Lung cancer organoids: a new strategy for precision medicine research

Yuan Meng[1,2,3#^], Xinyi Shu[1,2,3#], Jie Yang[1,2,3], Yangyueying Liang[1,2,3], Meiying Zhu[1,2,3], Xuerui Wang[1,2,3], Yue Li[4], Fanming Kong[1,2,3]

[1]Department of Oncology, First Teaching Hospital of Tianjin University of Traditional Chinese Medicine, Tianjin, China; [2]National Clinical Research Center for Chinese Medicine Acupuncture and Moxibustion, Tianjin, China; [3]Tianjin Cancer Institute of Traditional Chinese Medicine, Tianjin, China; [4]Tianjin Heping District Hospital of Traditional Chinese Medicine, Tianjin, China

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[#]These authors contributed equally to this work as co-first authors.

Correspondence to: Fanming Kong, MD, PhD. Department of Oncology, First Teaching Hospital of Tianjin University of Traditional Chinese Medicine, Changling Road No. 88, Xiqing District, Tianjin 300381, China; National Clinical Research Center for Chinese Medicine Acupuncture and Moxibustion, Tianjin, China; Tianjin Cancer Institute of Traditional Chinese Medicine, Tianjin, China. Email: kongfanming08@163.com.

Abstract: This article discusses new strategies for lung cancer organoids (LCOs) in precision medicine research. Precision medicine aims to identify and develop highly selective drugs targeted at specific disease markers for precise treatment. Given the genetic diversity among lung cancer cells, it is evident that different tumor cells may respond differently to various treatment regimens. LCOs can not only faithfully reproduce the pathological and genomic characteristics of samples, maintaining most variations, including driver gene mutations, but also preserve the cytological features of malignant tumor cells, showing a highly correlated in vitro drug screening response with the mutation spectrum in primary tumors. At this stage, several large-scale LCO biobanks have been established, providing ample sample resources for researchers. Based on this, the development of emerging technologies is expected to overcome limitations in the success rate, accuracy, and stability of the organoid culture process, significantly enhancing the level of precision medicine for lung cancer. This article mainly introduces the applications of LCO models in basic research, including the identification of drug targets, prediction of treatment efficacy, and overcoming drug resistance, assisting in the formulation of personalized treatment plans to improve treatment outcomes. Additionally, the article emphasizes the potential of cancer organoid co-culture models in the field of immunotherapy and their key role in advancing the evolution of precision medicine treatment strategies.

Keywords: Drug resistance; drug screening; lung cancer organoids (LCOs); precision medicine

Introduction

Precision medicine aims to identify and develop highly selective drugs targeting specific disease markers to achieve precise treatment. Due to the genetic heterogeneity of lung cancer cells, different tumor cells may respond differently to various therapeutic agents (1). The genetic heterogeneity of lung cancer is a key factor leading to acquired resistance (2), while intratumoral heterogeneity (ITH) is also a potential
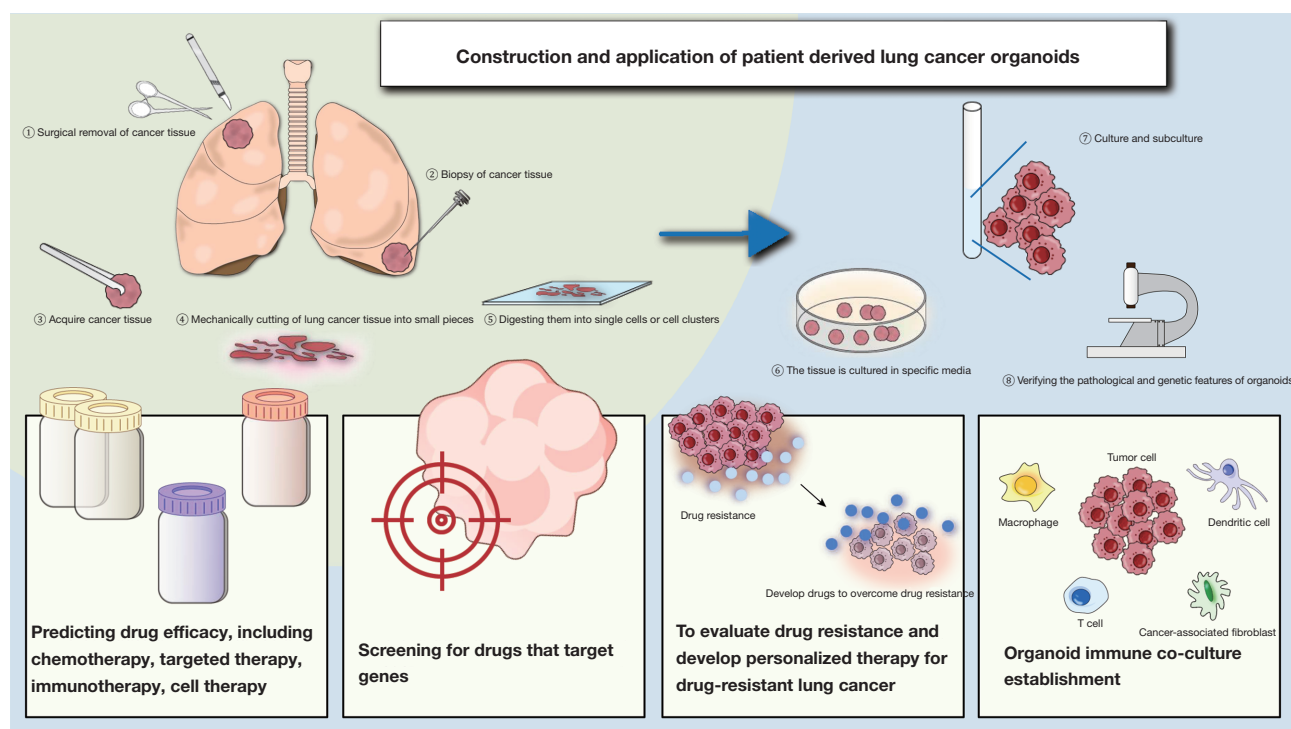
^ ORCID: 0009-0004-4634-1035.

Figure 1 Establishing LCOs from biopsy tissues and surgically resected tissue of lung cancer patients, for application in lung cancer drug development, target discovery, efficacy prediction, and other basic and clinical research. LCO, lung cancer organoid.

biomarker that can predict the efficacy of treatments in advanced non-small cell lung cancer (NSCLC) patients and other tumors receiving immune checkpoint inhibitors (ICIs) (3). Understanding tumor heterogeneity is fundamental to establishing the optimal precision treatment plan. Conventional two-dimensional (2D) cell lines, which are easy to obtain and culture, are widely used in basic research but fail to reflect tumor heterogeneity and the actual drug response of the parental tumor. In contrast, patient-derived xenograft (PDX) models from patients with lung cancer better preserve tumor heterogeneity. However, their use is limited by factors such as low success rates, high costs, and ethical issues (4), as well as some drawbacks common to animal models, including delayed implantation rates and interspecies differences (5). In particular, the differences in the immune system in mouse models make them inadequate for accurately replicating the complexity of the human immune microenvironment in cancer, leading to unsatisfactory predictions of the efficacy of immunotherapy (6). Organoid technology not only faithfully reproduces the pathological and genomic characteristics of samples (7,8), preserving most variations, including driver gene mutations (9), but also maintains the cytological features of malignant

tumors (10), with *in vitro* drug screening responses highly correlated with the mutation spectrum in primary tumors (11). Most studies produce organoids from surgical resection samples or biopsy-derived samples (*Figure 1*). After cutting lung cancer tissue into small pieces and digesting them into single cells or cell clusters, the tissue is cultured in specific media, regularly observed and the medium is changed, and the organoids' pathological and genetic characteristics are validated through staining and sequencing. Under this culture system, the genetic, proteomic, morphological, and pharmacological characteristics of tissue samples obtained from specific patients are largely retained in the *in vitro* cultured lung cancer organoids (LCOs) (12). Therefore, this novel system closely related to the original tumor provides valuable information for tumor biology, drug development, patient reactivity prediction, and clinical translation of high-throughput methods (13). This article focuses on the application of LCO models in basic research, including the discovery of drug targets, prediction of drug efficacy, and the development of new therapeutic strategies, which helps formulate personalized treatment strategies targeting specific tumor cell subpopulations to improve treatment outcomes. We also emphasize the potential value

Application of LCOs

The application of LCOs in the validation of tumor biomarkers

Organoids provide a distinct advantage over clinical lung cancer samples by delivering higher purity, thereby simplifying the exploration of tumor biomarkers. This is because the differences in gene expression between tumor cells and non-tumor cells in lung adenocarcinoma (LUAD) organoids are not influenced by stromal cells and immune cells (14). In LUAD organoid models, MEK has been identified as a promising target for LUADs with specific KRAS mutations (10). Glutathione S-transferase pi (GSTP1) has been shown to exert long-term control over LUAD by targeting cancer stem cells (CSCs) (15). Liu *et al.* generated patient-derived organoids (PDOs) from surgically resected LUAD specimens, confirming that elevated c-fos expression results in the upregulation of carcinoembryonic antigen cell adhesion molecule 6 (CEACAM6), thereby promoting tumor metastasis both *in vitro* and *in vivo* (16). Further research concluded the mechanism of LUAD metastasis induced by the BRMS1v2A273V/A273V mutation (16). Ample evidence has confirmed that the use of LCOs can facilitate the discovery of new therapeutic targets and accurately predict the therapeutic response by targeting these biomarkers. The expression status of NKX2-1 can predict responses to Wnt-targeted therapies (8). Inhibitors of another CSC marker, DCLK1, can reverse secondary resistance to tyrosine kinase inhibitions (TKIs) in both tumor cells and organoids (17). Lysine methyltransferase 2D (KMT2D) has been identified as a pivotal epigenetic regulator in the onset of lung squamous cell carcinoma (LUSC) (18). Han *et al.* found elevated CD73 (ecto-5'-nucleotidase) expression in organoids from NSCLC patients with epidermal growth factor receptor (EGFR), Kirsten rat sarcoma viral oncogene homolog (KRAS), or anaplastic lymphoma kinase (ALK) mutations, suggesting CD73 as a possible therapeutic target for these cancers (19). Pharmacological inhibitors of fascin (a pro-metastatic actin-bundling protein) can inhibit YAP1-PFKFB3 signaling and glycolysis in organoid models, and can be used to reprogram lung cancer metabolism (20). Fascin inhibitors can suppress both tumor growth and its spread to other parts of the body (20). Tyc *et al.* identified a 30-gene signature, "KDS30", which predicts the dependency on EGFR/ERBB2 (EGFR2) signaling, drug response, and survival in patients with mt KRAS mutations in lung and pancreatic cancers (21). In small cell lung cancer (SCLC) organoid models, the novel YES1 inhibitor CH6953755 or dasatinib induced significant antitumor activity, demonstrating that YES1 is a new druggable oncogenic target and biomarker (22). These methods that leverage LCOs to validate tumor biomarkers provide critical information and potential strategies for personalized treatment and the development of new drugs (*Table 1*).

The application of LCOs in predicting drug efficacy

Lung cancer exhibits significant tumor heterogeneity, necessitating extensive preclinical experiments to evaluate various treatment regimens. Drug sensitivity testing based on LCOs (LCO-DST) has been demonstrated to effectively capture the complexity of tumor heterogeneity and show a high degree of concordance with actual patient treatment outcomes (7). This strong correlation underscores the significant value of organoid models in predicting treatment responses and guiding clinical treatment decisions. In precision medicine, LCOs demonstrate a particular ability to select and predict drug treatments for specific patient populations (*Table 1*). This encompasses the identification of new therapeutic targets (23), the assessment of the effects of specific genetic mutations on drug responses (24,28), and the prediction of reactions to particular drugs (25), including both single-agent and combination therapies (21,24). Yun *et al.* studied organoids from patients with exon 20 mutations and found that Amivantamab can reduce EGFR-mesenchymal-epithelial transition (EGFR-MET) levels, boost immune responses against tumors, and increase IFN-γ production, thus inhibiting cell growth and serving as a potential TKI for this mutation (23). Early results from the CHRYSALIS I trial showed that Amivantamab was effective against tumors and safe for patients with EGFR exon 20 insertions who had received platinum chemotherapy (29). Amivantamab has also become the first Federal Drug Administration (FDA) approved bispecific molecule for the treatment of solid malignancies (29). Sachs *et al.* found that in airway organoid models, the 46T TP53 mutation is resistant to Nutlin-3a, the 52MET ERBB2 mutation is sensitive to EGFR/ERBB2 inhibitors, and the 65T ALK1 mutation responds to ALK/c-ros oncogene 1 (ALK/ROS) (28). Kim *et al.* demonstrated that PDOs are

Table 1 Summary of LCOs as personalized drug screening and efficacy analysis

LCOs based on subtypes	Organizational sources	Related drugs or targets	Main results in drug efficacy prediction/drug screening	References
LUAD, LUSC	Sputum, CTCs	MEK inhibitors (trametinib and selumetinib), FGFR inhibitor (BGJ398)	KRAS-mutated organoids exhibit high sensitivity to trametinib and selumetinib. Organoids with FGFR1 amplification display resistance to BGJ398; organoids with FGFR1 amplification exhibit synergistic effects when used in combination with trametinib	(10)
LUAD	Derived from patients	GSTP1 inhibitor (ezatiostat), ALK inhibitor (crizotinib)	GSTP1 is a promising therapeutic target in LUAD treatment. GSTP1 might mediate TKI resistance by enhancing the CSC resilience of LUAD	(15)
LUAD	Derived from patients	The c-fos pharmacological inhibitor (T5224)	T5224 can inhibit metastasis in BRMS1v2 A273V/A273V LUAD, suggesting that c-fos and CEACAM6 may be potential therapeutic targets for LUAD	(16)
LUAD, LUSC, SCLC, LCNEC	Surgery bronchoscopy; pleural effusion; CTCs; sputum	Wnt pathway inhibitor (T5224)	Wnt pathway inhibitor T5224 suppressed metastasis in NKX2-1-deficient LUAD organoids, suggesting the Wnt pathway is a potential target for lung adenocarcinoma therapy	(8)
Osimertinib-resistant lung cancer	Patient-derived lung cancer tissues	Gefitinib, osimertinib	Knockout of DCLK1 restores sensitivity to gefitinib and osimertinib in EGFR-TKI-resistant cells	(17)
NSCLC with EGFR mutations, KRAS mutations, and ALK rearrangements	Surgically removed tissue	CD73	Pharmacological or genetic inhibition of EGFR, MEK, KRAS, or ALK significantly reduced CD73 mRNA and protein expression in NSCLC cancer cells and PDOs	(19)
NSCLC	Derived from patients	EGFR/ERBB2 inhibitors, MEK inhibitors	The 'KDS30' assay can predict the response of lung and pancreatic cancer patients to combination therapy with EGFR/ERBB2 (neratinib, lapatinib, dacomitinib, gefitinib, osimertinib) and MEK inhibitors (cobimetinib, trametinib, selumetinib)	(21)
SCLC	Derived from patients	Dasatinib	YES1 (tyrosine kinase) is a novel druggable oncogenic target and biomarker	(22)
NSCLC	Hydrothorax	Amivantamab	Amivantamab demonstrates antitumor activity in multiple EGFR Exon20ins-driven NSCLC-PDOs	(23)
Advanced LUAD	Malignant effusions, brain metastasis, bone metastasis, lung primary tumor	Combine with dabrafenib and trametinib, afatinib, poziotinib, pralsetinib, gefitinib, erlotinib, osimertinib, lazertinib, dacomitinib	Demonstrated the clinical relevance of NSCLC-PDOs	(24)
NSCLC	Surgically obtained tissue	JAK inhibitor (TP-0903 and ruxolitinib)	PDO obtained from tumors with high AXL and JAK1 were sensitive to TP-0903 and ruxolitinib treatments, supporting the CyTOF findings	(25)

Table 1 (*continued*)

Table 1 (*continued*)

LCOs based on subtypes	Organizational sources	Related drugs or targets	Main results in drug efficacy prediction/drug screening	References
ALK-translocated LUAD	Surgical removal of tumor tissue, MPE	Crizotinib, alectinib, brigatinib	The CODRP index more accurately discriminates the sensitivity and resistance to ALK-targeted therapies in ALK-rearranged positive and negative patients, correlating with clinical patients' therapeutic responses to the drugs	(26)
LUAD, LUSC, SCLC, ASC, pulmonary sarcomatous carcinoma	MSE (pleural fluid, ascitic fluid, pericardial effusion), surgical removal of tumor tissue (primary or metastatic lesions); biopsy	Chemotherapy drugs and targeted drugs	LCO-DSTs can accurately predict the clinical response to treatment in advanced lung cancer patients	(7)
LUAD	–	Afatinib, T-DM1, lower dose pyrotinib, higher dose pyrotinib	Compared with afatinib, T-DM1 or lower dose pyrotinib, higher dose pyrotinib inhibited the growth of HER2-mutant cancer in both the HER2YVMA insertion PDOs	(27)

ALK, anaplastic lymphoma kinase; ASC, adenosquamous carcinoma; AXL, Axl receptor tyrosine kinase; CODRP, cancer organoid-based diagnosis reactivity prediction; CSC, cancer stem cell; CTC, circulating tumor cell; CyTOF, cytometry by time-of-flight; EGFR, epidermal growth factor receptor; Exon20ins, exon 20 insertion mutation; FGFR, fibroblast growth factor receptor; GSTP1, glutathione S-transferase pi; HER2, human epidermal growth factor receptor 2; JAK1, Janus kinase 1; KRAS, Kirsten rat sarcoma viral oncogene homolog; LCNEC, large cell neuroendocrine carcinoma; LCO, lung cancer organoid; LCO-DSTs, LCO-based drug sensitivity tests; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MEK, mitogen-activated protein kinases, extracellular signal-regulated kinases; MPE, malignant pleural effusion; MSE, malignant serous effusion; NSCLC, non-small cell lung cancer; PDO, patient-derived organoid; SCLC, small cell lung cancer; TKI, tyrosine kinase inhibition.

a significant diagnostic tool, with LUAD-PDOs harboring the EGFR L747P mutation being sensitive to afatinib (24). This aligns with clinical findings that the use of afatinib may significantly improve progression-free survival (PFS) in advanced LUAD patients with either the L747P or L747S EGFR mutations (30). Taverna *et al.* confirmed that PDOs with high Axl receptor tyrosine kinase (AXL) and Janus kinase 1 (JAK1) levels are sensitive to TP-0903 (Axl kinase inhibitor) and ruxolitinib (a JAK inhibitor) treatment through *in vitro* test (25). Combining neratinib, an EGFR/ERBB2 inhibitor, with cobimetinib, an MEK inhibitor, shows synergistic effects in PDOs with high KDS30 mt KRAS levels (21). Kim *et al.*'s experimental results showed that the combination therapy of dabrafenib/trametinib induces *in vitro* and clinical responses in NSCLC with EGFR exon 19 deletion and B-Raf proto-oncogene, serine/threonine kinase (BRAF) G464A mutation (24). A novel method for evaluating ALK-targeted therapies has been developed, which predicts patient responses to various drugs approximately 2 weeks prior to treatment initiation by integrating the growth rate of PDOs and the diagnostic stage of cancer (26). Lee *et al.*'s study indicates that, certain organoids, such as LC_02T, 03PE, and 04T, had increased resistance to crizotinib, while LC_03PE and 09PE were resistant to alectinib, and LC_04T was resistant to brigatinib, compared to ALK-negative organoids (26). In precision medicine, the significance of LCO-related research lies in their provision of a robust platform for predicting patient responses to specific treatments, evaluating the efficacy of new drugs and combination therapy regimens, and elucidating the impact of specific genetic mutations on drug responses. These models not only preserve key tumor cell variations but also maintain sensitivity to targeted therapies, playing a crucial role in precision medicine. Through these models, researchers can better understand tumor heterogeneity, predict treatment outcomes, and provide more personalized treatment plans for patients.

The application of LCOs in drug screening

LCO has been confirmed to have the potential to serve as a novel preclinical cancer model representative of individual patients (9), which can be employed to assess the therapeutic efficacy of specific drugs or drug combinations and is applicable in drug screening. In a real-world study, LCO-DST was conducted for both chemotherapy and targeted therapy (7). The results indicated that LCO-DST accurately predicted the clinical response to treatment in a cohort of advanced lung cancer patients (7). If the drug sensitivity test outcomes of LCO can be correlated with clinical outcomes, it suggests that these models can accurately predict patients' responses to treatment, which is of significant importance for drug screening and personalized medicine. Wang *et al.* demonstrated through *in vitro* experiments on LCOs with human epidermal growth factor receptor 2 (HER2) exon 20 mutations that high-dose pyrotinib can block the downstream signaling pathway of HER2, inhibit tumor growth, and be more effective than afatinib, T-DM1, or low-dose pyrotinib. Subsequently, this conclusion was validated in a phase II study with 15 patients with HER2 mutant NSCLC, showing an objective response rate (ORR) of 53.3% and a median PFS of 6.4 months (27). The LCO models established by Shi *et al.* not only retained the sensitivity to EGFR, FGFR, and MEK-targeted therapies that matched their parent tumors but also demonstrated the consistency of gene expression profiles between the organoids and the parent tumor tissues through whole-exome and RNA sequencing (10). LCO derived from various sources, such as surgically resected tumor tissues, biopsy samples, or malignant pleural effusions, can provide a comprehensive view of tumor heterogeneity, which is crucial for simulating the tumor response in the real world. Norman *et al.* provided a basic framework for selective growth of p53 mutant tumors in primary and metastatic NSCLC, summarizing tumor histology and mutation status, confirming that airway organoids can be used for *in vitro* drug screening (28). Drug experiments conducted by Peng *et al.* showed that in LCOs derived from malignant pleural effusion of EGFR⁺/MET amp⁺ patients, dual-targeted therapy was more effective than TKI monotherapy (31). To facilitate the widespread application of LCO in clinical drug screening, a crucial prerequisite is the optimization of workflows and the reduction of the turnaround time for organoid generation and drug sensitivity testing (32). This necessity stems partly from the heterogeneity of tumors, where different

cancers or even different parts of the same tumor may progress at varying rates. Recent studies have successfully identified methods for rapidly assessing drug responses in LCO, which holds the potential to enhance the efficiency and timeliness of clinical applications. Hu *et al.* developed a microarray chip-based drug sensitivity test using LCO that can predict drug responses within a week (13). Lee *et al.*'s drug sensitivity test based on the cancer organoid-based diagnosis reactivity prediction (CODRP) index not only reliably classified patients' responses to EGFR-TKI therapy within a clinical timeframe of 10 days but also demonstrated significant correlation with clinical outcomes (33). Takahashi *et al.* confirmed that the 384-well plate High Throughput Screening (HTS) system is suitable for *in vitro* evaluation of LCO sensitivity to molecular targeted drugs (including small molecule inhibitors, monoclonal antibodies, and antibody-drug conjugates) and chemotherapy drugs (34). Research conducted thus far has amply demonstrated the significance of LCOs as a crucial model for drug screening. However, there are currently variations in protocols for organoid culture and DST across different laboratories, which may lead to inconsistent and non-reproducible results (32). This poses a challenge to the reliability of clinical decision-making and research. Future efforts must establish stringent quality control standards to standardize the culture environment and reliability of organoids.

The application of LCOs in drug resistance

In recent years, extensive research has utilized LCOs to establish drug resistance models, aiming to explore the mechanisms of drug resistance and identify new strategies to reverse it (*Table 2*). A LUAD-PDOs model that preserves the biologic features of human metastases has been established to study the response of KRASG12C-mutated PDOs to sotorasib (35). It was found that sotorasib effectively inhibits metastasis and reduces cellular viability, confirming that PDOs can be a powerful tool for studying lung cancer metastasis and assessing resistance to targeted therapies (35). Research based on EGFR-TKI resistance models has shown that methanesulfonamide (MA; a natural PLA2 inhibitor) (50), compound Z11 (an effective CDK9 inhibitor) (36), and BI-4732 (a fourth-generation EGFR-TKI) (37), could be potential options for treating NSCLC patients with osimertinib-resistant. Li *et al.*, using an osimertinib-resistant LUAD model, discovered that RNA-binding motif protein 15 (RBM15) enhances the m6A

Table 2 Summary of LCOs as an enhanced model for personalized drug resistance

LCOs based on subtypes	Organizational sources	Therapy	Related drugs or targets	Main results in drug resistance	References
LUAD	Surgical removal of tumor tissue (primary or metastatic lesions); MPE	Targeted therapy	Osimertinib, sotorasib	The model can examine the efficacy of treatments to suppress metastasis and identify mechanisms of drug resistance	(35)
NSCLC	Derived from patients	Osimertinib-resistant	Compound Z11, a potent CDK9 inhibitor	Z11 served as a promising macrocycle-based CDK9 inhibitor for treating osimertinib-resistant NSCLC	(36)
NSCLC	MPE	Targeted therapy	BI-4732, a novel fourth-generation EGFR-TKI	BI-4732 is a novel and potent EGFR inhibitor targeting activating mutations and resistance to EGFR-targeted therapy in NSCLC	(37)
LUAD	Biopsy	Targeted therapy	RBM15	Early targeting of RBM15 during EGFR-TKI treatment could dramatically extend the patient response and benefit from TKI therapy	(38)
LUAD	MPE	Targeted therapy	Gefitinib, BYL719 (a PI3Kα inhibitor)	The combined effect and mechanism of gefitinib and BYL719 were confirmed in the NSCLC cells and PDOs	(39)
LUAD	Surgery samples, transbronchial biopsies	Cisplatin-resistant	HF, cisplatin	HF could sensitize the cisplatin-resistant LCOs	(40)
LUAD, LUSC	Surgical samples, transbronchial biopsies	Chemotherapy	Solamargine	Solamargine as a hedgehog pathway inhibitor and cisplatin-sensitizer	(41)
Lung cancer	Patient-derived tissue	Chemotherapy	DHA, cisplatin	Combining DHA with cisplatin presents a promising strategy to overcome chemoresistance in lung cancer patients	(42)
LUAD	LUAD tumor tissues	Chemotherapy	ALDH2, platinum	Targeting ALDH2 is a promising new strategy to enhance the sensitivity of platinum-based chemotherapy for the treatment of LUAD patients	(43)
NSCLC	Surgical removal of tumor tissue	Chemotherapy	Cisplatin, paclitaxel	PFKP can regulate the expression of ABCC2 through the activation of NF-κB, which promotes chemoresistance in NSCLC	(44)
LUAD, LUSC	Surgical removal of tumor tissue	Chemotherapy	Cisplatin, paclitaxel	AKR1B10 can be targeted by repurposing epalrestat to overcome chemoresistance in NSCLC	(45)
NSCLC	Patient-derived tumor tissue	Radiotherapy	NO	Targeting nitric oxide effectively reduced CSC populations within PDOs during radiotherapy	(46)
NSCLC	MPE	Targeted therapy, osimertinib-resistant	BLU-945, osimertinib	BLU-945 demonstrated in vivo tumor shrinkage in osimertinib-resistant models of NSCLC (osimertinib second line: EGFR_L858R/C797S and third line: EGFR_ex19del/T790M/C797S and L858R/T790M/C797S) both as monotherapy and in combination with osimertinib	(47)

Table 2 (*continued*)

Table 2 (*continued*)

LCOs based on subtypes	Organizational sources	Therapy	Related drugs or targets	Main results in drug resistance	References
NSCLC	Malignant effusions	Targeted therapy	BLU-945	BLU-945 is a potent, reversible, wild-type-sparing inhibitor of EGFR[+]/T790M and EGFR[+]/T790M/C797S resistance mutants that maintains activity against the sensitizing mutations, especially L858R	(48)
NSCLC	Malignant effusion	Targeted therapy	Dabrafenib, trametinib, osimertinib	EGFR/BRAF/MEK triple-targeted therapy is an effective and safe approach for treating EGFR-mutated NSCLC patients resistant to EGFR-TKIs with acquired BRAF V600E mutations	(49)

BRAF, B-Raf proto-oncogene, serine/threonine kinase; CSC, cancer stem cell; DHA, dihydroartemisinin; EGFR, epidermal growth factor receptor; HF, halofuginone; LCO, lung cancer organoid; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MEK, mitogen-activated protein kinase kinase; MPE, malignant pleural effusion; NO, nitric oxide; NSCLC, non-small cell lung cancer; PDO, patient-derived organoid; PFKP, phosphofructokinase, platelet; TKI, tyrosine kinase inhibition.

modification of SPOCK1 mRNA, activates bypass pathways, promotes epithelial-mesenchymal transition, and thereby mediates osimertinib resistance (38). It was concluded that RBM15 is a key factor in reversing EGFR-TKI resistance and, as a therapeutic target, has the potential to extend the benefits of TKI treatment for patients with LUAD (38). The research findings by Yu *et al.* demonstrate that PIK3CA mutations lead to resistance to EGFR-TKIs in NSCLC through the activation of the PI3K/AKT signaling pathway (39). The combined use of BYL719, a PI3Kα inhibitor, and gefitinib can overcome this resistance by dual inhibition of the signaling pathway (39). Yan *et al.* demonstrated that inhibiting DCLK1 expression can enhance the sensitivity of EGFR-TKI resistant cells and organoids to EGFR-TKIs, highlighting DCLK1 inhibitor (DCLKI-IN-1) as a potential targeted treatment option for wild-type EGFR patients post resistance development (17). In addition, DCLK1 plays a crucial role in maintaining the stem-like characteristics of tumor cells, which helps to avoid the targeted killing of EGFR-TKIs (17). Wang *et al.* utilized organoids derived from ALK-rearranged LUAD patients to show that combination therapy of ezatiostat (a specific GSTP1 inhibitor) and crizotinib (an ALK inhibitor) can modulate lung CSC activity and inhibit TKI-resistant LADC organoid proliferation (15). Furthermore, LCOs are employed to test the synergistic effects of various drug combinations in the quest for new therapies to overcome drug resistance. Research on cisplatin-resistant organoids indicates that halofuginone (HF; a natural small

molecule) (40), solamargine (a natural small molecule) (41), or dihydroartemisinin (DHA) (42) used in combination with cisplatin (DDP) individually represent promising strategies to overcome chemotherapy resistance in lung cancer patients. Targeting aldehyde dehydrogenase 2 (ALDH2) (43) or phosphofructokinase, platelet (PFKP) (44) can also enhance the sensitivity of LUAD patients to platinum-based chemotherapy. Suvilesh *et al.*, by establishing patient-derived tumor organoids (PDTO) from NSCLC, discovered that the use of the AKR1B10 inhibitor epalrestat can overcome chemoresistance in NSCLC (45). LCO can also be utilized to assess resistance to radiotherapy. Zhang *et al.* propose that nitric oxide (NO) promotes the S-nitrosylation and deubiquitination of Notch1 protein, thereby maintaining CSCs in NSCLC (46). Targeting NO can effectively reduce the CSC population within PDOs during radiotherapy, presenting a promising therapeutic target for overcoming radiotherapy resistance in NSCLC treatment (46). LCOs, capable of replicating the cellular traits and mutational heterogeneity of human cancers, have become essential tools for investigating resistance mechanisms and devising innovative treatment approaches, with initial clinical validation already achieved. Lim *et al.* have validated a novel EGFR-TKI inhibitor, BLU-945, for its antitumor activity against osimertinib-resistant NSCLC cells and organoids (47). Subsequently, the SYMPHONY phase I/II trial, a global first-in-human study, has demonstrated that BLU-945, as a promising next-generation EGFR-TKI, holds potential therapeutic value for

patients with EGFR-mutated NSCLC who have developed resistance following prior EGFR-TKI treatment (48). In a clinical retrospective study involving thirteen patients with advanced NSCLC who harbored the BRAF V600E mutation post-EGFR-TKI therapy, the combination therapy of dabrafenib, trametinib, and osimertinib yielded a median PFS of 13.5 months (95% confidence interval: 6.6–20.4) with an ORR of 61.5% (49). Validation through PDOs revealed that this treatment was highly effective, with significantly lower half-maximal inhibitory concentration (IC_{50}) values compared to other treatment regimens, achieving a 99.36% inhibition of tumor growth, confirming the efficacy of the triple therapy in patients with EGFR-mutated and BRAF V600E-resistant disease (49). Xie *et al.* reported a case of a patient with ceritinib-resistant lung cancer who maintained the original ALK-rearrangement without acquired mutations, and sequential treatment with brigatinib and lorlatinib resulted in PFS durations of 3.2 and 1.9 months, respectively, aligning with drug sensitivity testing outcomes from LCOs (51). Additionally, LCOs can exhibit cellular state transitions, a key mechanism in the development of drug resistance. Researchers, using a LUAD organoid model co-mutated for KRASG12C and STK11/LKB1, found that treatment with KRASG12C inhibitors, such as adagrasib, induced an adenosquamous transformation (AST) in KRAS/LKB1-mutated LUAD organoids, leading to drug resistance (52). The key factor ΔNp63 drives this process, and AST plasticity signatures along with Keratin 6A (KRT6A) expression levels may serve as predictive biomarkers for drug resistance (52). Recent advancements have witnessed a surge in research utilizing LCOs to establish drug resistance models and explore novel therapeutic strategies. However, the transition from preclinical to clinical applications remains a formidable challenge. It has been observed that the evidence linking clinical observational data with experimental results from LCOs is often limited by small sample sizes. This limitation is further compounded by the variability in LCO cultivation methods across different laboratories and the fact that the success rate of establishing LCO models is not 100%. Consequently, the immediate translation of experimental outcomes from organoid models into clinical practice is not feasible with the current experimental methods (24). Instead, the current strength of LCOs lies in their utility as an assessment tool for *in vitro* testing, which allows for the evaluation of the therapeutic compatibility between a specific individual or a cohort sharing common characteristics and a given drug.

Organoid-immune co-culture establishment

ICIs differ from cytotoxic chemotherapy and targeted therapy in that they activate anti-tumor immunity by blocking T cell co-inhibitory signals (53). However, tumor heterogeneity leads many patients to eventually develop acquired resistance to ICIs (54). Currently, our ability to predict the mechanisms of ICI resistance and treatment efficacy is limited, primarily relying on long-term follow-up and clinical data. Most PDOs lack a specific ratio of immune cell composition in culture (55), coupled with the short lifespan characteristic of immune cells (33), which prevents the reconstruction of the tumor microenvironment (TME) (56). Cancer organoid co-culture models have been proposed to address this issue. This system ingeniously integrates the cellular components of the TME, including T cells, into the cancer organoid cultures, better replicating the TME and thus providing a research platform closer to *in vivo* conditions (57,58). Specifically, within the context of either the extracellular matrix (ECM) or the liquid culture medium, fully developed organoids are supplemented with stimulated immune cells, including T lymphocytes and innate lymphoid cells (ILCs), to assess the interplay between these immune components and the epithelial cells (57). Co-culturing cancer organoids with patients' own peripheral blood mononuclear cells (PBMCs) to induce tumor-specific cytotoxic T cells is a widely adopted method (59,60). This includes maintaining and expanding natural immune cells within organoids or directly adding immune cells to the culture (61). Dijkstra *et al.* co-cultured colorectal cancer (CRC) or NSCLC organoids with autologous circulating T cells and successfully expanded tumor-reactive T cells paired with PBMC (59). Two weeks later, CD107a upregulation and IFN-γ secretion were observed in the NSCLC co-culture, with 2 out of 6 NSCLC patients producing LCO and 2 out of 6 NSCLC patients obtaining autologous tumor-reactive CD8^{+} T cells (59). Cattaneo *et al.* also established organoids from tumor samples of NSCLC and microsatellite unstable CRC patients, co-culturing them with peripheral blood lymphocytes for two weeks, using IFN-γ to stimulate tumor cells to expand tumor-reactive T cells (62). Results from T cell-organoid killing assays showed that altering the ratio of T cells to tumor cells could significantly change the observed level of tumor killing (62). Wang *et al.* used co-culture model to assess the immunobiological effects of serum amyloid A (SAA) secreted by CSCs (63). The experiment introduced

anti-programmed death-1 (α-PD-1) and SAA-neutralizing antibodies to evaluate T cell reactivity and killing ability against tumors (63). The results indicated that SAA promotes chemotherapy resistance, inhibits anti-tumor immunity, and exacerbates tumor fibrosis by driving a TH2-type immune response (63). To validate the therapeutic effect of inhibiting metastasis and determine resistance mechanisms, Liu *et al.* co-cultured pre-treated PDOs with chemotherapy or radiation therapy with autologous PBMCs (35). The results showed that the addition of the PD-1 inhibitor nivolumab could activate CD8+ T cells and increase tumor cell death levels (35). Notably, PDOs pre-treated with radiotherapy demonstrated more effective immune activation capabilities in co-culture (35). This finding provides theoretical support for the treatment of LUAD and aids in the development of personalized immunotherapy strategies. Macrophages are highly abundant in the innate immune infiltration of NSCLC TME and can be systematically incorporated into NSCLC organoids derived from wild-type tissues, generating dynamic crosstalk (64). The establishment of organoid-macrophage co-culture models contributes to the exploration of macrophage-targeted therapy in NSCLC immunotherapy, opening up new frontiers for NSCLC treatment (64). Chen *et al.* reported a three-dimensional (3D) co-culture system of LUSC epithelial cells with cancer-associated fibroblasts (CAFs) and ECM (65). This model can summarize the key pathological changes from tumor proliferation to dysplasia and ultimately invasion, providing fundamental evidence for the dynamic interaction between LUSC cells and the components of the TME (65). Patient-derived organotypic tissue cultures (PD-OTCs) of NSCLC offer a comprehensive model that captures the metabolic reprogramming observed in patient tumors and provides a platform to study immune metabolic responses to therapeutic modulators, as well as the emergence of resistance within the TME (66). Concurrently, recent studies have demonstrated the efficacy of organoid technology in the reprogramming of T lymphocytes, which significantly amplifies tumor cell vulnerability to T cell-mediated cytotoxic attacks, highlighting the dual utility of organoids in advancing our understanding of both metabolic and immunological dynamics in cancer (67). Although CD8+ T cells are the most powerful effectors in anti-cancer immune responses, reflecting the therapeutic effects of ICIs (68,69), this co-culture model also has limitations, as isolated immune cell populations, such as T cells or PBMCs, cannot fully represent the complexity and

heterogeneity of the entire immune system (70). Anti-cancer immune responses require the cooperation of tumor-infiltrating innate immune cells and adaptive immune cells [such as neutrophils, myeloid-derived suppressor cells (MDSCs), macrophages, natural killer (NK) cells, dendritic cells (DCs), and T cells] within the tumor tissue. New methods have been proposed to address the current challenges (71). Luan *et al.* proposed an agarose micropore platform specifically designed for PDO-based tumor and TME models, capable of rapid drug screening and resistance studies with small sample sizes (72). Various cell types, including cancer cells, fibroblasts, immune cells, and endothelial cells, have been integrated into various microfluidic co-culture platforms, successfully demonstrating cancer phenotype modeling and drug response outcomes (72). A novel air-liquid interface (ALI)-based organoid generation platform has also been successfully established and has been shown to accurately preserve T cells, CAFs, and CD31+ endothelial cells in renal cell carcinoma organoids (73). Therefore, the co-culture model needs further improvement to better reflect the interactions between tumors and immune cells (58). Although the current co-culture models cannot fully replicate the complex mechanisms and interactions found in the TME under cancerous conditions, it cannot be denied that they are a promising tool for simulating changes in the immune microenvironment of lung tissue under different pathological states. Studies have already utilized organoid culture technology to simulate the alveolar microenvironment (74,75). DJ-1 (an antioxidant protein) controls goblet cell metaplasia and eosinophil infiltration by affecting the interaction between airway progenitor cells and immune cells (75). During the critical period of alveolar epithelial cell (AEC2) proliferation, there is an accumulation of CD115+ and CCR2+ monocytes as well as M2-like macrophages in lung tissue (74). Furthermore, the co-culture study of mouse airway club progenitor cells and CD11b macrophages showed that the Lkb1 defect in mouse airway club progenitor cells promoted the expression of FIZZ1/RELM-α (resistin-like molecule-α), leading to airway goblet cell differentiation and lung macrophage recruitment (76). The process of simulating the alveolar microenvironment using organoid culture emphasizes the role of immune cells in promoting lung regeneration and repair. In contrast, in a cancerous state, organoids may exhibit different patterns of interaction between immune cells and tumor cells, such as immune suppression or signaling that promotes tumor growth. These differences

highlight the importance of organoids as research tools in simulating and understanding the changes in the immune microenvironment of lung tissue in different pathological states.

Cancer organoids co-cultured with immune cells provide a valuable model system for assessing the sensitivity of individual cancers to immunotherapy at any stage of treatment, which is crucial for evaluating new therapies. This innovative model has been shown to effectively and rapidly assess the impact of ICIs on activating cytotoxic lymphocytes and promoting T cell infiltration, especially in cases of T cell infiltration. Despite the promise of this model, obtaining sufficient high-quality tumor tissues and immune cells remains a challenge, which may limit the widespread application of the model. To overcome these challenges, it may be necessary to develop new techniques to improve the efficiency of sample acquisition and processing or explore alternative models, such as those based on cell lines or genetically engineered models, to enhance the reproducibility and scalability of research.

Discussion

PDO must accurately replicate the unique tumor characteristics of primary cancers, as this directly affects the clinical applicability of the model (77,78). Therefore, it is necessary to study the success rate and accuracy of cultured organoids (61,79). The success of LCO cultivation can be determined by several different factors. First is the aspect of tumor tissue sourced from patients. Current limitations include: (I) the proportion of tumor cells in the initial sample is often insufficient, which is especially common in tissue samples obtained through biopsies (9); (II) tumor tissue may be affected by excessive growth of surrounding normal tissue (80), resulting in almost no cancer cells in late passages of LCO (81). Previous studies have indicated that organoids can be generated from metastatic tissue or malignant effusions (33), thereby allowing for the exclusion of normal lung cells to address this limitation (82,83). Second, is the aspect of long-term amplification of organoids. Current limitations include: (I) the lack of medium formulations specifically for LCOs, leading to limited success rates and tumor purity (84); (II) the short lifespan of organoids is a major factor limiting their ability to reach later developmental stages and acquire mature phenotypic capabilities (12). Regarding the culture medium, researchers believe that the inducing factors for tumor organoids vary due to individual heterogeneity (12),

thus requiring screening of various cytokines and small molecules to optimize the culture medium for LCOs (78). Acosta-Plasencia *et al.* (84) evaluated three types of media, including permissive culture medium (PCM), limited culture medium (LCM), and minimal basal medium (MBM), showing that while PCM is associated with the highest success rate and aids in long-term amplification, MBM is the best choice for avoiding excessive growth of normal organoids in culture. Due to the high metabolic demands of organoid growth, necrotic cores form within the hydrogel structure; however, new methods are being researched to reconfigure the 3D environment of organoids, removing the necrotic centers of the hydrogel while retaining the outer layer containing viable organoids (85). When the LCO culture medium can successfully establish organoids, facilitate long-term amplification, and maintain the purity of cancer cells in culture, it is considered optimal (84). However, currently, no medium meets all requirements. Encouragingly, an increasing number of studies are emerging to propose more suitable culture methods for different subtypes. Malmros *et al.* (86) used four different methods to culture NSCLC cell lines in 3D, showing that adenocarcinoma cell lines (A549, NCI-H1975) retained their original immunohistochemical (IHC) characteristics, while squamous cell carcinoma (HCC-95, HCC-1588) lost their unique markers during culture. This suggests that different media may have varying impacts on the phenotypic and molecular characteristics of the cells. Choi *et al.* (77) validated WRNA culture conditions (including Wnt3A, R-spondin1, Noggin, and A8301) for the long-term culture of SCLC organoids. The study also indicated that although culture conditions are crucial for the long-term survival of organoids, they do not affect the genomic stability or representativeness of the organoids. For LADC organoids, the mechanical dissociation method using a 10 mL Stripette serum pipette to crush organoids into small fragments is a better choice compared to traditional enzymatic digestion methods (14). There is still a long way to go in the future, requiring the development of optimized culture protocols suitable for different subtypes of lung cancer. This not only necessitates proposing and conducting extensive experimental validations, including determining the optimal medium components, exploring processing methods for tumor tissues from different sources, long-term culture conditions, freezing and recovery conditions, etc., but also ensuring the genetic stability of phenotypes in organoids during long-term culture and passage, ultimately leading to the formulation of standardized LCO culture protocols.

At this stage, LCOs as disease models are developing rapidly and are unstoppable. The LCOs biobank has been established, which includes tumor tissue samples along with paired clinical information. This facilitates purposeful cryopreservation and revival during targeted experimental research, is applicable for high-throughput drug screening and drug efficacy prediction, and allows for targeted drug screening of specific mutations/subtypes of LCOs. In addition to serving as disease research models, LCOs can also act as platforms for new drug development. Naranjo *et al.* developed immunocompetent organoid-based models of KRAS, BRAF, and ALK mutant LUAD, aiding in the study of lung cancer immunotherapy (87). Liu *et al.* constructed a platform combining phenotype and transcriptomics, called FascRNA-seq, to analyze the dynamic response of the local immune microenvironment under immune checkpoint blockade (ICB) therapy (88). Notably, the concept of 'tumour assembloids' has recently been proposed, which is defined as self-organizing cellular systems composed of different organ or cell types (89). It emphasizes the interactions between various cells in a 3D space, particularly cancer cells and tumor-associated cells, aimed at creating more realistic simulations of PDTO within the TME (90). Another significant aspect is that LCOs provide researchers with the ability to assess the real-time effects of therapeutic interventions, which is especially important for studying lung cancer with high heterogeneity and contributes to the development of personalized treatment strategies. Additionally, the integration of organoid technology with 3D bioprinting and novel biomaterials, along with gene editing, multi-omics analysis, and artificial intelligence, will become future development trends. This interdisciplinary advancement also offers new opportunities for LCO-immunocyte co-culture models. It not only allows for the assessment of individual cancer sensitivity to immunotherapy at various stages of treatment but also provides an important platform for the development and evaluation of new immunotherapies, thereby promoting the progress of personalized precision medicine.

Conclusions

LCOs as a new strategy in precision medicine research, not only provide a powerful experimental platform for evaluating new treatment strategies and exploring biomarkers but also contribute to a deeper understanding of drug resistance mechanisms. Despite challenges in cultivation success rate and accuracy, significant progress has been made through technological innovation. This has not only improved the clinical relevance of the models but also provided new perspectives for personalized treatment. In the future, further optimization of LCO cultivation techniques and addressing the current limitations of cancer organoid co-culture models will be of great significance in promoting the development of precision medicine for lung cancer.

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Footnote

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