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Mini-patient-derived xenograft assay based on microfluidic technology promises to be an effective tool for screening individualized chemotherapy regimens for advanced non-small cell lung cancer

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Abstract

Patient-derived xenograft (PDX) assay has been widely used in preclinical research in patients with multidrug-resistant lung cancer. One hundred patients with non-small cell lung cancer (NSCLC) were divided into MiniPDX group and conventional group, with 50 cases in each group. The MiniPDX assay was established by enriching high-purity tumor cells using microfluidic technology to detect the drug sensitivity of NSCLC cells. All patients underwent conventional computed tomography (CT) scans of lung and mediastinum at baseline and during follow-up. Kaplan–Meier method was used to compare the overall survival and progression-free survival of two groups. The sensitivity of the same drug in different tumor xenograft varied greatly. The overall survival, progression-free survival, and clinical benefit rate of patients in the MiniPDX-guided chemotherapy group were significantly longer than those in the conventional chemotherapy group. MiniPDX assay may be an effective tool for screening chemotherapy regimens in NSCLC patients.

KEYWORDS
individualized chemotherapy regimens, microfluidic technology, mini-patient-derived xenograft assay, non-small cell lung cancer

1 | INTRODUCTION

Lung cancer is one of the most deadly malignant tumors in China, 80% of which are non-small cell lung cancer (NSCLC), which is generally diagnosed at an advanced stage (Siegel et al., 2018). Chemotherapy is an important means for the comprehensive treatment of advanced NSCLC. Gemcitabine is a novel cytosine derivative and plays a role in the G1/S phase (Qin et al., 2019). It has been reported that gemcitabine combined with cisplatin is a first-line treatment for Stages III and IV NSCLC, but its effective rate is only 20%–40% (Xiang Yong et al., 2017). Vinorelbine is a semi-synthetic vinca alkaloid with broad-spectrum antitumor activity and low toxicity, which produces cytotoxic effects by interfering with the accumulation of microtubules during cell mitosis (Nakanishi et al., 2018).

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Besides, carboplatin mainly acts on N_7 and O_6 atoms of DNA guanine, causing DNA chain and intrachain crosslinks, destroying DNA molecules, preventing their helical melting, interfering with DNA synthesis and producing cytotoxic effects (Gridelli et al., 2018). A Phase II study of first-line chemotherapy in advanced NSCLC patients clarified that the combination of carboplatin and irinotecan showed good activity and controlled toxicity characteristics in patients who did not receive chemotherapy (Kim et al., 2013). However, patients with different tumors have different sensitivities to different/identical chemotherapeutic drugs in clinical practice. Therefore, screening out the most sensitive chemotherapeutic drugs and dosages for each patient through drug sensitivity test may provide an effective reference for clinical individualized treatment.

Clinically, the acquisition of pleural fluids has the characteristics of minimally invasive, repeated and easy to operate features, making pleural fluids a substitute for tumor tissue for gene detection (He & Zeng, 2016). Nevertheless, the cancer cells in pleural fluids are few in number, low in purity, and mixed with inflammatory cells and mesothelial cells. In the traditional immunomagnetic separation method, the enriched cells observed and counted by microscopy need to be cleaned, centrifuged, resuspended and transferred, and these complicated processes may cause the loss of target cells. Thus, it is of great urgency to find an efficient and specific enrichment separation method.

Emerging microfluidic technology refers to the control of microvolume fluids for flow, energy exchange, and biochemical reactions by applying external forces (He & Zeng, 2016). With the introduction of the Precision Medicine program, microfluidic technology has been widely used in single-cell research, disease-specific diagnosis, and genetic analysis because of its integration, automation, and high-throughput (He & Zeng, 2016).

In recent years, there has been an increasing interest in developing patient-derived xenograft (PDX) assays for cancer research (Gandara et al., 2015). Byrne et al. (2017) concluded that unlike cell line-derived tumor assays, the PDX assay retains the major histological and genetic characteristics of the donor tumor and remains stable throughout the passage (Byrne et al., 2017). However, Ben-David et al. (2017) argue that the genomic instability of PDX is underestimated and they found that PDX models show dynamic alterations in tumor genetics over time (Ben-David et al., 2017). There are other limitations in the direct application of traditional PDX assay to NSCLC patients. Isler et al. (2014) have reported that the PDX assay may be used to predict the clinical response to drug therapy in NSCLC patients, but the implantation rate is only 57%. Moreover, the established PDX assay usually takes 4–8 months to assess drug sensitivity, which is too long for NSCLC patients who begin treatment with drug sensitivity guidance (Hidalgo et al., 2014; Zhan et al., 2018). MiniPDX assay is a technique for isolating primary cells from tumor specimens in vitro and conducting drug sensitivity assays in vivo (Zhan et al., 2018). Compared with traditional PDX assay, MiniPDX assay can obtain the results of drug sensitivity assays for patients within 7 days, which can meet the urgent needs of drug selection for patients with advanced tumors, providing guidance for rapid personalized drugs for each patient (Zhan et al., 2018).

In this study, we hypothesized that detection of the response of the most commonly used chemotherapeutic drugs (vinorelbine, docetaxel, gemcitabine, nab-paclitaxel, pemetrexed, and carboplatin) and a combination of drugs to NSCLC cells via personalized MiniPDX assay can benefit NSCLC patients receiving advanced chemotherapy.

2 | MATERIALS AND METHODS

2.1 | The selection of subjects

Clinical samples were taken from NSCLC patients with admitted to the Shanghai Lung Cancer Center of Shanghai Chest Hospital between September 2014 and September 2016. The inclusion criteria were as follows: (1) Stage IV NSCLC confirmed by histopathology or cytology in patients aged 18–75 years, with adequate hematological and end-organ function, as well as expected survival time ≥ 3 months; (2) tumors of patients lacked EGFR-sensitive mutations (Exon 19 deletion, or Exon 21 L858R, Exon 21 L861Q, Exon 18 G719X, or Exon 20 S7681 mutation) and ALK rearrangements, or failed to respond to targeted drug therapy; (3) failure of first-line platinum-containing chemotherapy; (4) participants could provide fresh tumor tissue or pleural fluid samples; (5) computed tomography (CT) scan revealed at least one lesion with a maximum diameter of 10 mm (the short axis of lymph nodes must be 15 mm) and no prior radiotherapy, which could be measured repeatedly and accurately; (6) the Eastern Cooperative Oncology Group (ECOG) physical status score was 0 or 1; (7) signed informed consent. The exclusion criteria were as follows: (1) patients refused to provide clinical samples and to accept the treatment and follow-up plan provided by researchers; (3) the overall survival (OS) time of patients was insufficient to complete at least one course of drug treatment, resulting in the inability to evaluate drug effect; (4) patients were unable to receive treatment according to the established treatment plan owing to a deteriorating condition or serious complications. We excluded 17 patients who did not meet the incision criteria, 3 patients who refused to accept the treatment and follow-up plan provided by researchers, and 11 patients withdrew from the study during the treatment. Finally, 279 participants were included in the final analysis. A computer-generated sequence of numbers was used to randomly assign patients on a 1:1 basis. Two hundred and seventy nine participants randomly divided into MiniPDX group and conventional group, with 140 in MiniPDX group and 139 in the conventional chemotherapy group.

2.2 | Enrichment of NSCLC cells by microfluidic technology

Pleural fluids 50 ml was taken from each patient and centrifuged at 1000*g* for 10 min. The fabrication and modification of microfluidic

nanochips was performed as previously described (Shen et al., 2016). Briefly, malignant tumor cells were isolated from the patient's pleural fluid using the ClearCell FX1 system (Clearbridge BioMedics Pte Ltd) according to the manufacturer's protocol. Streptavidin 250 μl (250 μg/ml) was added into each chip and placed in a refrigerator at 4°C overnight. Then, chips were assembled and rinsed once with PBS 0.5 ml at a flow rate of 0.5 ml/h, followed by addition of epithelial cell adhesion molecule 500 μl (200 μg/ml). The chips were incubated at room temperature for 45 min and washed once with 0.5 ml of PBS. Finally, 1 ml of pleural fluids at a rate of 0.5 ml/h flowed over the chips, and rinsed once with 0.5 ml of PBS at a flow rate of 0.5 ml/h. The chip was unloaded and stored in a 4°C refrigerator.

2.3 | MiniPDX assay

The chemotherapy regimens for patients in MiniPDX group was based on the results of drug sensitivity assay in mice. MiniPDX assay was performed using the OncoVee™ MiniPDX kit (LIDE Biotech Co., Ltd). Each patient used two mice per dosing regimen implanted in three capsules each, corresponding to six replicates per dosing regimen. The conventional chemotherapy group does not require mouse models and are administered directly following clinical experience or guideline recommended methods. In the MiniPDX group, a blank control group was set up for each patient's different regimens, which was also two mice without the administration operation. Briefly, the NSCLC cell suspension enriched by microfluidic technology was transferred to Hank's balanced salt solution-washed capsules made of a hollow fiber membrane with an aperture of less than 500 kDa. The fiber system delivered the media to cells in a manner similar to blood delivery through the capillary network in vivo.

BALB/c (concatenation of Bagg and Albino) nude mice (4-6 weeks of age) (SLARC Inc.) weighing 15–20 were used for subcutaneous implantation. A small skin incision was made and the capsule was embedded in the subcutaneous tissues. One day after inoculation of tumor cells, the tumor-bearing mice were given different treatment regimens for 7 days each, including monotherapy (docetaxel, gemcitabine, nab-paclitaxel, pemetrexed, and carboplatin) and combination therapy (vinorelbine, docetaxel, gemcitabine, nab-paclitaxel, and pemetrexed combined with carboplatin, respectively). The specific medication regimen is as follows, vinorelbine, 6.7 mg/kg, intraperitoneally (ip), every 1 days; gemcitabine, 60 mg/kg, ip, every 4 days; docetaxel, 10 mg/kg, ip, every 4 days; nab-paclitaxel, 20 mg/kg, intravenously (iv), every 4 days; pemetrexed, 50 mg/kg, ip, every 2 days; carboplatin, 25 mg/kg, ip, every 4 days. Normal saline was used as a control. Tumor cell viability was assessed based on relative fluorescence units (RFU) using CellTiter-Glo® Luminescent Cell Viability Assay (Promega) to demonstrate the antitumor activity of each drug. The equation for calculating proliferation rate was as follows:

$$\text{Proliferationrate} = (\text{RFU}^{D7} - \text{RFU}^{D0})_{\text{drug}}/(\text{RFU}^{D7} - \text{RFU}^{D0})_{\text{placebo}}.$$

The research flow chart is shown in Figure 1. All procedures were performed in accordance with the guidelines for the Care and Use of Laboratory Animals of the National Institutes of Health under specific pathogen-free conditions. The experiment was approved by the Ethics Committee of Shanghai Jiao Tong University Affiliated Chest Hospital.

2.4 | Conventional chemotherapy

Patients in the conventional group were treated with chemotherapy regimens according to National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology, version 2.2013 (Ettinger et al., 2013). Treatment regimens were decided by at least two independent medical professionals.

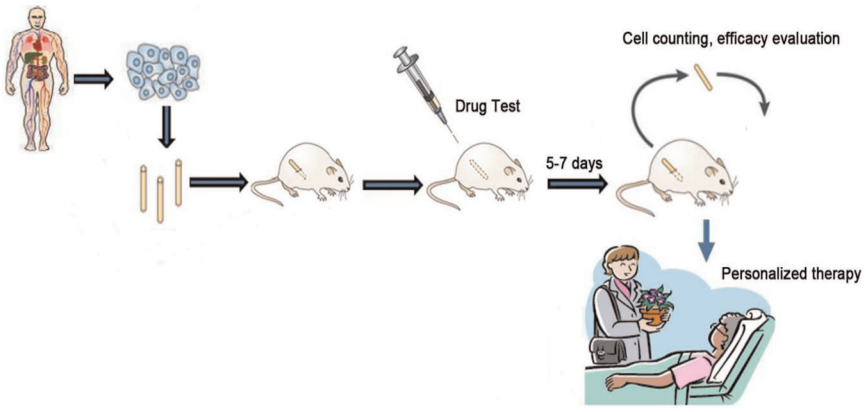

FIGURE 1 An overview of the establishment of MiniPDX assay. Tumor cells enriched from pleural fluid samples from NSCLC patients using microfluidic technology were transferred to the HBSS-washed capsules and then subcutaneously implanted into BALB/c nude mice. Drugs were injected via the tail veins or intraperitoneally. After 6–7 days, the capsules were taken out and the cell viability was evaluated using CellTiter-Glo® Luminescent Cell Viability Assay. Based on the antitumor activity data of the MiniPDX assay, the optimal chemotherapy regimens were selected for different NSCLC patients. HBSS, Hank's balanced salt solution; NSCLC, non-small cell lung cancer

2.5 | CT scans

All patients underwent conventional CT scans of lung and mediastinum by Somatom PLUS-S CT scanner (Siemens Medical Systems) at baseline and during follow-up. CT images were processed using 3D slice software package (Version 4.7). Two chest radiologists with more than 10 years of work experience and an assistant researcher completed the entire process together. They all blinded to the study arm. Radiographic assessments of short-term efficacy were performed every two cycles until disease progression or death during chemotherapy as per RECIST v1.1, and patients were classified into four subgroups: complete remission (CR), partial remission (PR), stable disease (SD), and progressive disease (PD). CR is defined as disappearance of all target lesions. Any pathological lymph nodes must have reduction in short axis of 10 mm. PR is defined as at least a 30% decrease in the sum of diameters. SD is defined as neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study. PD is defined as at least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study. In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. The appearance of one or more new lesions is also considered progression.

2.6 | Outcomes

Patients were followed up for a long-term survival until death. They were followed up every 3 months for the first year, then every 6 months for the following 2 years, and annually thereafter. The follow-up evaluations consisted of history, physical examination, hematology and blood chemistry panels, including serum tumor markers. Progression-free survival (PFS) and OS were measured as the time between treatment initiation and documented disease progression (PFS) or death (OS). of target lesions, taking as reference the baseline sum diameters.

2.7 | Statistical analysis

Plots were constructed using GraphPad Prism 7 (GraphPad Software, Inc.), and the statistical analysis was performed using SPSS 19.0 software (IBM Corp.). Normally distributed data were presented as the mean ± *SD*. The non-normally distributed data are presented as the mean ± interquartile range or as the median. Normally distributed continuous variables were analyzed using unpaired Student's *t* test. For multiple comparisons, the Tukey–Kramer honestly significant difference test was applied following analysis of variance. OS refers to the time from treatment initiation to death. PFS is the time from treatment initiation to disease progression or death. The OS and PFS were analyzed using the Kaplan–Meier method and log-rank test, and the correlation between clinical pathological variables and drug sensitivity were analyzed using the Pearson χ^2 test. $p < .05$ was considered to indicate a statistically significant difference.

3 | RESULTS

3.1 | MiniPDX assay predicts drug sensitivity patterns in NSCLC patients

According to the results of cell viability assay, the average proliferation rates of NSCLC cells treated with docetaxel, gemcitabine, nab-paclitaxel, pemetrexed, carboplatin, docetaxel plus carboplatin (Doc + Carbo), gemcitabine plus carboplatin (Gem + Carbo), pemetrexed plus carboplatin (Pem + Carbo), vinorelbine plus carboplatin (Vin + Carbo), and nab-paclitaxel plus carboplatin (Pa c+ Carbo) were 68.3% ± 30.8%, 69.6% ± 28.3%, 59.1% ± 39.1%, 69.0% ± 36.2%, 64.8% ± 45.9%, 24.0% ± 64.2%, 37.3% ± 73.5%, 40.5% ± 81.6%, 72.0% ± 38.2%, and 40.8% ± 33.4%, respectively (Figure 2). The sensitivity of the same drug varied considerably in mice inoculated with tumors from different patients. For example, mice injected with carboplatin had the highest relative tumor proliferation rate at 189% and the lowest at −42% in, and mice injected with nab-paclitaxel had the highest relative tumor proliferation rate at 123% and the lowest at −61% in, implying the need for individualized therapy.

3.2 | MiniPDX-guided chemotherapy is superior to conventional chemotherapy in clinical efficacy

The cohort included 63 males and 37 females with an average age of 60.95 ± 10.41 years. The MiniPDX-guided chemotherapy group included 31 males (62%) and 19 females (38%) with a median age of 63 years (range: 34–82 years). There were no statistically significant differences in demographic and baseline characteristics between the MiniPDX group and the conventional chemotherapy group (Table 1).

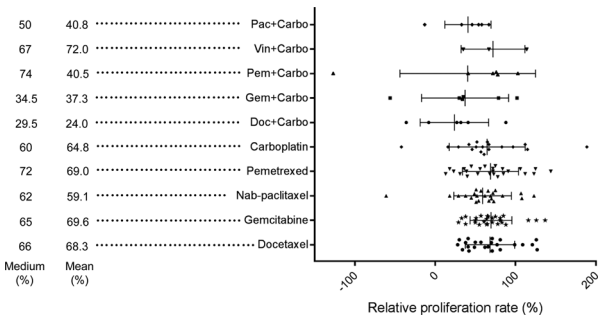

FIGURE 2 MiniPDX responses to chemotherapeutic and targeted regimens. Scatter plot showed the relative proliferation rate of the drugs tested via MiniPDX assay among the 140 NSCLC patients. Growth of MiniPDX in mice treated with docetaxel (23 patients), gemcitabine (24 patients), nab-paclitaxel (23 patients), pemetrexed (25 patients), carboplatin (18 patients), docetaxel + carboplatin (Doc + Carbo, 6 patients), gemcitabine + carboplatin (Gem + Carbo, 6 patients), pemetrexed + carboplatin (Pem+Carbo, 6 patients), vinorelbine + carboplatin (Vin + Carbo, 3 patients), and nab-paclitaxel + carboplatin (Pac+Carbo, 6 patients). NSCLC, non-small cell lung cancer

TABLE 1 Patient demographic and baseline characteristics

Characteristic	All (*n* = 279)	Conventional chemotherapy (*n* = 139)	PDX-guided chemotherapy (*n* = 140)	*p* value
Gender, *n* (%)				
Male	176 (63.08)	89 (64.03)	87 (62.14)	.744
Female	103 (36.92)	50 (35.97)	53 (37.86)	
Age (years) *n* (%)				
≥65	109 (39.07)	58 (41.73)	51 (36.43)	.364
<65	170 (60.93)	81 (58.27)	89 (63.57)	
TNM stage				
IIIB	29 (10.39)	18 (12.95)	11 (7.85)	.163
IV	250 (89.61)	121 (87.05)	129 (92.14)	
Therapy				
First-line therapy	0 (0)	0 (0)	0 (0)	-
Second-line therapy	279 (100)	139 (49.82)	140 (50.18)	
Tumor size, cm, *n* (%)				
≤2	76 (27.24)	37 (26.62)	39 (27.85)	.123
>2, ≤4	109 (39.07)	62 (44.60)	47 (33.57)	
>4	94 (33.69)	40 (28.78)	54 (38.57)	
Clinical diagnosis, n (%)				
SCC	67 (24.01)	28 (20.14)	39 (27.86)	.117
ADC	193 (69.18)	104 (74.82)	89 (63.57)	
Low differentiation NSCLC	19 (6.81)	7 (5.04)	12 (8.57)	
Chemotherapy regimen, *n* (%)				
Docetaxel	50 (17.92)	27 (19.42)	23 (16.43)	.760
Gemcitabine	57 (20.43)	33 (23.74)	24 (17.14)	
Nab-paclitaxel	37 (13.26)	14 (10.07)	23 (16.43)	
Pemetrexed	46 (16.49)	21 (15.11)	25 (17.86)	
Carboplatin	40 (14.34)	22 (15.83)	18 (12.86)	
Docetaxel plus carboplatin	11 (3.94)	5 (3.60)	6 (4.29)	
Gemcitabine plus carboplatin	10 (3.58)	4 (2.88)	6 (4.29)	
Pemetrexed plus carboplatin	10 (3.58)	4 (2.88)	6 (4.29)	
Vinorelbine plus carboplatin	7 (2.51)	4 (2.88)	3 (2.14)	
Nab-paclitaxel plus carboplatin	11 (3.94)	5 (3.60)	6 (4.29)	
History of smoking, *n* (%)				
Never	188 (67.38)	87 (62.59)	101 (72.14)	.089
Smoker	91 (32.62)	52 (37.41)	39 (27.86)	

Note: Tumor stage was defined according to the American Joint Committee on Cancer (AJCC) TNM staging system (AJCC 7th edition).
Abbreviations: ADC, adenocarcinoma; NSCLC, non-small cell lung cancer; PDX, patient-derived xenograft; SCC, squamous cell carcinoma.

TABLE 2 Evaluation of clinical efficacy in the MiniPDX-guided chemotherapy and conventional chemotherapy

Efficacy assessment	Conventional chemotherapy (n = 50)	Mini-PDX-guided chemotherapy (n = 50)	*p* value
CR	0 (0)	0 (0)	–
PR	6 (12%)	17 (34%)	.009
SD	29 (58%)	28 (56%)	.840
PD	15 (30%)	5 (10%)	.012
CBR	35 (70%)	45 (90%)	.012

Abbreviations: CBR, clinical benefit rate; CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.

As shown in Table 2, patients in MiniPDX-guided chemotherapy group had higher clinical benefit rate (CBR) and partial response (PR) and lower PD than those in conventional chemotherapy group (90% vs. 70% for CBR, 34% vs. 12% for PR, and 10% vs. 30% for PD; $p = .012$, .009 and .012, respectively). In addition, patients with MiniPDX-guided chemotherapy (18.30 months; 95% confidence interval [CI] = 14.55–22.05 months) had significantly longer median OS than those in the conventional chemotherapy group (14.27 months; 95% CI = 12.79–15.75 months) (Figure 3a). The median PFS of patients in the MiniPDX-guided chemotherapy group (18.06 months; 95% CI: 13.17–22.94 months) was also significantly longer compared with the conventional chemotherapy group (13.37 months; 95% CI :11.83–14.91 months) (Figure 3b). The final survival rate was 38% in the MiniPDX group and 16% in the conventional chemotherapy group.

3.3 | CT showed improvement in nodal and tumor size in patients from MiniPDX group

We selected several representative CT images from patients receiving individualized MiniPDX-guided chemotherapy and the conventional chemotherapy. After two cycle of trial chemotherapy regimen, tumors of patients who receiving individualized MiniPDX-guided chemotherapy shrank, from 18 to 11 mm in the first tumor and from 23 to 19 mm in the second tumor, and the other tumors also shrank significantly (Figure 4a). The tumor changes in patients receiving conventional chemotherapy were not as significant as in the MiniPDX group. (Figure 4b).

3.4 | Association analysis between clinicopathologic characteristics and chemosensitivity

Table 3 provides an overview of correlation between clinicopathologic characteristics and chemosensitivity. Gemcitabine sensitivity was associated with nerve invasion, and vinorelbine sensitivity was related with lymph node metastasis, while carboplatin efficacy was related to tumor size and TNM stage.

4 | DISCUSSION

In this study, tumor cells were enriched from pleural fluids samples of 50 NSCLC patients by using microfluidic technology, followed by the establishment of a MiniPDX assay and the formulation of

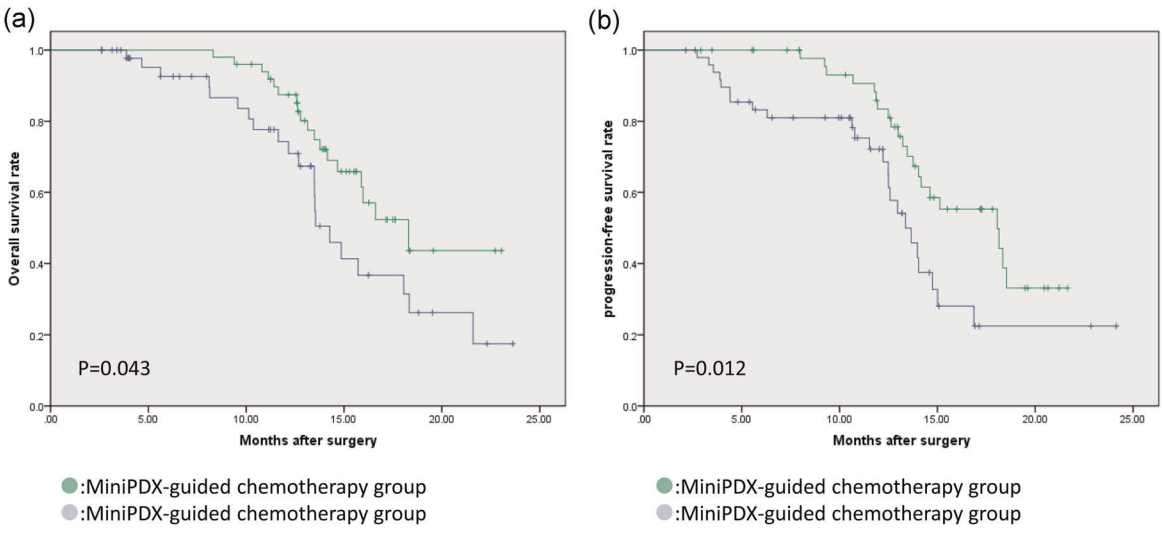

FIGURE 3 Comparison of the prognosis of NSCLC patients between conventional chemotherapy group and MiniPDX-guided chemotherapy group. The NSCLC patients who received agents based on the MiniPDX results had higher (a) overall survival and (b) progression-free survival rates than those in conventional chemotherapy group. NSCLC, non-small cell lung cancer

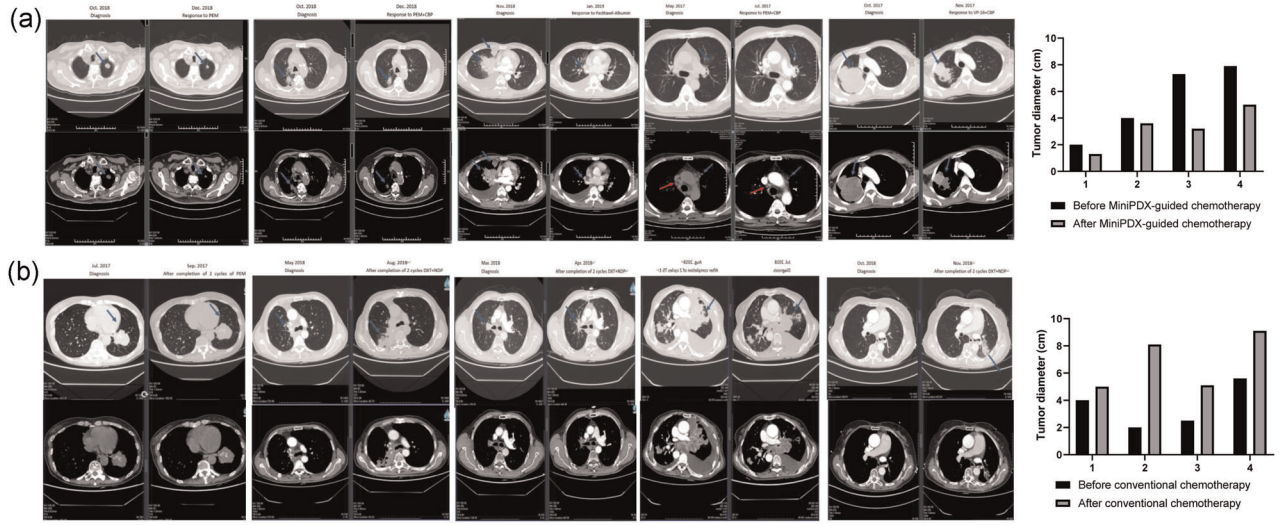

FIGURE 4 Chest CT scans before and after chemotherapy. (a) Tumors before and after MiniPDX-guided chemotherapy; (b) tumors before and after the conventional chemotherapy; (c) change in tumor diameter before and after chemotherapy. The upper and lower images showed the nodules in the lung and mediastinal windows, respectively. The blue arrow indicated tumor in lung, and the red arrow indicated lymph node.CT, computed tomography

individualized chemotherapy regimens based on drug sensitivity test results. The results confirmed that MiniPDX-guided chemotherapy was more beneficial to NSCLC patients than conventional chemotherapy, which might have some implications for oncologists making informed decisions about individualized chemotherapy.

In recent years, microfluidic technology is on the rise. This noninvasive detection technology has been widely used in early cancer screening, tumor marker detection, personalized diagnosis and treatment, and the study of tumor metastasis mechanism (Rana et al., 2018). To our knowledge, microfluidic technology enables precise processing of Micro-Quantity Liquid in micro or nanoscale low-dimensional channel structures, saving pleural fluids samples and assembling the samples on one chip for pretreatment, transportation, mixing, reaction, separation, collection and detection, with the advantages of simple operation, fast sorting speed, high specificity and high purity (Pei et al., 2020). As the main development direction of single cell research, tumor cell detection and analysis have gradually brought microfluidic technology into one of the important research methods. In the current research, we used microfluidic technology to enrich high-purity tumor cells from pleural fluid samples for subsequent studies.

Accurate chemotherapeutic drug sensitivity testing is the prerequisite for the development of individualized treatment regimes, and most transformed cancer research requires effective preclinical assays (Hu et al., 2017; Zhou et al., 2016), including NSCLC (Xingsheng et al., 2016). The lack of preclinical assays that can reliably predict the efficacy of novel compounds on cancer patients has hampered the development of new cancer drugs. The preclinical PDX assay breaks through the limitations of conventional cell line-based assays, which have been proved to predict clinical outcomes and are used for preclinical drug assessment, biomarker recognition, biological research, and personalized drug strategy (Bissig-Choisat et al., 2016; Chapuy et al., 2016; Nicolle et al., 2016). However, PDX assay has a low success rate and requires a large amount of tumor tissues and 4–8 months to determine an effective therapy for specific cancer patient, which limit its clinical application in more aggressive cancers, such as NSCLC (Byrne et al., 2017; Hidalgo et al., 2014). The MiniPDX is a platform with reduced complexity and faster result turn-around that overcomes some of the limitations of PDX analysis. Although MiniPDX still consists of shortcomings due to the use of animal hosts and possible interactions with mouse biology, it is overall superior to PDX. Therefore, MiniPDX assay was selected in this study to enable NSCLC patients to receive treatment within a clinically relevant time frame.

As a heterogeneous cell population with varying degrees of differentiation, tumor has obvious individual differences in response to various chemotherapeutic drugs (Lorz et al., 2015). Thus, it is necessary to select different chemotherapeutic drugs for different patients and adopt individualized treatment plans. It has been the focus of researchers to explore a better second-line chemotherapy regimen, which combine two single drugs to synergistically catalyze different mechanisms of action to kill tumor cells more effectively and improve drug efficiency and patient survival (Durm & Hanna, 2017). For the time being, the combination of two chemotherapy drugs as second-line therapy in advanced NSCLC patients in clinic is relatively common in China, mainly for patients with chemotherapy tolerance. In this study, the MiniPDX assay examined the inhibitory effects of monotherapy (docetaxel, gemcitabine, nab-paclitaxel, pemetrexed, and carboplatin) and combination therapy (vinorelbine, docetaxel, gemcitabine, nab-paclitaxel, and pemetrexed combined with carboplatin, respectively) on NSCLC. The results of this study showed that CBR and PR were higher and PD was lower in the MiniPDX group than in the conventional chemotherapy group, and median OS and median PFS were also significantly longer than in

TABLE 3 Correlation between clinicopathological variables and chemosensitivity

Proliferation rate (%)	Vinorelbine	*p*	Docetaxel	*p*	Gemcitabine	*p*	Nab-paclitaxel	*p*	Pemetrexed	*p*	Carboplatin	*p*
Tumor size, cm												
< 4	86.5 ± 31.6	.668	72.0 ± 20.5	.769	52.3 ± 18.5	.335	68.3 ± 20.4	.284	83.8 ± 33.3	.617	65.2 ± 26.4	.037
≥ 4	35.7 ± 89.7	56.8 ± 26.8	36.8 ± 58.6	20.3 ± 88.2	68.5 ± 44.4	20.4 ± 57.6						
Tumor differentiation												
Well and moderate	36.7 ± 78.6	.215	53.5 ± 24.8	.133	41.2 ± 68.6	.701	45.7 ± 66.2	.271	64.8 ± 26.3	.257	52.1 ± 66.8	.431
Poor	57.4 ± 48.1	76.8 ± 38.6	47.8 ± 63.7	76.3 ± 64.1	69.5 ± 31.2	60.3 ± 67.2						
Nerve invasion												
No	86.2 ± 21.5	.631	79.5 ± 23.8	.627	9.2 ± 64.4	.428	73.1 ± 85.4	.315	67.9 ± 34.1	.381	42.0 ± 43.8	.681
Yes	67.2 ± 34.2	64.7 ± 40.4	79.6 ± 23.5	66.3 ± 24.6	35.4 ± 24.6	46.7 ± 41.3						
Lymph node metastasis												
No	21.4 ± 36.3	.026*	53.5 ± 37.3	.227	39.6 ± 74.8	.421*	23.7 ± 62.4	.137	67.1 ± 31.2	.151	22.3 ± 65.4	.209
Yes	61.2 ± 35.3	52.7 ± 41.2	71.1 ± 31.8	62.2 ± 22.3	81.5 ± 62.4	81.3 ± 53.2						
TNM stage												
I–IIIA	32.8 ± 43.5	.602	62.5 ± 32.7	.581	23.5 ± 62.7	.317	19.4 ± 65.3	.158	61.2 ± 33.7	.213	11.2 ± 41.7	.010*
IIIB–IV	72.7 ± 55.1	78.3 ± 28.6	58.4 ± 39.7	84.7 ± 56.2	71.4 ± 16.2	57.6 ± 22.8						

Note: Tumor stage was defined according to the American Joint Committee on Cancer (AJCC) TNM staging system (AJCC 7th edition).

Abbreviation: NSCLC, non-small cell lung cancer.

*$p < .05$.

the conventional chemotherapy group. It indicated the MiniPDX-guided chemotherapy regimen selected the most effective combination of drugs to treat NSCLC patients and could effectively improve patient outcomes.

However, the present study maintains limitations. Although our experiment had six replicates (two mice and three replicates each), the intergroup error is unavoidable. Therefore, it could potentially have an impact on the experimental results.

5 | CONCLUSION

This study provides the first preclinical and clinical evidence for the utility of an optimized MiniPDX assay in guiding adjuvant chemotherapy in NSCLC patients. Tumor cells from patients maintain tumorigenicity, and the drug-susceptibility pattern of MiniPDX can summarize the response of patients from their source, which allows MiniPDX assay to better improve the efficacy of chemotherapy regimens in NSCLC patients. In summary, MiniPDX assay provides an important reference for oncologists to develop individualized chemotherapy regimens to maximize efficacy and minimize side effects. Additionally, the potential mechanism of chemoresistance requires larger sample size studies to further demonstrate the relationship between clinicopathological features, biomarkers, and drug efficacy.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

DATA AVAILABILITY STATEMENT

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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