparib and olaparib, within ascites-derived primary cell cultures (AsPCs) derived from patients diagnosed with HGSOC. An agarose-based protocol for AsPC preparation enabled efficient isolation and propagation of monolayer and 3D AsPCs. The results showed a distinct sensitivity difference to PARPis, with monolayer AsPCs exhibiting 88 and 52% sensitivity to niraparib and olaparib, respectively, compared with 66 and 38% sensitivity in 3D AsPCs. The latter aligns with prior homologous recombination deficiency (HRD) estimates in EOC (40-60%), emphasizing the relevance of 3D models.

Application of OC organoid models in individualized therapy. The application of organoids in personalized medicine has also begun to be implemented (74,75). The development of personalized medicine is about preventing, diagnosing and treating pathological conditions based on individual characteristics (38). The characteristics of the individual include the entire body, tissues and cells, including genetic, epigenetic, transcriptomic, proteomic and metabolomic markers (76). In addition, gene expression profiles can be studied and changes associated with tumors can be identified (77). Personalized medicine obtains patient-specific disease information to select drugs and the correct dosage, and to optimize therapeutic procedures (78). Personalized medicine also influences preclinical studies of drugs, not just regarding efficacy and safety, but also to reduce drug development costs (38). OC encompasses a diverse array of histotypes, each characterized by distinct origins, underlying genetic and epigenetic alterations, and varying survival outcomes. Moreover, the differing prevalence of these histotypes across various ethnicities adds another layer of intricacy (79). In light of these multifaceted variables, the use of traditional 2D cell lines, albeit immortalized and homogeneous, as *in vitro* models of OC, are inherently limited in their capacity to capture the complexity of the disease at any single time point or in a microenvironment that accurately mirrors the *in vivo* scenario. Consequently, organoid technology has emerged as a promising avenue for advancing cancer research and facilitating the development of tailored therapeutic strategies (80). In clinical practice, OC organoid models have demonstrated their unique value in personalized therapy. For example, Lui *et al* (81) provided prospective and retrospective evidence from ≥18 cases, which indicated that organoid drug screening can accurately predict clinical response to chemotherapy and targeted therapy. This previous study also reported on a patient with platinum-resistant plasma OC who responded to ibrutinib therapy after screening, with this drug identified as having an excellent response in their organoid. After 3 months of treatment, the CA125 levels of the patient had decreased from 250 to 125 U/ml.

Preclinical studies. It is becoming increasingly apparent that standard treatment does not apply to every patient and that it would be helpful to use pretreatments to provide a more individualized regimen. Drug testing of PDOs can be executed in a timely manner; in addition, PDOs have the potential to identify the most efficacious drug in each case, and to offer a promising preclinical platform for tailoring personalized cancer treatment strategies in patients with gynecological malignancies (69).

For example, in 2019, Phan *et al* (82) pioneered the use of PDOs from HGSOC and ovarian sarcoma, using an automated screening system to assess the unique responses of these organoids to a panel of 240 kinase inhibitors. The high-throughput methodology not only identified sensitive signaling pathways but also facilitated the selection of the most efficacious drugs within specific molecular classes. This previous study presented a scalable and functional precision medicine platform titled Drug Efficacy Testing in 3D Culture, which can quantify the responsiveness of patient-derived cells to various drugs and drug combinations through live-cell imaging. Employing this platform, 27 tailored drug combinations were evaluated, achieving results within 10 days post-surgery. Notably, the findings revealed that the combination of carboplatin and a Bcl-xL inhibitor exhibited synergistic effects in 4 out of 8 OC samples tested, whereas afatinib and the Bcl-xL inhibitor A-1331852 demonstrated synergism in 5 of 7 OC models. These results suggested that combining the Bcl-xL inhibitor A-1331852 with either afatinib or carboplatin may hold promise as a potential future therapeutic strategy for patients with OC (83).

Biomarkers. Predictive biomarkers of therapeutic response are vital for assessing clinical benefit in patients with OC, yet they remain an unmet need (84). Notably, the potential of RAD51 foci as predictive biomarkers in HGSOC warrants validation in clinical trials (85). RAD51 has emerged as a prime candidate biomarker for such assessments due to several reasons. Firstly, upon the occurrence of DNA double-strand breaks, the kinase ATM swiftly phosphorylates histone H2AX (γH2AX), initiating a cascade that leads to the formation of single-stranded DNA with 3' projections. These, in turn, engage replication protein A, which RAD51 subsequently displaces via BRCA1 and BRCA2, facilitating homologous recombination (HRec). Given the multitude of upstream events required for RAD51 binding and its central role in HRec, RAD51 provides a holistic readout of the intricate steps of HRec. Secondly, RAD51 binding results in the formation of nucleoprotein filaments that can be visualized as foci under a microscope, with RAD51 focus deficiency serving as a functional marker for

HRD (86-88). Thirdly, RAD51 foci detection has been shown to possess predictive value for platinum chemotherapy and PARPi responses in OC xenografts (89-91), correlating with *in vitro* platinum responses in established and primary OC cell lines (Pearson r=0.96; P=0.01). Tumor tissue cells that did not respond to platinum have been reported to have significantly higher RAD51 scores than those that responded to platinum (P<0.001). Notably, tumors with low RAD51 scores exhibited heightened platinum sensitivity, with a significantly higher likelihood of achieving pathologic complete response [relative risk (RR) 5.28; P<0.001] and platinum sensitivity (RR, ∞; P=0.05). Furthermore, the RAD51 score could accurately predict chemotherapy response (AUC, 0.90; 95% CI, 0.78-1.0; P<0.001), with an automated quantification system mirroring manual test results at 92% accuracy. In the validation cohort, RAD51-low tumors displayed enhanced platinum sensitivity (RR, ∞; P<0.001), a 100% positive predictive value for platinum sensitivity, and improved progression-free survival (HR, 0.53; 95% CI, 0.33-0.85; P<0.001) and OS (HR, 0.43; 95% CI, 0.25-0.75; P=0.003) compared with RAD51-high tumors (85). Advancements have focused on assessing HRec proficiency through RAD51 foci formation at DNA damage sites, exemplified by the repair capability (RECAP) test (92). Initially devised for breast cancer HRD assessment, this test has been validated using tumor sections and has demonstrated applicability in OC (93), underlining the versatility of tumor sections and diagnostic/therapeutic biomarker potential. Additionally, functional HRec status in PDOs has been evaluated using the RECAP test, correlating with PDO drug sensitivity (including platinum chemotherapy and PARPis) (37,42) and clinical response (41). However, as evidenced by *in vivo* studies, HRD alone does not guarantee a response to platinum or PARPis (41), pointing to a more intricate landscape requiring further exploration.

Genomics. Precision healthcare represents a strategy that tailors therapeutic interventions by comprehensively assessing the interplay of an individual's genetic makeup, environmental factors and lifestyle patterns. Since genomics alone is insufficient to determine treatment options for most patients with advanced diseases, the creation of live biological specimens of tumor-like organs can facilitate the integration of genomic profiles and drug screening within an iterative framework, enabling the identification of personalized therapeutic strategies tailored to each patient (38). In comparison to traditional 2D cultures, 3D organoid cultures exhibit a broader spectrum of drug responses that more accurately mirror genomic variations (39), rendering them an ideal platform for drug sensitivity assessments in translational medicine and precision healthcare endeavors. PDX models of OC also reflect the heterogeneity of tumor genomic profiles and serve as a valuable tool for drug evaluation (94,95). Nevertheless, organoids emerge as a superior 3D culture system, surpassing PDX in terms of i) reduced tumor sample requirements, ii) expedited engraftment timelines and iii) enhanced engraftment success rates (96). Notably, de Witte *et al* (97) conducted *in vitro* drug screening utilizing 36 genome-wide profiled PDOs from 23 patients with OC, demonstrating the ability of OC PDOs to faithfully recapitulate individual patient responses to first-line chemotherapeutics. Notably, variations in sensitivity were observed, with low responsiveness to carboplatin/paclitaxel, PARPis and specific tyrosine kinase inhibitors (afatinib, adavotinib), contrasted by high responsiveness to gemcitabine, flavopiridol (a cell cycle-dependent kinase inhibitor) and vimofenib (a BRAF V600E kinase inhibitor). Therefore, OC organoid models may be mainly applied for drug screening, sensitivity and individualized therapy (Fig. 2).

4. Deficiencies and prospects of OC organoid models in chemotherapy

Deficiencies

Technical limitations. PDO cultures may not be representative of tumor settings because of the absence of stroma, vasculature and immune cells (35); therefore, the organoid model cannot mimic the response to immunotherapy and antiangiogenic therapy (98). Heterogeneity within PDO culture tumors is also incomplete (97). The ability of organoids to maintain biological properties and functions similar to those of the primary tumors in an *in vitro* environment holds paramount significance for developing clinical applications.

In vivo, tissue development is constrained by external stimulation delivered in an exact space and time sequence. Usually, this does not occur in conventional 3D culture systems, where cells are embedded in an isotropic matrix and uniformly flooded with biochemical and ecological niche signals. This limitation can be overcome by a 3D culture matrix that can release or present biomolecules under spatiotemporal control (99,100). Achieving accurate control of the growth and differentiation of organoids is a critical task in organic research, which carries profound significance across diverse areas, such as disease modeling, pharmaceutical screening and regenerative medical research.

Individual differences. Because of the significant individual differences in patients with OC and the wide variety of tumor mutant types (e.g. Trp53, Brca1, KRAS, BRAF, PTEN, PIK3CA), it is not possible to fully simulate each patient's individual conditions. This may result in some bias in the screening and evaluation of chemotherapeutic drugs, and also increases the difficulty and cost of organoid culture.

Tumor characteristics and physiological barriers, such as the blood-brain barrier (BBB), vary from patient to patient, affecting drug penetration and metabolism in cancer tissues and thus altering therapeutic efficacy. Therefore, the use of human equivalent dose (the *in vivo* concentration of a drug in tumor tissue) is recommended in cancer organ tissue therapeutic trials to more accurately assess drug efficacy. The development of microfluidics and an artificial BBB based on microchip organ technology may advance this field (101,102).

Cost. Compared with monolayer cell cultures, organoid cultures are more costly because of the need for specific media, including ECM (e.g., Matrigel) and cofactors (e.g., growth factors and hormones) (103,104). In addition, the existence of individual differences and the difficulty of standardization further increases the cost of experiments, as different laboratories and research groups may need to use different methods and materials to conduct experiments, leading to higher costs of duplicating experiments and validation.

The key to improving the efficacy and comparability of organoid techniques is standardization; however, there are currently notable differences in organoid culture protocols

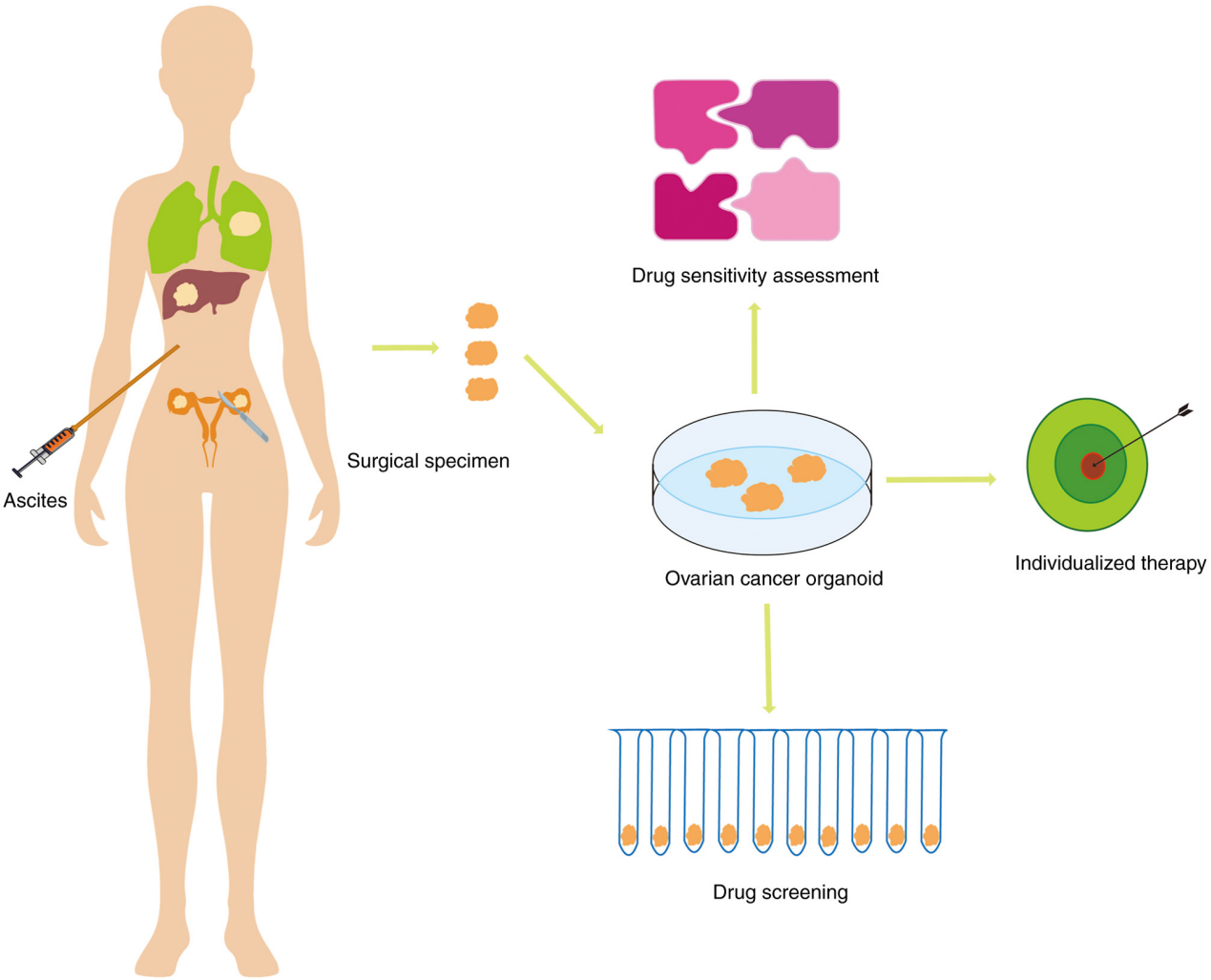

Figure 2. Application of organoids for chemotherapy of OC. OC organoid models may be mainly applied for drug screening, sensitivity and individualized therapy. OC, ovarian cancer.

from different investigators (34,103), which may lead to inconsistent results. To achieve standardization, key factors such as tissue manipulation procedures, culture media and growth factors need to be harmonized (103). However, standardization is challenged by individual differences, unknown composition of animal-derived scaffolds and poor tunability (105). In addition, the animal-derived Engelbreth-Holm-Swarm substrates used in organoid cultures vary widely between batches and contain unknown contaminants (106), which also limits standardization. Next-generation organoid models, such as co-culture models that incorporate different cells, may partially address these issues but have variable success rates and have also not been standardized (107).

Prospects

3D bioprinting. 3D bioprinting is a promising method for the construction of heterogeneous and reproducible cancer models with controlled shape and structure. The 3D-bioprinted structure can be used to simulate the TME. Different cell layers can be produced using bioprinting, including normal tissue-specific cells, connective tissue and cancer cells (108). This technology can accurately replicate the complex structure and microenvironment of cancer cells, creating organoid models that are more similar to the real-world situation of the tumor (109). Baka *et al* (110) described a 3D-bioprinted ovarian carcinoma model that combined cancer cells (SKOV-3) and cancer-associated fibroblasts (CAFs). The resulting tumor models demonstrated their ability to maintain cell viability and proliferation, and cells were observed to self-assemble in heterogeneous aggregates. Furthermore, it was observed that CAFs were recruited and multiplied in the surrounding tumor cells in an *in vivo* process occurring in the TME. Notably, this approach has also demonstrated an ability to produce a large number of reproducible tumor models, which can undergo conventional experimental methods (cell viability and metabolism assays, histology and immunology, and real-time imaging). 3D bioprinting of tumor constructs as personalized *in vitro* models may be a useful tool for screening for anticancer drugs and establishing accurate treatment regimens. As an example, MRC-5 fibroblasts and human OC cells (OVCAR-5) were previously printed on Matrigel by means of droplet-based bioprinting to form high-throughput, reproducible multi-cellular structures in a controlled space. This approach was shown to provide a platform for minimizing the size of 3D culture models at the macro scale; this could be considered an innovative platform for high-throughput

and robust personalized drug screening, including tumor and stromal cell microenvironments (111).

Gene editing technologies. Gene editing techniques, such as CRISPR-Cas9, have the potential to introduce or modify mutations that mimic tumor-forming processes, and potentially identify and modify driver genes in cancer organoids (112). Through CRISPR-Cas9, organoids can be artificially altered by mutation of function or loss of function to induce malignancy (113). Notably, these gene editing techniques may be used to modify specific genes in organoid models to investigate the role of these genes in the occurrence, progression and treatment of OC. This could help to improve the understanding of the mechanisms of OC and to develop new therapeutic agents. Lõhmussaar *et al* (114) previously introduced mutations into common HGSOC genes, including Trp53, Brca1, Nf1 and Pten, using the genome editing method of CRISPR-Cas9. Organoids have also been artificially modified by gain-of-function or loss-of-function mutations to induce malignancy via CRISPR-Cas9 (115). Furthermore, Kopper *et al* (37) demonstrated the feasibility of this approach in OC using normal Fallopian tube organoids from high-risk OC donors that could be efficiently genome-edited via CRISPR-Cas9 and clonally amplified. In addition, in this previous study, CRISPR-Cas9 genome editing was used to introduce common HGSOC gene defects into mouse oviducts and the ovarian surface epithelium cell-like organs, showing that both organ types are potentially carcinogenic.

Genomics. Single-cell sequencing has emerged as an advanced biotechnological technique capable of decoding the OC landscape at single-cell resolution. It works at the gene, transcriptome, protein, epigenome and metabolism levels, and provides detailed information that differs from bulk sequencing methods, which only provide average data, representing the overall or average characteristics of the cell population in the entire sample or lesion area on specific lesions. Single-cell sequencing techniques provide detailed insights into the immune and molecular mechanisms that underlie tumorigenesis, progression, resistance and immune escape. These insights can be used to develop innovative diagnostic markers, treatment strategies and prognostic indicators (116). Wan *et al* (117) reported that HGSOC-like organoid co-cultures were treated with bispecific anti-PD-1 or PD-L1 antibodies, and then underwent single-cell RNA-sequencing. The results showed that this treatment reprogrammed inert natural killer cells and T cells into highly active cytotoxic states, thus indicating the potential benefit of bispecific antibody treatment in HGSOC. Gonzalez *et al* (118) performed an in-depth single-cell phenotypic characterization of HGSOC by multiparametric mass cytometry. The features of HGSOC biology were examined using a cytometry antibody panel and rare tumor subtypes of HGSOC were identified. It was shown that one of these rare subtypes was enriched in epithelial-mesenchymal transition signaling and was associated with increased tumor metastasis.

5. Conclusion

In summary, OC organoids maintain high tumor cell heterogeneity and are cultured in small samples, which can be maintained for long-term expansion and can be cryopreserved. OC organoids have been successfully developed from multiple stages and subtypes (56 organoid lines) (37). Thus far, OC organoid models have been widely used in drug screening, biomarker identification and personalized therapy; however, there are challenges to the use of these organoid models, including technical limitations, individual differences, cost issues and issues of standardization. To overcome these shortcomings, a further exploration into the application of technologies, such as 3D bioprinting, gene editing and genomics, is recommended in the optimization of organoid models to improve the future stability and reproducibility of these models. The development of these technologies is expected to make organoid models more stable and reproducible, which will better simulate the *in vivo* environment, and improve the accuracy and efficiency of drug screening. Therefore, the OC organoid model has a broad potential for application in chemotherapy. Along with the development of the technique, it is hoped that this model will be a more precise and efficient method to predict the prognosis of OC, and to improve therapeutic effects and the quality of life in patients with OC. In addition, research in this area may provide valuable information for other cancer treatments.

Acknowledgements

Not applicable.

Funding

This work was supported by the Natural Science Foundation General Project of Hubei Provincial (grant no. 2024AFB1044), the Development Fund Project of Yangtze University (grant no. WJ2019-22) and the Start Fund of First Affiliated Hospital of Yangtze University (grant no. 2022DIF01).

Availability of data and materials

Not applicable.

Authors' contributions

WZ designed and conceived the study, wrote the manuscript and acquired funding. YD designed and conceived the study and wrote the manuscript. HH and KC conducted the literature analysis. QZ and XC were involved in visualization. HZ and YX revised the manuscript. Data authentication is not applicable. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Kuroki L and Guntupalli SR: Treatment of epithelial ovarian cancer. BMJ 371: m3773, 2020.
2. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I and Jemal A: Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 74: 229-263, 2024.
3. Matulonis UA, Sood AK, Fallowfield L, Howitt BE, Sehouli J and Karlan BY: Ovarian cancer. Nat Rev Dis Primers 2: 16061, 2016.
4. American Cancer Society: Cancer Facts and Figures 2023. American Cancer Society, Atlanta, GA, 2023.
5. Liberto JM, Chen SY, Shih IM, Wang TH, Wang TL and Pisanic TR II: Current and emerging methods for ovarian cancer screening and diagnostics: A comprehensive review. Cancers (Basel) 14: 2885, 2022.
6. Kikuchi Y, Kita T, Takano M, Kudoh K and Yamamoto K: Treatment options in the management of ovarian cancer. Expert Opin Pharmacother 6: 743-754, 2005.
7. Bookman MA: First-line chemotherapy in epithelial ovarian cancer. Clin Obstet Gynecol 55: 96-113, 2012.
8. Ozols RF, Bundy BN, Greer BE, Fowler JM, Clarke-Pearson D, Burger RA, Mannel RS, DeGeest K, Hartenbach EM and Baergen R: Phase III trial of carboplatin and paclitaxel compared with cisplatin and paclitaxel in patients with optimally resected stage III ovarian cancer: A gynecologic oncology group study. J Clin Oncol 41: 4077-4083, 2023.
9. Kyrgiou M, Salanti G, Pavlidis N, Paraskevaidis E and Ioannidis JP: Survival benefits with diverse chemotherapy regimens for ovarian cancer: Meta-analysis of multiple treatments. J Natl Cancer Inst 98: 1655-1663, 2006.
10. Kim SI, Cho J, Lee EJ, Park S, Park SJ, Seol A, Lee N, Yim GW, Lee M, Lim W, *et al*: Selection of patients with ovarian cancer who may show survival benefit from hyperthermic intraperitoneal chemotherapy: A systematic review and meta-analysis. Medicine (Baltimore) 98: e18355, 2019.
11. Ozols RF: Challenges for chemotherapy in ovarian cancer. Ann Oncol 17 (Suppl 5): v181-v187, 2006.
12. Fung-Kee-Fung M, Oliver T, Elit L, Oza A, Hirte HW and Bryson P: Optimal chemotherapy treatment for women with recurrent ovarian cancer. Curr Oncol 14: 195-208, 2007.
13. Pokhriyal R, Hariprasad R, Kumar L and Hariprasad G: Chemotherapy resistance in advanced ovarian cancer patients. Biomark Cancer 11: 1179299x19860815, 2019.
14. Cornelison R, Llaneza DC and Landen CN: Emerging therapeutics to overcome chemoresistance in epithelial ovarian cancer: A mini-review. Int J Mol Sci 18: 2171, 2017.
15. Baker BM and Chen CS: Deconstructing the third dimension: How 3D culture microenvironments alter cellular cues. J Cell Sci 125: 3015-3024, 2012.
16. Zhang Z, Bédard E, Luo Y, Wang H, Deng S, Kelvin D and Zhong R: Animal models in xenotransplantation. Expert Opin Investig Drugs 9: 2051-2068, 2000.
17. Bertotti A, Migliardi G, Galimi F, Sassi F, Torti D, Isella C, Corà D, Di Nicolantonio F, Buscarino M, Petti C, *et al*: A molecularly annotated platform of patient-derived xenografts ('xenopatients') identifies HER2 as an effective therapeutic target in cetuximab-resistant colorectal cancer. Cancer Discov 1: 508-523, 2011.
18. DeRose YS, Wang G, Lin YC, Bernard PS, Buys SS, Ebbert MT, Factor R, Matsen C, Milash BA, Nelson E, *et al*: Tumor grafts derived from women with breast cancer authentically reflect tumor pathology, growth, metastasis and disease outcomes. Nat Med 17: 1514-1520, 2011.
19. Sachs N, de Ligt J, Kopper O, Gogola E, Bounova G, Weeber F, Balgobind AV, Wind K, Gracanin A, Begthel H, *et al*: A living biobank of breast cancer organoids captures disease heterogeneity. Cell 172: 373-386.e310, 2018.
20. Zanoni M, Cortesi M, Zamagni A, Arienti C, Pignatta S and Tesei A: Modeling neoplastic disease with spheroids and organoids. J Hematol Oncol 13: 97, 2020.
21. Graham O, Rodriguez J, van Biljon L, Fashemi B, Graham E, Fuh K, Khabele D and Mullen M: Generation and culturing of high-grade serous ovarian cancer patient-derived organoids. J Vis Exp 6: 191, 2023.
22. Yani W, Qi J, Yuchen Z and Haiyan Z: Application of organoids technology in drug sensitivity test of ovarian cancer. J Int Obstet Gynecol 49: 181-185, 2022.
23. Yujie S, Hong Y, Jia L and Ying X: Application prospects on organoid culture system in drug screening and treatment target for ovarian cancer. J Chin Oncol 28: 1042-1045, 2022 (In Chinese).
24. Jianjun G, Wei Q, Hao W and Xiangyu Z: Application and prospect of organoid technique in cancer research. Chin J Tissue Engineering Res 23: 1136-1141, 2019 (In Chinese).
25. Aihara A, Abe N, Saruhashi K, Kanaki T and Nishino T: Novel 3-D cell culture system for in vitro evaluation of anticancer drugs under anchorage-independent conditions. Cancer Sci 107: 1858-1866, 2016.
26. Ben-David U, Ha G, Tseng YY, Greenwald NF, Oh C, Shih J, McFarland JM, Wong B, Boehm JS, Beroukhim R and Golub TR: Patient-derived xenografts undergo mouse-specific tumor evolution. Nat Genet 49: 1567-1575, 2017.
27. Byrne AT, Alférez DG, Amant F, Annibali D, Arribas J, Biankin AV, Bruna A, Budinská E, Caldas C, Chang DK, *et al*: Interrogating open issues in cancer medicine with patient-derived xenografts. Nat Rev Cancer 17: 632, 2017.
28. Sachs N and Clevers H: Organoid cultures the analysis of cancer phenotypes. Curr Opin Genet Dev 24: 68-73, 2014.
29. Bleijs M, van de Wetering M, Clevers H and Drost J: Xenograft and organoid model systems in cancer research. EMBO J 38: e101654, 2019.
30. Wensink GE, Elias SG, Mullenders J, Koopman M, Boj SF, Kranenburg OW and Roodhart JML: Patient-derived organoids as a predictive biomarker for treatment response in cancer patients. NPJ Precis Oncol 5: 30, 2021.
31. Perkhofer L, Frappart PO, Müller M and Kleger A: Importance of organoids for personalized medicine. Per Med 15: 461-465, 2018.
32. Rossi G, Manfrin A and Lutolf MP: Progress and potential in organoid research. Nat Rev Genet 19: 671-687, 2018.
33. Tsang SI, Hassan AA, To SKY and Wong AST: Experimental models for ovarian cancer research. Exp Cell Res 416: 113150, 2022.
34. Yang J, Huang S, Cheng S, Jin Y, Zhang N and Wang Y: Application of ovarian cancer organoids in precision medicine: Key challenges and current opportunities. Front Cell Dev Biol 9: 701429, 2021.
35. Aboulkheyr Es H, Montazeri L, Aref AR, Vosough M and Baharvand H: Personalized cancer medicine: An organoid approach. Trends Biotechnol 36: 358-371, 2018.
36. Maenhoudt N, Defraye C, Boretto M, Jan Z, Heremans R, Boeckx B, Hermans F, Arijs I, Cox B, Van Nieuwenhuysen E, *et al*: developing organoids from ovarian cancer as experimental and preclinical models. Stem Cell Reports 14: 717-729, 2020.
37. Kopper O, de Witte CJ, Lõhmussaar K, Valle-Inclan JE, Hami N, Kester L, Balgobind AV, Korving J, Proost N, Begthel H, *et al*: An organoid platform for ovarian cancer captures intra- and interpatient heterogeneity. Nat Med 25: 838-849, 2019.
38. Pauli C, Hopkins BD, Prandi D, Shaw R, Fedrizzi T, Sboner A, Sailer V, Augello M, Puca L, Rosati R, *et al*: Personalized in vitro and in vivo cancer models to guide precision medicine. Cancer Discov 7: 462-477, 2017.
39. Jabs J, Zickgraf FM, Park J, Wagner S, Jiang X, Jechow K, Kleinheinz K, Toprak UH, Schneider MA, Meister M, *et al*: Screening drug effects in patient-derived cancer cells links organoid responses to genome alterations. Mol Syst Biol 13: 955, 2017.
40. Maru Y, Tanaka N, Itami M and Hippo Y: Efficient use of patient-derived organoids as a preclinical model for gynecologic tumors. Gynecol Oncol 154: 189-198, 2019.
41. Hill SJ, Decker B, Roberts EA, Horowitz NS, Muto MG, Worley MJ Jr, Feltmate CM, Nucci MR, Swisher EM, Nguyen H, *et al*: Prediction of DNA repair inhibitor response in short-term patient-derived ovarian cancer organoids. Cancer Discov 8: 1404-1421, 2018.
42. Nanki Y, Chiyoda T, Hirasawa A, Ookubo A, Itoh M, Ueno M, Akahane T, Kameyama K, Yamagami W, Kataoka F and Aoki D: Patient-derived ovarian cancer organoids capture the genomic profiles of primary tumours applicable for drug sensitivity and resistance testing. Sci Rep 10: 12581, 2020.
43. Hoffmann K, Berger H, Kulbe H, Thillainadarasan S, Mollenkopf HJ, Zemojtel T, Taube E, Darb-Esfahani S, Mangler M, Sehouli J, *et al*: Stable expansion of high-grade serous ovarian cancer organoids requires a low-Wnt environment. EMBO J 39: e104013, 2020.
44. Kondo J and Inoue M: application of cancer organoid model for drug screening and personalized therapy. Cells 8: 470, 2019.
45. Antoni D, Burckel H, Josset E and Noel G: Three-dimensional cell culture: A breakthrough in vivo. Int J Mol Sci 16: 5517-5527, 2015.

46. Kimlin LC, Casagrande G and Virador VM: In vitro three-dimensional (3D) models in cancer research: An update. Mol Carcinog 52: 167-182, 2013.
47. Shoemaker RH: The NCI60 human tumour cell line anticancer drug screen. Nat Rev Cancer 6: 813-823, 2006.
48. Rizvanov AA, Yalvaç ME, Shafigullina AK, Salafutdinov II, Blatt NL, Sahin F, Kiyasov AP and Palotás A: Interaction and self-organization of human mesenchymal stem cells and neuro-blastoma SH-SY5Y cells under co-culture conditions: A novel system for modeling cancer cell micro-environment. Eur J Pharm Biopharm 76: 253-259, 2010.
49. Enmon RM Jr, O'Connor KC, Lacks DJ, Schwartz DK and Dotson RS: Dynamics of spheroid self-assembly in liquid-overlay culture of DU 145 human prostate cancer cells. Biotechnol Bioeng 72: 579-591, 2001.
50. Westhouse RA: Safety assessment considerations and strategies for targeted small molecule cancer therapeutics in drug discovery. Toxicol Pathol 38: 165-168, 2010.
51. Wong CC, Cheng KW and Rigas B: Preclinical predictors of anticancer drug efficacy: Critical assessment with emphasis on whether nanomolar potency should be required of candidate agents. J Pharmacol Exp Ther 341: 572-578, 2012.
52. Ravi M, Paramesh V, Kaviya SR, Anuradha E and Solomon FD: 3D cell culture systems: Advantages and applications. J Cell Physiol 230: 16-26, 2015.
53. Jamieson LE, Harrison DJ and Campbell CJ: Chemical analysis of multicellular tumour spheroids. Analyst 140: 3910-3920, 2015.
54. Beningo KA, Dembo M and Wang Yl: Responses of fibroblasts to anchorage of dorsal extracellular matrix receptors. Proc Natl Acad Sci USA 101: 18024-18029, 2004.
55. Sambale F, Lavrentieva A, Stahl F, Blume C, Stiesch M, Kasper C, Bahnemann D and Scheper T: Three dimensional spheroid cell culture for nanoparticle safety testing. J Biotechnol 205: 120-129, 2015.
56. Jarockyte G, Dapkute D, Karabanovas V, Daugmaudis JV, Ivanauskas F and Rotomskis R: 3D cellular spheroids as tools for understanding carboxylated quantum dot behavior in tumors. Biochim Biophys Acta Gen Subj 1862: 914-923, 2018.
57. Mehta G, Hsiao AY, Ingram M, Luker GD and Takayama S: Opportunities and challenges for use of tumor spheroids as models to test drug delivery and efficacy. J Control Release 164: 192-204, 2012.
58. Souza AG, Silva IBB, Campos-Fernandez E, Barcelos LS, Souza JB, Marangoni K, Goulart LR and Alonso-Goulart V: Comparative assay of 2D and 3D cell culture models: Proliferation, gene expression and anticancer drug response. Curr Pharm Des 24: 1689-1694, 2018.
59. Breslin S and O'Driscoll L: The relevance of using 3D cell cultures, in addition to 2D monolayer cultures, when evaluating breast cancer drug sensitivity and resistance. Oncotarget 7: 45745-45756, 2016.
60. Verjans ET, Doijen J, Luyten W, Landuyt B and Schoofs L: Three-dimensional cell culture models for anticancer drug screening: Worth the effort? J Cell Physiol 233: 2993-3003, 2018.
61. Hirst J, Pathak HB, Hyter S, Pessetto ZY, Ly T, Graw S, Koestler DC, Krieg AJ, Roby KF and Godwin AK: Licofelone enhances the efficacy of paclitaxel in ovarian cancer by reversing drug resistance and tumor stem-like properties. Cancer Res 78: 4370-4385, 2018.
62. Cavarzerani E, Caligiuri I, Bartoletti M, Canzonieri V and Rizzolio F: 3D dynamic cultures of HGSOC organoids to model innovative and standard therapies. Front Bioeng Biotechnol 11: 1135374, 2023.
63. Samson DJ, Seidenfeld J, Ziegler K and Aronson N: Chemotherapy sensitivity and resistance assays: A systematic review. J Clin Oncol 22: 3618-3630, 2004.
64. Brooks EA, Galarza S, Gencoglu MF, Cornelison RC, Munson JM and Peyton SR: Applicability of drug response metrics for cancer studies using biomaterials. Philos Trans R Soc Lond B Biol Sci 374: 20180226, 2019.
65. Brodeur MN, Simeone K, Leclerc-Deslauniers K, Fleury H, Carmona E, Provencher DM and Mes-Masson AM: Carboplatin response in preclinical models for ovarian cancer: Comparison of 2D monolayers, spheroids, ex vivo tumors and in vivo models. Sci Rep 11: 18183, 2021.
66. Thorel L, Morice PM, Paysant H, Florent R, Babin G, Thomine C, Perréard M, Abeilard E, Giffard F, Brotin E, *et al*: Comparative analysis of response to treatments and molecular features of tumor-derived organoids versus cell lines and PDX derived from the same ovarian clear cell carcinoma. J Exp Clin Cancer Res 42: 260, 2023.
67. Loessner D, Stok KS, Lutolf MP, Hutmacher DW, Clements JA and Rizzi SC: Bioengineered 3D platform to explore cell-ECM interactions and drug resistance of epithelial ovarian cancer cells. Biomaterials 31: 8494-8506, 2010.
68. Tofani LB, Abriata JP, Luiz MT, Marchetti JM and Swiech K: Establishment and characterization of an in vitro 3D ovarian cancer model for drug screening assays. Biotechnol Prog 36: e3034, 2020.
69. Bi J, Newtson AM, Zhang Y, Devor EJ, Samuelson MI, Thiel KW and Leslie KK: Successful patient-derived organoid culture of gynecologic cancers for disease modeling and drug sensitivity testing. Cancers (Basel) 13: 2901, 2021.
70. Garcia J, Hurwitz HI, Sandler AB, Miles D, Coleman RL, Deurloo R and Chinot OL: Bevacizumab (Avastin®) in cancer treatment: A review of 15 years of clinical experience and future outlook. Cancer Treat Rev 86: 102017, 2020.
71. Cohen MH, Gootenberg J, Keegan P and Pazdur R: FDA drug approval summary: Bevacizumab plus FOLFOX4 as second-line treatment of colorectal cancer. Oncologist 12: 356-361, 2007.
72. Govindaraju S and Yun K: Synthesis of gold nanomaterials and their cancer-related biomedical applications: An update. 3 Biotech 8: 113, 2018.
73. Oliva P, Decio A, Castiglioni V, Bassi A, Pesenti E, Cesca M, Scanziani E, Belotti D and Giavazzi R: Cisplatin plus paclitaxel and maintenance of bevacizumab on tumour progression, dissemination, and survival of ovarian carcinoma xenograft models. Br J Cancer 107: 360-369, 2012.
74. Yang H, Wang Y, Wang P, Zhang N and Wang P: Tumor organoids for cancer research and personalized medicine. Cancer Biol Med 19: 319-332, 2021.
75. Seidlitz T, Koo BK and Stange DE: Gastric organoids-an in vitro model system for the study of gastric development and road to personalized medicine. Cell Death Differ 28: 68-83, 2021.
76. Rivenbark AG, O'Connor SM and Coleman WB: Molecular and cellular heterogeneity in breast cancer: Challenges for personalized medicine. Am J Pathol 183: 1113-1124, 2013.
77. Verma M: Personalized medicine and cancer. J Pers Med 2: 1-14, 2012.
78. Offit K: Personalized medicine: New genomics, old lessons. Hum Genet 130: 3-14, 2011.
79. Peres LC, Risch H, Terry KL, Webb PM, Goodman MT, Wu AH, Alberg AJ, Bandera EV, Barnholtz-Sloan J, Bondy ML, *et al*: Racial/ethnic differences in the epidemiology of ovarian cancer: A pooled analysis of 12 case-control studies. Int J Epidemiol 47: 1011, 2018.
80. Nero C, Vizzielli G, Lorusso D, Cesari E, Daniele G, Loverro M, Scambia G and Sette C: Patient-derived organoids and high grade serous ovarian cancer: From disease modeling to personalized medicine. J Exp Clin Cancer Res 40: 116, 2021.
81. Lui G, Richardson A, Chatterjee P, Pollastro M, Lints M, Peretti D, Rosati R, Appleyard L, Durenberger G, Diaz R, *et al*: Functional drug screening of organoids from ovarian cancer patients demonstrates clinical and genomic concordance and identifies novel therapeutic vulnerabilities. Cancer Res 81: 534-534, 2021.
82. Phan N, Hong JJ, Tofig B, Mapua M, Elashoff D, Moatamed NA, Huang J, Memarzadeh S, Damoiseaux R and Soragni A: A simple high-throughput approach identifies actionable drug sensitivities in patient-derived tumor organoids. Commun Biol 2: 78, 2019.
83. Åkerlund E, Gudoityte G, Moussaud-Lamodière E, Lind O, Bwanika HC, Lehti K, Salehi S, Carlson J, Wallin E, Fernebro J, *et al*: The drug efficacy testing in 3D cultures platform identifies effective drugs for ovarian cancer patients. NPJ Precis Oncol 7: 111, 2023.
84. Clark J, Fotopoulou C, Cunnea P and Krell J: Novel ex vivo models of epithelial ovarian cancer: The future of biomarker and therapeutic research. Front Oncol 12: 837233, 2022.
85. Compadre AJ, van Biljon LN, Valentine MC, Llop-Guevara A, Graham E, Fashemi B, Herencia-Ropero A, Kotnik EN, Cooper I, Harrington SP, *et al*: RAD51 foci as a biomarker predictive of platinum chemotherapy response in ovarian cancer. Clin Cancer Res 29: 2466-2479, 2023.
86. Ceccaldi R, Rondinelli B and D'Andrea AD: Repair pathway choices and consequences at the double-strand break. Trends Cell Biol 26: 52-64, 2016.
87. Ito K, Murayama Y, Kurokawa Y, Kanamaru S, Kokabu Y, Maki T, Mikawa T, Argunhan B, Tsubouchi H, Ikeguchi M, *et al*: Real-time tracking reveals catalytic roles for the two DNA binding sites of Rad51. Nat Commun 11: 2950, 2020.

88. Wilson AJ, Stubbs M, Liu P, Ruggeri B and Khabele D: The BET inhibitor INCB054329 reduces homologous recombination efficiency and augments PARP inhibitor activity in ovarian cancer. Gynecol Oncol 149: 575-584, 2018.
89. Pellegrino B, Herencia-Ropero A, Llop-Guevara A, Pedretti F, Moles-Fernández A, Viaplana C, Villacampa G, Guzmán M, Rodríguez O, Grueso J, *et al*: Preclinical in vivo validation of the RAD51 test for identification of homologous recombination-deficient tumors and patient stratification. Cancer Res 82: 1646-1657, 2022.
90. Mukhopadhyay A, Elattar A, Cerbinskaite A, Wilkinson SJ, Drew Y, Kyle S, Los G, Hostomsky Z, Edmondson RJ and Curtin NJ: Development of a functional assay for homologous recombination status in primary cultures of epithelial ovarian tumor and correlation with sensitivity to poly(ADP-ribose) polymerase inhibitors. Clin Cancer Res 16: 2344-2351, 2010.
91. Shah MM, Dobbin ZC, Nowsheen S, Wielgos M, Katre AA, Alvarez RD, Konstantinopoulos PA, Yang ES and Landen CN: An ex vivo assay of XRT-induced Rad51 foci formation predicts response to PARP-inhibition in ovarian cancer. Gynecol Oncol 134: 331-337, 2014.
92. Meijer TG, Verkaik NS, Sieuwerts AM, van Riet J, Naipal KAT, van Deurzen CHM, den Bakker MA, Sleddens HFBM, Dubbink HJ, den Toom TD, *et al*: Functional ex vivo assay reveals homologous recombination deficiency in breast cancer beyond BRCA gene defects. Clin Cancer Res 24: 6277-6287, 2018.
93. van Wijk LM, Vermeulen S, Meijers M, van Diest MF, Ter Haar NT, de Jonge MM, Solleveld-Westerink N, van Wezel T, van Gent DC, Kroep JR, *et al*: The RECAP test rapidly and reliably identifies homologous recombination-deficient ovarian carcinomas. Cancers (Basel) 12: 2805, 2020.
94. Liu JF, Palakurthi S, Zeng Q, Zhou S, Ivanova E, Huang W, Zervantonakis IK, Selfors LM, Shen Y, Pritchard CC, *et al*: Establishment of patient-derived tumor xenograft models of epithelial ovarian cancer for preclinical evaluation of novel therapeutics. Clin Cancer Res 23: 1263-1273, 2017.
95. Cybulska P, Stewart JM, Sayad A, Virtanen C, Shaw PA, Clarke B, Stickle N, Bernardini MQ and Neel B: A genomically characterized collection of high-grade serous ovarian cancer xenografts for preclinical testing. Am J Pathol 188: 1120-1131, 2018.
96. Hidalgo M, Amant F, Biankin AV, Budinská E, Byrne AT, Caldas C, Clarke RB, de Jong S, Jonkers J, Mælandsmo GM, *et al*: Patient-derived xenograft models: An emerging platform for translational cancer research. Cancer Discov 4: 998-1013, 2014.
97. de Witte CJ, Valle-Inclan JE, Hami N, Lõhmussaar K, Kopper O, Vreuls CPH, Jonges GN, van Diest P, Nguyen L, Clevers H, *et al*: Patient-Derived ovarian cancer organoids mimic clinical response and exhibit heterogeneous inter- and intrapatient drug responses. Cell Rep 31: 107762, 2020.
98. Zhou Z, Cong L and Cong X: Patient-Derived organoids in precision medicine: Drug screening, organoid-on-a-chip and living organoid biobank. Front Oncol 11: 762184, 2021.
99. Narita Y, Kitazoe Y, Kurihara Y, Okuhara Y, Takamatsu K, Saito N and Doi Y: Increase or decrease of HDL-cholesterol concentrations during pravastatin treatment depending on the pre-treatment HDL cholesterol levels. Eur J Clin Pharmacol 52: 461-463, 1997.
100. Mosiewicz KA, Kolb L, van der Vlies AJ, Martino MM, Lienemann PS, Hubbell JA, Ehrbar M and Lutolf MP: In situ cell manipulation through enzymatic hydrogel photopatterning. Nat Mater 12: 1072-1078, 2013.
101. Verduin M, Hoeben A, De Ruysscher D and Vooijs M: Patient-derived cancer organoids as predictors of treatment response. Front Oncol 11: 641980, 2021.
102. Ahn SI, Sei YJ, Park HJ, Kim J, Ryu Y, Choi JJ, Sung HJ, MacDonald TJ, Levey AI and Kim YT: Microengineered human blood-brain barrier platform for understanding nanoparticle transport mechanisms. Nat Commun 11: 175, 2020.
103. Nagle PW, Plukker JTM, Muijs CT, van Luijk P and Coppes RP: Patient-derived tumor organoids for prediction of cancer treatment response. Semin Cancer Biol 53: 258-264, 2018.
104. Psilopatis I, Sykaras AG, Mandrakis G, Vrettou K and Theocharis S: Patient-derived organoids: The beginning of a new era in ovarian cancer disease modeling and drug sensitivity testing. Biomedicines 11: 1, 2022.
105. Kenny PA, Lee GY, Myers CA, Neve RM, Semeiks JR, Spellman PT, Lorenz K, Lee EH, Barcellos-Hoff MH, Petersen OW, *et al*: The morphologies of breast cancer cell lines in three-dimensional assays correlate with their profiles of gene expression. Mol Oncol 1: 84-96, 2007.
106. Hughes CS, Postovit LM and Lajoie GA: Matrigel: A complex protein mixture required for optimal growth of cell culture. Proteomics 10: 1886-1890, 2010.
107. Chan WS, Mo X, Ip PPC and Tse KY: Patient-derived organoid culture in epithelial ovarian cancers-Techniques, applications, and future perspectives. Cancer Med 12: 19714-19731, 2023.
108. Mandrycky C, Wang Z, Kim K and Kim DH: 3D bioprinting for engineering complex tissues. Biotechnol Adv 34: 422-434, 2016.
109. Annett S, Moore G, Short A, Marshall A, McCrudden C, Yakkundi A, Das S, McCluggage WG, Nelson L, Harley I, *et al*: FKBPL-based peptide, ALM201, targets angiogenesis and cancer stem cells in ovarian cancer. Br J Cancer 122: 361-371, 2020.
110. Baka Z, Godier C, Lamy L, Mallick A, Gribova V, Figarol A, Bezdetnaya L, Chateau A, Magne Z, Stiefel M, *et al*: A coculture based, 3D bioprinted ovarian tumor model combining cancer cells and cancer associated fibroblasts. Macromol Biosci 23: e2200434, 2023.
111. Xu F, Celli J, Rizvi I, Moon S, Hasan T and Demirci U: A three-dimensional in vitro ovarian cancer coculture model using a high-throughput cell patterning platform. Biotechnol J 6: 204-212, 2011.
112. Driehuis E and Clevers H: CRISPR/Cas 9 genome editing and its applications in organoids. Am J Physiol Gastrointest Liver Physiol 312: G257-G265, 2017.
113. Neal JT and Kuo CJ: Organoids as models for neoplastic transformation. Ann Rev Pathol 11: 199-220, 2016.
114. Lõhmussaar K, Kopper O, Korving J, Begthel H, Vreuls CPH, van Es JH and Clevers H: Assessing the origin of high-grade serous ovarian cancer using CRISPR-modification of mouse organoids. Nat Commun 11: 2660, 2020.
115. Dumont S, Jan Z, Heremans R, Van Gorp T, Vergote I and Timmerman D: Organoids of epithelial ovarian cancer as an emerging preclinical in vitro tool: A review. J Ovarian Res 12: 105, 2019.
116. Li Z, Gu H, Xu X, Tian Y, Huang X and Du Y: Unveiling the novel immune and molecular signatures of ovarian cancer: Insights and innovations from single-cell sequencing. Front Immunol 14: 1288027, 2023.
117. Wan C, Keany MP, Dong H, Al-Alem LF, Pandya UM, Lazo S, Boehnke K, Lynch KN, Xu R, Zarrella DT, *et al*: Enhanced efficacy of simultaneous PD-1 and PD-L1 immune checkpoint blockade in high-grade serous ovarian cancer. Cancer Res 81: 158-173, 2021.
118. Gonzalez VD, Samusik N, Chen TJ, Savig ES, Aghaeepour N, Quigley DA, Huang YW, Giangarrà V, Borowsky AD, Hubbard NE, *et al*: Commonly occurring cell subsets in high-grade serous ovarian tumors identified by single-cell mass cytometry. Cell Rep 22: 1875-1888, 2018.