Application of organoids in precision immunotherapy of lung cancer (Review)

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Abstract. In immunotherapy, the immune system is modulated in order to treat cancer. Traditional two dimensional *in vitro* models and *in vivo* animal models are insufficient to simulate the complex tumor microenvironment (TME) in the original tumor. As tumor immunotherapy involves the immune system, additional tumor mimic models, such as patient-derived organoids, are required for the evaluation of the efficacy of immunotherapy. Furthermore, non-tumor components and host tumor cells in the TME may interact to promote cancer incidence, progression, drug resistance and metastasis. It is possible to produce organoid models for lung cancer by retaining endogenous stromal components (e.g., multiple immune cell types), supplying cancer-associated fibroblasts and exogenous immune cells, constructing tumor vasculature and adding other biological or chemical components that emulate the TME. Therefore, the lung cancer organoid culture platform may facilitate preclinical testing of immunotherapy drugs for lung cancer by mimicking immunotherapy responses. The present review summarizes current lung cancer organoid culture methods for TME modeling and discusses the use of lung cancer-derived organoids for the detection of lung cancer immunotherapy and individualized cancer immunotherapy.

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Abbreviations: TME, tumor microenvironment; PDO, patient-derived organoids; NSCLC, non-small cell lung cancer; PD-1, programmed cell death protein-1; PD-L1, programmed cell death ligand-1; ICI, immune checkpoint inhibitor; TMB, tumor mutational burden; MSI, microsatellite instability; PDTX, patient-derived tumor xenograft; 3D, three-dimensional; ALI, air-liquid interface; SAG, smoothened agonist; FGF, fibroblast growth factor; EGF, epidermal growth factor; PDOTS/MDOTS, patient-derived organotypic tumor spheroids/murine-derived organotypic tumor spheroids; LCOs, lung cancer organoids; NK, natural killer; TCR, T cell receptor; CTLA-4, cytotoxic T lymphocyte antigen-4; PBMCs, peripheral blood mononuclear cells; CEA, carcinoembryonic antigen

Key words: lung cancer, organoids, immunotherapy, tumor microenvironment, precision therapy

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1. Introduction

Lung cancer is the leading cause of cancer deaths. In the last decade, patients with lung cancer have demonstrated continuously improved overall survival rates, mainly due to the development of effective advanced treatment regimens and precision medicine in the field of oncology (1). Precision medicine can treat tumors by matching a patient's unique clinical and biological characteristics with an optimal treatment or combination of treatments, with the intent to maximize clinical benefit with minimal side effects and ultimately achieve an effective and long-lasting impact on immune response and tumor cell escape (2-4). Advances in immunotherapy have demonstrated that certain beneficial immune responses are triggered in patients with cancer. For example, in patients with non-small cell lung cancer (NSCLC) who demonstrate overexpression of programmed cell death ligand-1 (PD-L1), treatment regimens using immune checkpoint inhibitors (ICIs) are currently one of the mainstays of immunotherapy (3,4). The detection of certain biomarkers, including tumor mutational burden (TMB) (5) and microsatellite instability (MSI) (6), can improve the outcomes of checkpoint blockade-based immunotherapy by identifying patients with the best response. Nonetheless, the majority of patients with relatively high MSIs or TMBs fail to respond to immunotherapy or will develop a mechanism for adaptive resistance (7). Therefore, there is an urgent need for advanced treatment sensitivity predictors that can guide therapeutic regimens and prevent the unnecessary exposure of patients

to ineffective treatments. Static biomarkers detected using genomic (8), transcriptome (8) or proteomic (9) analysis can support the selection of a suitable choice among treatment regimens. However, analysis of certain biomarkers, such as TMB and PD-L1 expression (5) and the content of certain immune cells, such as CD8+ cells, fails to adequately capture inter- and intra-tumor heterogeneity alongside the variation demonstrated between individuals (10). Compared with the aforementioned static tumor biomarkers, *in vivo* animal models can directly evaluate how immunotherapy may affect patient-derived materials (PDMs), thereby providing a functional readout of tumor response to certain treatments. While murine models are a powerful tool for the investigation of the efficacy of classical drugs, they are not applicable to all types of immunotherapy, as the murine immune system differs from the human immune system (11). Developing alternative models that can replicate human tumors and preserve the characteristics of the human immune system is therefore necessary to improve immunotherapy research. Currently, the interactions of cancer cells with their environment have been successfully modeled using patient-derived tumor xenografts (PDTX) and human cell lines. PDTX cost, productivity and comprehensive immuno-compatibility are currently issues with this form of model (10,11). The construction of effective PDTX models is laborious, taking 4-8 months to develop. As a consequence, these models are not currently a feasible option for targeted cancer therapy research (12). In previous years, both tumor organoids and complex tumor-immune organoids have been considered promising models which mimic the human TME and migration, extravasation, and Angiogenesis) of the human immune system. In the present article, the current developments in lung cancer organoid (LCO) technology will be reviewed and its application in precision immunotherapy approaches will be discussed. Comparison of different types of lung cancer models were list in Table I.

2. Lung organoids and LCOs

Organoids are multicellular spheroids originally derived from healthy organ tissue with the aim of reconstructing and miniaturizing the multicellular architecture of the organ (13). Organoids are three dimensional (3D) structures which can be cultured in embedded 3D matrices to imitate original tissues (14-16). In 2008, Eiraku *et al* (17) reported the production of one self-organized formation of apico-basally polarized cortical tissues from embryonic stem cells using an efficient 3D aggregation culture, the cortical neurons were both functional and transplantable. In 2009, the creation of organoids from mouse intestinal stem cells was first described by Sato *et al* (18) and this work served as the basis for subsequent organoid cultivation techniques in other murine and human epithelial tissues. Organoids have the capacity to self-organize and can be produced from human stem cells to simulate disease progression or tissue homeostasis, or from pluripotent embryonic stem cells or induced pluripotent stem cells to imitate embryonic development.

Lung organoids. To evaluate the application of organoids in lung diseases, numerous studies first explored the use of lung organoids. Lung organoids have the potential to aid in the development of advanced treatments for a number of lung diseases, such as lung cancer (19,20), idiopathic pulmonary fibrosis (21), cystic fibrosis (22) and asthma (23). Notably, *in vitro* organoid models for human distal pulmonary infectious diseases, such as coronavirus disease 2019, have also previously been established (24). Since organoids are derived from cells with progenitor potential, adult lung epithelial stem or progenitor cells, including basal cells, alveolar type II cells and airway secretory cells can be used as the required primary cell source to establish lung organoids (25). Rock *et al* (26) cultured airway basal cells in a 3D air-liquid interface system and reported that basal cells could differentiate into tracheal spheroids in the absence of mesenchymal stem cells (MSCs). The differentiation of lung progenitor cells into airway and alveolar structures can be promoted when co-cultured with MSCs (27,28). Moreover, the ability to alter human pluripotent stem cells (hPSCs) using the CRISPR-Cas9 system enables researchers to modify and investigate human genes linked to lung organoid development and disease, is an additional benefit of this type of organoid model (21).

In a previous study, certain techniques used for creating human or mouse lung-derived organoids failed to achieve the long-term goal of constructing single lung organoids derived from lung basal cells cultured in culture for 2 weeks shows abnormal differentiation occurs) (25). However, with development of lung organoid culture technology, this may no longer be a limitation in the future. Sachs *et al* (29) previously described the conditions required for long-term lung organoid culture. In the aforementioned study, human lung-derived organoids were passaged every 2 weeks for >1 year, maintaining similar proportions of basal, rod, multiciliated and secretory cells. Furthermore, hPSC-derived lung organoids can be cultivated for up to 170 days (28). Notably, Salahudeen *et al* (24) reported distal human lung progenitors as organoids derived clonally from single adult human alveolar epithelial type II (AT2) or KRT5+ basal cells) for the long-term growth of human distal airway and alveolar organoids.

LCOs. Similar to organs, tumors are composed of numerous cell types with the addition of cancer cells (5). The clonal heterogeneity and mutational status of donor tumors can be substantially retained in organoids (30). The development of patient-derived tumor organoid (PDTO) cultures presents a novel form of *in vitro* model to effectively mimic human tumors (31). To date, tumor tissues of certain types of cancer have been successfully cultured into PDTOs, most of which are derived from epithelial carcinoma (32), including lung adenocarcinoma. LCOs are 3D structures derived from processed lung tumor tissue that contain different cell types and grow in a standardized manner (33). Numerous lung cell types, including stromal cells, as well as cancer cells at various stages of disease, can be cultured in lung cancer cell lines derived from patient tumor tissue (34). Furthermore, cancer cells at the most advanced stage of disease can be modeled by short-term cultured cells (PDX/PDO), which may serve as a genetic depiction of the primary tumor (33). Although certain types of patient-derived lung cell lines can be cultured in monolayers, the original 3D organ architecture

Table I. Advantages and disadvantages of certain types of lung cancer models.

Model type	Advantages	Disadvantages
Cell lines	Easy to obtain. Easy to cultivate. Low cost. Widely available.	Heterogeneity exists between laboratories. Long-term cultures are prone to genetic drift and loss of key features of the primary tumor.
Patient-derived xenograft tumor	Preserves the genome and phenotype of patient's tumor tissue.	Expensive and time-consuming. Cannot be passaged. Lacks the patient native tumor microenvironment.
Patient-derived organoid model	Preserves the genome and phenotype of the patient's tumor tissue. Simulates the original tumor microenvironment. Saves time compared with the patient-derived xenograft tumor model.	Lack of standardized culture methods. Long-term culture is difficult.

and heterogeneity of a cancerous organ cannot be retained in a monolayer differentiated environment. The cooperative interplay of numerous cell types that are dispersed and arranged in a 3D structure is necessary for human organs to operate in a disease-free state. Originating from healthy organs, lung tumors are a complex cell community. The intricate extracellular matrix and tumor microenvironment (TME) also aid in the formation of tumors (35).

3. LCO culture technology for immunotherapy research

Certain LCO culture methods have been used to model suitable TMEs for the testing of immunotherapy, including reconstructive methods. Reconstructive methods involve the culture of organoids composed solely of epithelial cells in submerged Matrigel (36). Then, exogenous immune cells and various stromal cells are added for the investigation of immunotherapy and TME that rely on this method (33). Holistic approaches, such as microfluidic 3D culture and air-liquid interface (ALI), have also been used to model TMEs suitable for immunotherapy research. This can involve production of aggregates of 3D spheroids, in suspension or implanted in 3D matrices (Fig. 1) (34). Notably, ALI can be applied through explant culture, which can be used to preserve tissue structures. With these techniques, small fragments of native TME and tumor tissue are preserved as a complete unit (35).

Submerged Matrigel culture. The submerged Matrigel technique is used to culture tumor cells isolated from tumor biopsies in tissue culture media mixed with 3D Matrigel in domed or flat gels (36-38). This technique requires growth factor supplementation and the use of small-molecule inhibitors, such as Y27632 and Rho-associated kinase, or activators, such as smoothened agonist (SAG), the use of which may differ between laboratories (39,40). Small-molecule activators and inhibitors can promote or block multiple biological responses, which is helpful to maintain organoid growth and phenotype. For instance, a previous study by Huo *et al* (41) used common media formulations such as DMEM/F12, HEPES, penicillin-streptomycin and Glutamax, with N-acetyl-L-cysteine and B-27 supplements for the maintenance of certain properties (genetic markers such as EGFR)/protein markers (e.g., TTF-1, p63, cytokeratin 5)) of lung cancer cells derived from stem cells or patients. A number of small molecule activators or inhibitors, including A83-01, CHIR 99021, Noggin, Y-27632 and SAG were also added to the culture medium. With this tailor-made 'cocktail', which also included certain growth factors such as epidermal growth factor (EGF), fibroblast growth factor (FGF) 4 and FGF10, the successful organoid culture period exceeded 3 months (19). By contrast, another previous study produced a simpler organoid culture medium with fewer components (18). This medium consisted of EGF, βFGF, Y-27632, N2 and B-27 supplements and basic cell culture components such as penicillin-streptomycin and DMEM/F12. In the aforementioned study, organoids derived from lung tumors could be effectively cultured for >6 months. However, the addition of media components is only part of the reason for the success of organoid cultures.

During LCO culture, issues with healthy epithelial cell overgrowth have been documented (40). The genetic instability and high mortality rate of cancer cells may be one reason why healthy lung cells display a growth advantage during organoid culture compared with cancer cells (42). Another reason may be a large amount of stem cells in the medium formulation (43). Bleijs *et al* (32)supplemented growth medium with a variety of small-molecule signal regulators and growth factors, including A83-01, Noggin, SB 202190, R-spondin, Y-27632, FGF7 and FGF10 and also added Nutlin-3α to induce the senescence or apoptosis of TP53 wild-type cells, which can slow the increased proliferation rate observed in healthy epithelial cells. Utilizing the Nutlin-3a selection method, the aforementioned study enlarged the selected LCOS carrying p53 mutations and produced pure LCOs derived from several tumor tissues belonging to different histological subtypes (mucinous/acinar/lepidic). However, the effective incubation time of these organoids was not detailed in the study.

The aim of the aforementioned organoid culture strategies is to construct pure LCOs while largely inhibiting the growth of healthy lung cells. Other culture techniques, including ALI, have also used these supplements (44). It should be noted that the traditional method of submerged Matrigel enriches only epithelial cancer cells but does not maintain components

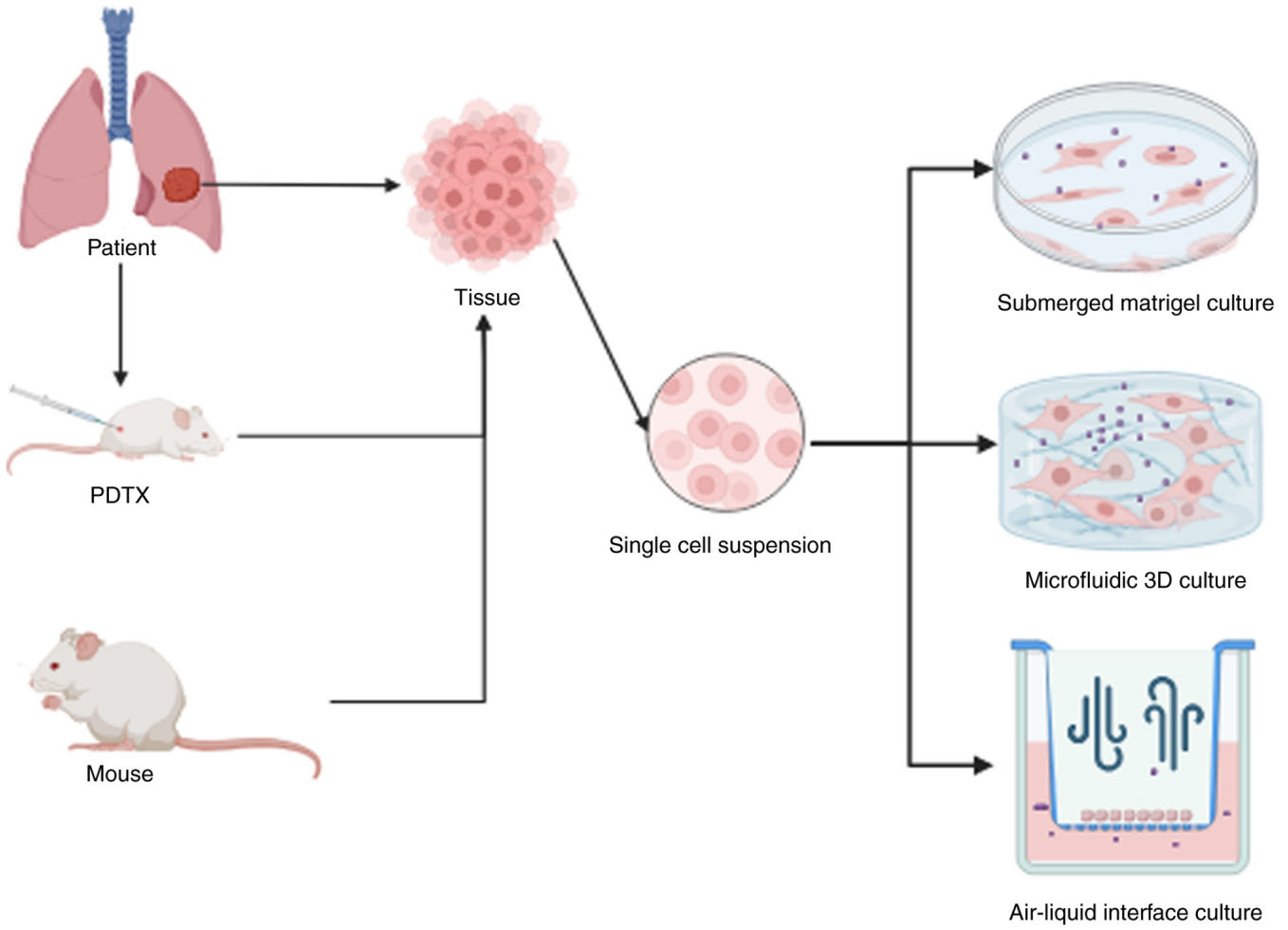

Figure 1. Lung cancer organoid culture technology for immunotherapy research.

(lymphocyte/tumor-associated macrophages) (17). Therefore, co-culture of patient-derived organoids (PDOs) with exogenous immune cells is necessary for temporal modeling in these methodologies. A pure tumor organoid was produced by Dijkstra *et al* (45) using Nutlin-3a, a mouse double minute 2 homolog inhibitor, to culture NSCLC organoids with p53 mutations, which was compared with patient autologous peripheral blood mononuclear cells (PBMCs) in Geltrex basement membrane. PBMCs were co-cultured with colorectal cancer cell and induced tumor-reactive CD8+ population expansion and showed specific anti-tumor responsiveness in the TME. A previous study by Takahashi *et al* (46) also used this method to culture LCOs called 'Fukushima' organoids and assess the efficacy of lung cancer immunotherapy by simulating the complex interaction between immune cells and malignant cells. However, the TME of the cultivated organoid is still relatively monolithic, which is a drawback of this strategy, but this problem can be solved by producing a comprehensive model of native TME.

Microfluidic 3D culture. In a microfluidic 3D device, a patient- or murine-derived organotypic tumor spheroid (PDOTS/MDOTS) is cultivated in collagen gel (type IV collagenase and HEPES) (47). In PDOTS/MDOTS cultures, tumor tissue specimens are obtained from patients and separated enzymatically and mechanically (48). This process ultimately yields a heterogeneous mixture of spheroids, single cells and macroscopic tumor fragments. Next, this mixture is filtered via 100 and 40 mm aperture filters in order to retain 40-100 mm diameter spheres. These spheres are then combined with collagen gel, pelleted in ultra-low attachment plates and seeded into the middle of regional microfluidic device. The medium is injected into the medium channels flanking the central channel to nurture the spheroids. In addition, Jung *et al* (49) developed an all-in-one microfluidic system that continuously flows medicated medium through the system to deliver nutrients and oxygen to LCOs and can also deliver drugs to LCOs for drug susceptibility testing. The aforementioned study also reported that after induction using cisplatin and etoposide, cells in the peripheral region of LCOs died, while cells in the core region continued to survive for 72 h. This suggested that the core region of LCOs contained chemo-resistant cells, which indicated that this system could aid in predicting the chemotherapy response of lung cancer cells and could be used to choose the most effective treatment plan. Notably, in this approach, spheroids preserve the cellular diversity and complexity of native cancer tissues, such as autologous bone marrow cell populations (dendritic cells, myeloid-derived suppressor cells, monocytes and tumor-associated macrophages), lymphocytes (T and B cells) and non-reconstituted

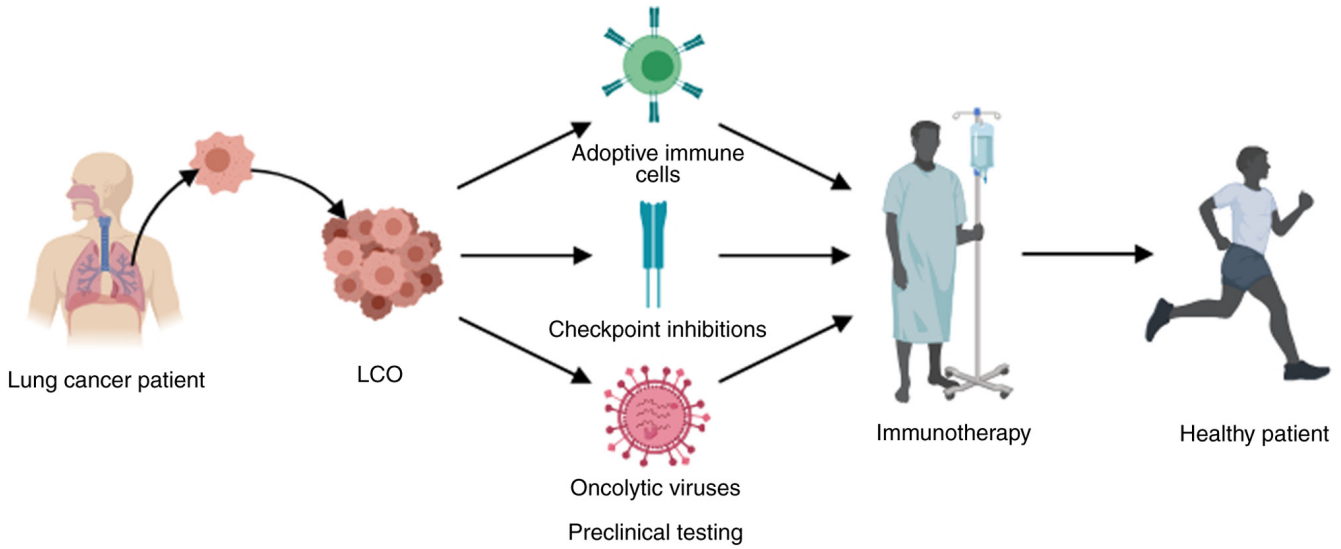

Figure 2. Application of LCOs in immunotherapy. LCO, lung cancer organoid.

cancer cells (50). By adding exogenous T cells to the media channels, Kitajima *et al* (51) evaluated T cell infiltration into LCOs and LCO interactions and crosstalk with immune cells in these devices.

ALI culture. The ALI culture method relies on a set of inner and outer disks, with the inner disk being made of two layers (52). First, a collagen gel matrix is added to the inner disk to prepare the bottom layer (53). After obtaining primary tissue samples, samples are immediately placed into ice-cold culture medium for further preparation (53). Next, the tissue is rinsed, physically broken down into small pieces, then incubated and mixed into collagen gel. This mixture is then poured onto the underlying gel matrix of the inner dish, to produce the top layer inside the inner dish (53). The gel in the inner dish is allowed to harden by being placed into the outer dish and moved to a 37˚C incubator (53). The culture medium is subsequently poured into the outer disk, where it can permeate into the inner disk via the porous membrane. Tissues and organs may efficiently receive oxygen from the top layer as the culture is directly exposed to the air (53-55). In contrast to submerged Matrigel cultures, ALI enables the development of larger multicellular fragments that maintain original tissue structure, such as resection-sourced cancer cells co-cultured with non-reconstituted immune cells and indigenous stroma (56). According to a previously published study by Finnberg *et al* (57), endogenous CD45+ immune cells of human colorectal organoids and LCOs can persist for up to 10 days using the ALI culture technique, despite a significant reduction in the number of CD3+ cells. Additionally, the PDO cultured with ALI can retain native tumor genetic changes in addition to the TME's complex cellular components and structural organization (44). Compared with submerged Matrigel cultures, ALI's characteristics make this technique an appropriate TME model (58). The ALI technique was utilized to develop PDOs from several types of surgically removed tumors, including lung cancer (44). Cultures retain tumor epithelium and its stromal microenvironment for 30 days along with fibroblasts and immune cells, such as tumor-associated T helper cells, B cells, cytotoxic T lymphocytes, natural killer (NK) cells, NK T cells and macrophages. Furthermore, the T cell receptor (TCR) heterogeneity present in the initial tumor is retained in these cultures (59).

ALI-cultured organoids derived from murine or human tumors are notably different in many aspects. The doubling times and serial passaging of cell line-derived murine organoids are rapid and reproducible (60). Comparatively, the growth and reproduction rates of PDOs are highly variable and are associated with the initial conditions present during tumor biopsy, such as sampling condition, preservation duration, tumor viability, pre- or post-treatment and tumor histology grade (high or low) (61). Thus, in the culture plate where organoids are grown, necrotic tissue is various proportions of the organoids. In immunogenic murine-derived organotypic tumor spheroid (MDOTS), cytotoxic responses as well as activation and expansion of tumor infiltrating lymphocytes (TILs) are fixed, but a very different picture exists in human PDOs, given the patient-intrinsic differences, and many well-documented tumors and immune components are resistant to checkpoint inhibition to various extents. Immunity declined and fibroblast stroma in murine- and human-derived organoids deteriorated during a 1 year period. Though anti-CD3/anti-CD28 or IL-2 supplementation can slow TIL loss, further optimization is required to preserve TIL above the current 60 day limit (40).

4. LCO application in immunotherapy

An optimal *ex vivo* model is needed for immunotherapy screening and research to accurately reproduce the heterogeneity of original TMEs. PDO is considered to have great potential because it saves time and represents the patient's stage and treatment history. This section will discuss the immunotherapeutic application of organoids (Fig. 2).

Immune checkpoint inhibitors. In the field of lung cancer treatment, particularly for NSCLC, a transformation occurred following the identification of immunological checkpoints and the discovery of ICIs, work which was recognized with

a Nobel Prize (62). Among several types of tumors that are known to use immune checkpoints to evade the host immune system, cytotoxic T lymphocyte antigen-4 (CTLA-4) and programmed cell death protein-1 (PD-1)/PD-L1 are the most widely clinically used (63). Once these pathways are inhibited, cytotoxic T-cell priming and antitumor activity can occur. Successful breakthroughs with ICIs in the immunotherapeutic treatment of NSCLC include PD-1 inhibitors, such as nivolumab (64) and pembrolizumab (65), PD-L1 inhibitors durvalumab (66) and atezolizumab (67) and the CTLA-4 inhibitor ipilimumab (68). Compared with the increased application of ICIs in the treatment of NSCLC, the frequency of the use of this type of treatment in small cell lung cancer (SCLC) remains low (69). Over the past 30 years, first-line therapy that combines platinum-based chemotherapy with a PD-L1 inhibitor (atezolizumab or durvalumab) has been the sole advancement in the treatment of extensive-stage SCLC (70). However, though ICIs have achieved unprecedented success in clinical trial sites in the treatment of lung cancer, breast cancer, ovarian cancer and other fields, the *in vivo* mechanisms of action and possible drug resistance factors are still unclear. First, ICI-based treatment does not provide long-term benefits for ~70% of patients with advanced NSCLC and ~80% of patients with SCLC (71). Secondly, efforts are currently underway to identify a suitable biomarker of the ICI response. Finally, SCLC is difficult to treat and the addition of existing ICIs to therapeutic modalities has seen limited success due to the immunosuppressive TME (59). In order to overcome these obstacles and combat the propensity for SCLC to persist, new strategies for harnessing the potential of the immune system must be developed.

Tumor immunity arises from the synergy of active communication between peripheral and intratumoral components (72-74). However, current *in vivo* systems are unable to break down the distinct contributions made by peripheral immune cells to intra-tumor immunological responses, rather than immune cells that are inherent residents of the TME (75). The recognition of such local events is currently enabled by the basic mechanism of existing PDOs. It remains unclear whether anti-PD-1 antibodies can enlarge intratumorally depleted CD8+ T cells through acting on peripheral and tumor-infiltrating populations (76) and also increase the proliferation of peripheral blood PD-1+ CD8+ T cells (77,78). Therefore, PD-1 axis inhibition inside the TME is effective for the induction of growth and activation of TILs, as anti-PD-1 and anti-PD-L1 can activate TILs in human and murine PDOs. According to a previous study reported by Neal *et al* (79), expanded clear cell renal cell carcinoma tumor infiltrating lymphocytes(ccRCC TIL) TCR clonotypes are enriched in depleted T cells, which may aid in the development of tumors.

A number of cancers, including renal cell carcinoma (RCC) (77), cutaneous squamous cell carcinoma (78), melanoma (80), head and neck cancer (81) and NSCLC (82) have been effectively treated through the use of ICI targeting PD-1/PD-L1 and CTLA-4. With the efforts of certain organizations, such as the Human Cancer Models Initiative, specific organoid biobanks for epithelial tissues from a variety of cancers are publicly accessible. However, the immunotherapeutic applications of these types of PDOs are limited by the absence of immune compartments (83). A previous study has been performed to address this issue. To evaluate T cell cytotoxicity and TIL-to-organoid migration, Kong *et al* (84) co-cultured epithelial-only organoids with autologous TILs in an immersed Matrigel matrix. The aforementioned study also reported that TIL function is restored following immune checkpoint blockade (ICB). However, as a recombinant approach, co-culture of epithelial-only PDO with foreign immune cells cannot accurately mimic the connections and cross-talk that occur between various cell populations in the TME, particularly when immunomodulatory medications are applied. Tumor immune microenvironment (TIME) modeling can be utilized to overcome this problem using integrated culture techniques like 3D microfluidics and ALI culture methodologies (85). In short-term 3D microfluidic cultures, organotypic spheroids can be used to maintain autologous bone marrow and lymphoid cells which are comparable to the original donor tumors and can mimic dynamic responses and resistance to ICBs, such as PD-1 inhibition (47). Likewise, the donor tumor cytokine secretion profile can be matched in PDOTS/MDOTS (50). Thus, PDOTS/MDOTS analysis enables the cultivation of models that utilize ICB which are relevant to clinical settings. Neal *et al* (79) used the ALI approach to cultivate lung cancer patient-derived organoids and implanted tumor epithelial cells together with autologous immune cells (T, B and NK cells and macrophages) into the organoids. The original tumor T cell receptor profile was retained by PDO TILs (TCR). PDOs successfully mimic ICB and induce tumor cytotoxicity through the expansion of antigen-specific TIL activation, anti-PD-1 and/or anti-PD-L1. Additionally, there is a potential impact of the design and material of the device used in organoid cultivation on the effectiveness of immunotherapy, as observed previously in ICB research (86).

Adoptive cell transfer therapy. Recently, immune cell-based treatment has been used as a potential immunotherapy strategy to combat lung cancer (87). Building on the success of previously published oncology studies, the aforementioned study harnessed the innate ability of immune cells to destroy cancer cells and generate a robust immune response by bringing in additional cells to the TME. The genetic engineering of T or NK cells enables these cells to target certain antigens produced on lung cancer cells and reprogram the behavior of immune cells to enhance their function, increasing the specificity of immune detection of cancer cells.

Most immune cell-based lung cancer targeting studies have reported the use of chimeric antigen receptor (CAR)-T cells, although a growing body of research is using the allogenic nature of NK cells to provide infused CAR-T cells, which is a potentially safer alternative (NK cells have a limited life span in circulation and less produce memory cells) (88,89). Despite the success of cell-based approaches to treat hematological malignancies, solid tumors, such as lung cancers, consistently demonstrate poor response rates to these treatments (90). This may be related to immune tolerance and TME heterogeneity in the presence of immune cells in adoptive cell transfer therapy. In lung cancer, the TME forms a complex barrier to immune cell activity, often leading to resistance to therapy. In the lung cancer TME, CAR-T cells display poor ability to aggregate (91). In addition, unfavorable intra-tumoral immunometabolic conditions (enhanced glycolysis and lactate

production, reduced mitochondrial respiration, and alterations in lipid and amino acid metabolism) can lead to the dysfunction of infiltrating immune cells. CAR-T cell dysfunction can involve fatigue, senescence or weakness (92). Systemic drug toxicity caused by CAR-T cells is also a possibility (93). Adoptive cell transfer immunotherapy involves the collection of circulating lymphocytes or TILs and the selection or genetic engineering of high-affinity TCRs that detect tumor antigens. These cells are subsequently activated and expanded *in vitro* before infusion into patients (94,95). CAR-T cells produce synthetic TCRs that target certain antigens on the tumor cell surface and can overcome major histocompatibility complex (MHC) limitations such as polymorphism (96). Previous studies have reported that PDO is an effective platform to assess the cytotoxicity of T cells (TCR or CAR-T cells) for specific tumors (97,98). PDO was utilized to assess the outcome of CAR-T cell therapy combined with the apoptosis antagonist birinapant in a study by Michie *et al* (99). The aforementioned study reported that CAR-T cells alone were insufficient, whereas a combination of birinapant and CAR-T cells inhibits PDO development in a manner which relied on tumor necrosis factor. A preclinical model (luciferase-based measurement), 3D PDO, was created by Schnalzger *et al* (100) to enable the identification of CAR-mediated cytotoxicity in a natural TIME model. Additionally, the aforementioned study developed a procedure for confocal live-cell imaging to dynamically monitor cytotoxic activity against individual organoids. In co-cultures of NK cells with regular cancer organoids on colorectal cancer (CRC) or extracellular matrix layers, co-cultures demonstrated durable effector-target cell connections. Additionally, tumor antigen-specific cytotoxicity of FRIZZLED or EGFRvIII receptor-targeted NK-92 cells engineered by CAR was monitored using CRC organoids. In conclusion, a framework was developed to analyze CAR effectiveness and tumor selectivity in an individualized manner (101). In addition, although epithelial-only PDO is devoid of immunological and stromal components, it can be utilized to select T cells that are reactive to tumors (45). Tumor-reactive lymphocyte enrichment, stimulation and efficacy assessments may be performed using this co-culture method (45). To produce tumor-reactive CD8+ communities, autologous circulating T cells (PBMCs) and CRC or NSCLC organoids were co-cultured in medium containing IL-2, anti-CD28 and anti-PD1. MHC-dependent cytotoxicity and T cell-mediated killing effects on autologous tumor organoids were observed after 2 weeks of co-culture, along with an elevation in the production of IFNg and CD107a in CD8+ T cells. However, the survival of matching healthy organoids was unaffected by CD8+ T cells that could react to tumor cells. As a result, a previous study created a platform for developing tumor-reactive T cells and testing the sensitivity and precision of autologous T cells to eradicate cancer cells at the individual level (102). In addition, it is possible to extract tumor-reactive T cells from TILs and re-infuse them into patients, which is a more targeted treatment than the use of non-infiltrating lymphocytes (103).

Other immunotherapy applications. The cytotoxicity and infectivity of oncolytic viruses alone or in conjunction with chemotherapy can be studied using PDO (104). Oncolytic adenovirus demonstrated high replication selectivity in PDAC organoids in a study by Raimondi *et al* (104) but not in organoids from healthy pancreatic tissue. Additionally, patient-specific responses were observed, which indicated that PDO was a useful *in vitro* tumor model for evaluating early oncolytic viral responses. The efficacy and specificity of antibody-based immunotherapies can also be studied using tumor organoids. Previous studies focused on antibody-based ICB treatment have utilized organoid models (42,50,105). Courau *et al* (106) reported that the infiltration of activated/memory T and NK cells into organoids involves both the NK Group 2A-Human Leukocyte Antigen E (NKG2A-HLA-E) and NKG2D-Major Histocompatibility Complex Class I Chain-Related Molecule A/B (MICA/B) pathways and these activated cells may subsequently destroy 3D structures and kill cancer cells. The aforementioned study demonstrated that during co-culture with autologous TIL, anti-MICA/B antibodies and an antibody cocktail consisting of anti-MICA/B and anti-NKG2A might trigger immune-mediated death in colorectal tumor organoids. Gonzalez-Exposito *et al* (107) constructed eight PDOs, seven of which were derived from refractory metastatic CRC, while one was derived from untreated primary CRC tumor to study the mechanisms of resistance and sensitivity to cibisatamab, a compound which binds tumor cells and CD3 Carcinoembryonic antigen (CEA) bispecific monoclonal antibody on T cells. In order to assess the efficacy of cibisatamab, co-cultures of organoids and allogeneic CD8+ T cells were produced. This method demonstrated that CEA-low PDO is resistant to cibisatamab whereas CEA-high PDO is vulnerable to this treatment. CEA-low cells support tumor cell proliferation and through the use of RNA sequencing, it was reported that CEA-low cells demonstrate increased WNT/β-catenin pathway activity (108). To increase the efficacy of this therapy, the aforementioned study proposed the use of a possible combination of cibisatamab with an inhibitor of the WNT/β-catenin pathway.

5. Conclusion

The study of the dynamic interplay between the tumor and immune system using PDO has gained increasing attention over recent years. Moreover, advances in TME modeling could facilitate the testing of novel immunotherapies in preclinical settings. Organoid-focused techniques have limitations when used to analyze the effects of TIME on the behavior of immunotherapy medications in cancer, due to the absence of stroma and a vascular network. Complex organoids can be created by co-culturing source or progenitor cells with cancer-associated fibroblasts, mesodermal progenitor cells and immune cells to negate these constraints. Furthermore, the cancer-immune cycle, composed of effector T cell initiation or activation, T cell trafficking or infiltration into cancer tissue and T cell killing or recognition of cancer cells, may be mimicked through organoid co-culture with additional immune cells sourced from lymph nodes or PBMCs. IL-2, anti-CD3 and anti-CD28 antibodies are among the extra supplements that are advised for the long-term protection of immune cells. The composition of the growth medium should also be adjusted such that it promotes the

development of all clones equally rather than favoring the growth of any particular colony. The modeling of native TME can be enhanced by the reproduction of mechanical stressors, such as physiological shear flow. The repeatability of drug screening findings can be enhanced by the use of scaffolds with a certain shape, cell quantity, regulated size, relative arrangement and customized composition of the various cell types inside the organoid. Animal models require more time to develop a research platform than organoid models, whereas effective human organoid cultures may be established in weeks or months while enabling high-throughput screening and circumventing possible ethical implications. question. Thus, PDO can be utilized in precision medicine to provide reliable data about individual drug responses and mutation profiles (109). At present, researchers have begun to use LCO models to evaluate the efficacy of chemotherapy and targeted drug therapy in a real clinical scenario (110). Certain clinical studies, such as NCT03778814, NCT04951115 and NCT05332925, have been registered to explore the effect of PDO in different stages of lung cancer immunotherapy, so as to reduce the current treatment time spent in the clinical-laboratory-clinical cycle(mainly for the time of drug screening model construction). Immunotherapy may also be used for additional practical applications (tumor vaccine) in the future.

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Availability of data and materials

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Authors' contributions

YJ and HT designed the project and wrote, reviewed and edited the manuscript. JR and RM constructed figures and tables. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

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