Organoids from Patient Biopsy Samples can Predict the Response of BC Patients to Neoadjuvant Chemotherapy

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Research Article

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Abstract

Background

Neoadjuvant chemotherapy (NAC) has been widely used to treat locally advanced and inflammatory breast cancer. The initial purpose of neoadjuvant chemotherapy in operable breast cancer is to reduce the tumor stage, achieve local control, and increase the conservative operation rate. Another significant advantage of using neoadjuvant chemotherapy is the early identification of unresponsive tumors, predicting the sensitivity of new drugs and promoting the development of new medicines and clinical trials. It is generally believed that complete pathological response (pCR) after neoadjuvant chemotherapy treatment predicts survival. The overall survival rate of patients who achieve pCR is much higher than that of patients who do not. Therefore, it is essential to predict the response of patients to neoadjuvant chemotherapy. There is increasing evidence that patient-derived organoids (PDOs) can be used in cancer research and drug development and for the personalized in vitro evaluation of drug responses.

Method

We generated a living organoid biobank from locally advanced breast cancer patients receiving neoadjuvant chemotherapy. These patients had three clinically common molecular types of breast cancer (luminal-type, triple-negative and HER2+ breast cancer). When the patient received clinical neoadjuvant chemotherapy, the organoids were treated with similar drugs, thereby simulating the situation of the patient receiving treatment

Result

We successfully constructed organoids from breast cancer biopsies to simulate clinical treatment, demonstrating that viable tumoroid lines can be generated from a small sample of tissue. The phenotype of breast cancer organoid often agreed with the original breast cancer according to the blinded histopathological analysis of H&E stain tissue and organoid sections. Our clinical trial data confirm that the patient's response to chemotherapy closely matches the organoids' response to drugs. The patient-derived organoids may identify breast cancer patients who respond to neoadjuvant chemotherapy and thereby improve initial care.

Conclusion

Our data indicate that patient-derived organoids can be used to predict the clinical response of breast cancer patients to neoadjuvant chemotherapy in vitro and to screen drugs that have different effects on different patients.

Introduction

Breast cancer (BC) is the most commonly diagnosed cancer globally and has the highest death rate among cancers affecting females.[1, 2] BC can be divided into three subtypes based on the hormone

receptor status and the expression of human epidermal growth factor receptor 2 (HER2) and proliferation marker Ki67 as follows: hormone receptor-positive luminal BC, primarily treated with endocrine therapy; HER2-positive BC, mainly treated with adjuvant chemotherapy combined with anti-HER2 antibodies such as trastuzumab and patolizumab; and estrogen receptor(ER), progesterone receptor[PR], and human epidermal growth factor receptor 2 (HER2) expression-negative (triple-negative; TNBC) BC, mainly treated with adjuvant chemotherapy[3–6]. TNBC does not respond well to targeted therapy and endocrine therapy.[7] Surgery and chemotherapy are the main treatments for most TNBC patients.

Compared with traditional postoperative adjuvant therapy, systemic adjuvant therapy before surgery, namely, neoadjuvant therapy, is a new treatment model. The initial purpose of neoadjuvant chemotherapy (NAC) for operable BC is to reduce the tumor stage, achieve local control and increase the conservative operation rate. Another significant advantage of using NAC is the early identification of unresponsive tumors, predicting the sensitivity of new drugs in the body and promoting the development of new medicines and clinical trials[8]. According to reports, after NAC, the overall survival rate of BC patients who achieve a complete pathological response (pCR) is much higher than that of patients with residual disease. Whether patients achieve pCR after neoadjuvant chemotherapy is closely related to the survival rate[9]; however, the drug resistance mechanism of BC patients with residual disease after neoadjuvant chemotherapy is not fully understood. In recent decades, the survival rate of BC patients has increased significantly. In addition to early diagnosis, surgical treatment, radiotherapy, and chemotherapy, individualized and precise comprehensive treatment has led to high cure and survival rates[10]. Despite the differences between different species and differences between in vivo and in vitro environments, research into the biological behavior of tumors remains at the level of traditional tumor cell lines and animal models, which complicates the application of high-throughput genomics in clinical practice and research.

Over the past few decades, our understanding of the origin of cancer has dramatically increased. Although significant progress has been made in treating certain types, cancer remains a significant health problem worldwide.[11] Screening examinations and early detection methods can reduce the number of deaths from cancer. In addition, new and more targeted individualized therapies provide patients with more effective treatment opportunities. However, one of the main obstacles to developing new treatment options is the challenge of translating scientific knowledge from experimental platforms into clinical practice. This complication mainly arises to the failure of many cancer models to recapitulate patient tumors[12]; therefore, many drugs that perform well in cancer models eventually fail in clinical trials [13]. Although animal-derived cancer models provide many opportunities for basic cancer research, developing these models is time-consuming. Additionally, studies have shown that these models usually do not faithfully reflect the course of the disease in humans.[14] For example, the complexity and genetic heterogeneity of human cancer histology are generally not reflected in genetically modified mouse models.[15]

Recently developed 3D cell culture technology has generated more novel and physiologically meaningful human healthy tissue and cancer models. Tissue-derived human stem cells can be used to efficiently

grow tissue-derived organ-type structures, referred to as organoids, in a 3D culture matrix. In 2009, Sato et al. [13]demonstrated that 3D epithelial organoids can be established from a single G protein-coupled receptor 5+ (LGR5) intestinal stem cell rich in leucine repeats[16–18]. Using this culture method, a variety of organoids were cultivated, including colon[19, 20],liver, pancreas[21],prostate[22, 23],stomach[24], fallopian tube[25], taste bud[17], salivary gland[26], esophagus[27],lung[28], and endometrium [29]and breast organoids.[30] Studies have shown that organoids can be cultured for a long time, undergo freezing and genetic modification, and remain genetically and phenotypically stable. This characteristic of organoids allows them to be widely applied in cancer research[31].

To date, many organoids have been developed from patient-derived tumors and matched healthy tissues, and an organoid library has been constructed. These organoids can be used to predict whether individual patients will respond to a drug.[30, 32, 33]

Methods And Materials

Experimental design

The biopsies obtained from patients hospitalized at the First Affiliated Hospital of Chongqing Medical University who met the criteria of neoadjuvant chemotherapy after ultrasound or mammography and pathological examinations were used to establish a biobank of PDOs.

The patient’s neoadjuvant chemotherapy regimen is shown in Table 1.

Patient	Sex	Age (years)	Histopathology	Molecular typing	ER	PR	HER-2	Ki-67	Neoadjuvant treatment	Clinical response
BCP20	female	63	Invasive carcinoma Non-special type	TNBC	-	-	2+	40%、+	TAC	pCR
BCP21	female	51	Invasive carcinoma Non-special type	Her2	50%、+	10%、+	3+	20%、+	TCbH	non-pCR
BCP22	female	74	Invasive lobular carcinoma	her2	-	-	3+	20%、+	TCb-HP	pCR
BCP27	female	45	Invasive carcinoma Non-special type	TNBC	10%、weak	-	1+	30%、+	TAC	non-pCR
BCP30	female	37	Invasive carcinoma Non-special type	Luminal B	60%、+	2%、+、weak	-	40%、+	TAC	non-pCR

The histopathology and morphology of PDOs were characterized in detail and compared to their parental tumors, demonstrating high similarities between the two. It mainly reaches whether PDO, as a “carcinoid model,” is consistent with the patient’s clinical response in terms of chemotherapy response or how much consistency is it? The researchers were blinded to the patients’ responses to avoid biases.

Establishment and culture of PDOs from BC samples

BC biopsy samples were obtained with informed consent from patients suspected of having BC who required neoadjuvant treatment from Jun 2020 to December 2020. A total of 17 biopsy samples were

collected (Figure 1A). Given that most patients with triple-negative breast cancer or HER2-positive breast cancer and patients with axillary lymph node metastasis usually receive neoadjuvant treatment before surgical resection, the PDO biobank is a valuable resource. It is worth noting that at the time of writing this manuscript, 15 patients had undergone surgery, and tumor pathology assessments were available. One patient was lost to follow-up. One patient was treated with an NP (gemcitabine combined with cisplatin) regimen due to bone and liver metastasis and did not receive surgery.

The collected biopsy specimens were placed in a 15 ml centrifuge tube containing 10 ml AdDF++ (Thermo Fisher Scientific), transported to the laboratory in an icebox for tumor cell isolation and culture, aspirated in a 15 ml centrifuge tube, transferred to an empty dish, and weighed (x). The sample was then transferred into a small dish containing 10 ml AdDF++, and the weight of the empty dish (y) was used to determine the weight of the tumor ($x-y=$ ___).

The tumor was cut into small pieces with a diameter of 1-3 mm. The tumor block was divided into three parts. Two parts were placed into liquid nitrogen immediately after harvest for subsequent whole-exome sequencing and transcriptome sequencing. The other parts were used for tumor organoid construction. A Pasteur pipette was used to transfer the small piece of tissue into a 15 ml centrifuge tube containing 10 ml AdDF++; the tube was then centrifuged (300 g, 5 min) and the fragments were digested in digestion medium (advanced DMEM/F12 with 1 M Hepes (Invitrogen) and 1X GlutaMax (Invitrogen), Pen/Strep (Invitrogen), 1 mg/ml collagenase (Sigma) and 5 mM RHOK inhibitor ly27632 (Sigma-Aldrich, Y0503) at 37°C with shaking for 60 or 90 minutes. Samples derived from resected tumors were filtered through a 70 µm cell strainer and centrifuged at 300 g for 5 min. Dissociated cells were collected in Advanced DMEM/F12 (Gibco), pelleted (1,200 rpm, 5 minutes), resuspended in 40 µl Cultrex Reduced Growth Factor Basement Membrane Extract (BME), Type 2 (R&D Systems), and seeded into Matrigel in a well of a pre-warmed 24-well flat-bottom cell culture plate (Corning). Then, the cells were incubated for 20 minutes in a 37°C and 5% CO₂ cell incubator to solidify the Matrigel and were subsequently overlaid with 400 µl of complete human organ culture medium. The complete medium was refreshed every four days. PDOs were observed and photographed as required.

Organoids were typically passaged every 1–2 weeks. Passaging of PDOs was performed using TrypLe (Gibco). The PDO supernatant was aspirated, PBS was added to wash the PDO, and 1x TrypLe (including 1x Y27) was used to mechanically harvest the PDO from the Matrigel (pipetting) followed by incubation at 37°C for 5–10 minutes. Then, the PDO was dissociated into smaller cell clusters by applying mechanical force (pipetting), precipitated (300 g, 5 minutes) with AdF+++10% FBS and resuspended in BME Matrigel according to the cell growth state to adjust the passaging ratio.

Human PDO culture media

Human PDOs were cultured in Advanced DMEM/F12 medium containing HEPES, penicillin/streptomycin, Glutamax, B27, R-spondin 1, neuregulin 1, FGF7, FGF10, Noggin, n-acetylcysteine, nicotinamide, primocin, Y-27632, a selective inhibitor of TGF-βRI, ALK4, ALK7, p38 inhibitor, and EGF. Detailed information regarding the cell culture medium is shown in table2.

Reagent name	Company	Cat No.	Stock solution	Solvent	Final concentration
Advanced DMEM/F12	Gbico	10565018.00	-	-	1x
Penicillin/Streptomycin	themo	15140122	100X	-	100 U·ml ⁻¹ / 100 mg·ml ⁻¹
Hepes	Sigma	H3784-500G	-	ddH2O	10 mM
GlutaMax 100x	Invitrogen	12634034(35050061)	100X	-	1x
B27 supplement	Gibco	17504044	50X	-	1x
R-Spondin 1	Peprtech	120-38-100	250 ng·ul ⁻¹	PBS	250 ng·ml ⁻¹
Neuregulin 1, recombinant human Heregulin beta 1	Peprtech	100-03-50	5 uM	PBS	5 nM
FGF 7, recombinant human KGF	Peprtech	100-19-50	5 ng·ul ⁻¹	PBS	5 ng·ml ⁻¹
FGF 10	Peprtech	100-26-50	20 ng·ul ⁻¹	PBS	20 ng·ml ⁻¹
Noggin	Peprtech	120-10C-50	100 ng·ul ⁻¹	PBS	100 ng·ml ⁻¹
N-Acetylcysteine	Sigma	A9165-5g	1.25 M	PBS	1.25 mM
Nicotinamide	Sigma	N0636	5 M	PBS	5 mM
Primocin	Invivogen	ant-pm-1	50 ng·ul ⁻¹	PBS	50 mg·ml ⁻¹
Y-27632	stemcell	72304	5 M	PBS	5 mM
A83-01	Stemcell	72022	5 M	DMSO	500 nM
SB202190	Sigma	S7067	5 M	DMSO	500 nM
EGF,human epidermal growth factor	Peprtech	AF-100-15-100	5 ng·ul ⁻¹	PBS	5 ng·ml ⁻¹

3D PDO drug assays

PDOs were harvested according to the above passage procedure and digested to an appropriate size as opposed to dissociating into a single-cell suspension. The cell pellet was resuspended in 500 µl of advanced DMED/F12, the cells were counted with a Countess automatic cell counter (Thermo Fisher Scientific), and appropriate cell dilutions were made in BME Matrigel. Twenty microliters of 50% BME Matrigel (diluted with organoid complete culture medium) containing 3,500–5,000 cells was seeded in standard 96-well cell culture plates (Corning), and the plates were incubated for 20 min in a 37 °C and 5% CO₂ cell culture incubator to solidify the Matrigel. Then, the Matrigel was covered with 50 µl of complete human organoid medium. The complete media was replaced once after two days.

Two days after inoculation, the medium was removed and replaced with 50 µl of whole human organoid medium containing the drug. A total of 3 drugs were used in this experiment, docetaxel, carboplatin, and epirubicin. The concentration gradient of the different drugs was set based on pre-experimental results from breast cancer cell lines. Both HER2-positive and HER2-negative BC patients receive preoperative

adjuvant treatment with docetaxel. In the organoid drug sensitivity test, the concentration gradient of docetaxel as a single agent was 0 μ M, 0.001 μ M, 0.01 μ M, 0.1 μ M, 1 μ M, and 10 μ M. The concentration of docetaxel used when the drug was combined with carboplatin or anthracycline was 0.1 μ M. The concentration gradient of carboplatin used for HER2-positive organoids was as follows: 0 μ M, 0.001 μ M, 0.01 μ M, 0.1 μ M, 1 μ M, 2 μ M, 4 μ M, 8 μ M, 16 μ M, and 32 μ M. The concentration gradient of epirubicin used for HER2-negative organoids was: 0 μ M, 0.001 μ M, 0.01 μ M, 0.1 μ M, 1 μ M, 2 μ M, 4 μ M, 8 μ M, 16 μ M, and 32 μ M. Based on the growth rate of various organoid cultures, the drug-containing medium was changed every three days to provide the cytokines/additives required for organoid growth. At the end of the treatment (after six days), the medium was removed and replaced with 100 μ l of complete human organoid medium containing 50% CellTiter-Glo®3D Cell Viability Assay (Promega). The contents were mixed vigorously for 5 minutes to induce cell lysis and then incubated at room temperature for an additional 25 minutes to stabilize the luminescent signal. Luminescence was recorded using a microplate reader for chemiluminescence. The drugs used in the in vitro experiments and their concentrations are shown in table3.

Treatment programs	Composition	concentration in vitro	Dose in vivo
TCb-HP	Docetaxel	0、0.001、0.01、0.1、1、10uM	75mg/m2
	Carboplatin	0、0.001、0.01、0.1、1、2、4、8、16、32uM	AUC6
	Trastuzumab	-	The first dose is 8mg/kg, then 6mg/kg
	Pertuzumab	-	The first dose is 840mg, then 460mg
THP	Docetaxel	0、0.001、0.01、0.1、1、10uM	75mg/m2
	Trastuzumab	-	The first dose is 8mg/kg, then 6mg/kg
	Pertuzumab	-	The first dose is 840mg, then 460mg
TCbH	Docetaxel	0、0.001、0.01、0.1、1、10uM	75mg/m2
	Carboplatin	0、0.001、0.01、0.1、1、2、4、8、16、32uM	AUC6
	Trastuzumab	-	The first dose is 8mg/kg, then 6mg/kg
TAC	Docetaxel	0、0.001、0.01、0.1、1、10uM	75mg/m2
	Anthracyclines	0、0.001、0.01、0.1、1、2、4、8、16、32uM	50-100mg/m2
	Cyclophosphamide	-	600mg/m2

Pathological evaluation system after NAC

Currently, commonly used pathological assessment systems include the Miller–Payne (MP) system, the RCB system, the Sataloff system, and AJCC ypTNM staging. The Pathology Department of the First Affiliated Hospital of Chongqing Medical University mainly uses MP grading to evaluate NAC treatment effects

(MP grade) Evaluation of surgical specimens. The definition of tumor grade according to the MP system is listed in table 4:[34]

Miller and Payne	
Grade 1	No change or some alteration to individual malignant cells but no reduction in overall cellularity
Grade 2	A minor loss of tumor cells but overall cellularity still high; up to 30% loss.
Grade 3	Between an estimated 30% and 90% reduction in tumor cells.
Grade 4	A marked disappearance of tumor cells such that only small clusters or widely dispersed individual cells remain; more than 90% loss of tumor cells.
Grade 5	No malignant cells identifiable in sections from the site of the tumor; only vascular fibroelastotic stroma remains often containing macrophages. However, ductal carcinoma in situ (DCIS) may be present.

RECIST 1.1

We evaluated the efficacy of adjuvant chemotherapy based on the postoperative pathological examination of the patient and simultaneously assessed the change in tumor burden in conjunction with the RECIST 1.1 system shown in table 5.

RECIST 1.1		
Grade	Full name	Description
CR	Complete Response	Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10mm
PR	Partial Response	At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.
PD	Progressive Disease	At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study) . In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression) .
SD	Stable Disease	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

Complete pathological response (pCR)

Complete pathological response (pCR) can be used as a surrogate marker to predict the prognosis of BC patients after NAC. The FDA (Food and Drug Administration) recommends yPT0/Tis yPN0 (i.e., absence of invasive tumor cells in the breast and the nodes) as the definition of pCR. The definition of pCR is based on the absence of invasive tumors at the primary site, even if ductal carcinoma in situ is present. [35]

PDO/tissue histology, immunohistochemistry

To maintain the 3D structure of PDOs and dissolve the Matrigel, we incubated PDOs with 1 ml of Cell Recovery Solution (Corning) at 4°C for 30 minutes. Then, the PDOs were collected in cold PBS, precipitated (300 g, 4 minutes, 4°C), and fixed in 4% paraformaldehyde (Sigma-Aldrich) overnight. After fixation, the PDOs were washed with PBS, precipitated (300 g, 4 minutes, 4°C), then resuspended in 40 µl

histogel (Thermo scientific). The histogel containing the PDO precipitate was transferred to a cylindrical grinding tool, and after the tissue had solidified, 100 µl 1% agarose was added to coat the histogel. The agarose pellet was dehydrated with ethanol (EtOH) and embedded in paraffin using standard histological protocols. These deparaffinized organoid sections were then analyzed using HE and immunohistochemistry staining.

Before HE and immunohistochemistry staining, 4-mm thick sections were dewaxed in xylene, rehydrated in decreasing ethanol concentrations, and washed with water. For histological analysis, the sections were stained with hematoxylin and eosin. Immunohistochemistry was performed to measure Ki-67, ER, PR, and Erb-B2 expression. Cells were then incubated with EDTA (sodium citrate) antigen retrieval solution (Solabio C1032). Endogenous peroxidase blockers were used for blocking, and the cells were incubated in a humid chamber at room temperature for 10–15 min (generally 15 min). Then, the sections were incubated with primary antibodies diluted with antibody diluent (ZSGB-BIO ZLI-9028) overnight at 4C. The primary antibodies and their concentrations used for immunohistochemistry are listed in table 6.

Target	Company	Cat NO.	Source	Dilution
ERα(Estroge Receptors)	Santa Cruz Biotechnology	sc-71064	Mouse	1:50
PR(Progesterone Receptor)	Proteintech	66300-1-Ig	Mouse	1:1000
HER-2/c-erb-B2	Cell Signaling	4290	Rabbit	1:400
Ki-67	Proteintech	27309-1-AP	Rabbit	1:8000

After rinsing with PBS, the sections were incubated with secondary antibodies (ZSGB-BIO SP-9002, SP-9001) for 30 minutes at room temperature. Then, sections were stained with hematoxylin. Finally, the areas were dehydrated and made transparent in increasing concentrations of ethanol and xylene and covered with 60% neutral gum (diluted with xylene). HE and immunohistochemistry images were obtained with an upright microscope.

Statistics

Quantitative variables are presented as the mean ± SD. Data analyses were conducted using GraphPad Prism 8.4.0.

Result

Result 1. Establishing BC organoids from biopsy samples.

With the patient’s informed consent, we obtained hollow needle biopsy samples from patients expected to receive neoadjuvant chemotherapy, removed the normal tissue, and separated the breast cancer cells through physical and mechanical disruption and chemical and enzymatic digestion. The isolated cells were seeded into BME droplets in pre-treated 24-well plates and covered with 400 µl complete breast cancer organoid medium (Figure 1A).

From June 2020 to December 2020, a total of 17 BC biopsy specimens were collected. The patients included those who did not require modified radical mastectomy and NAC due to benign pathological findings, those who needed modified radical mastectomy but not NAC, and those who were needed to receive NAC. Approximately 1-2 cm of tissue is taken for biopsy depending on the size of the tumor (Figure 1B); according to the clinical guidelines of the Chinese Society of Clinical Oncology, breast cancer patients who meet one of the following five conditions can choose preoperative neoadjuvant drug treatment: large mass (>5 cm); axillary lymph node metastasis; HER-2 positive tumor; triple-negative tumor and tumor too large to preserve breast in a patient with a desire to undergo breast-preserving surgery.

Among the 17 patients whose biopsy tissues were collected, one patient had a benign tumor that did not require modified radical mastectomy. Five patients did not require NAC before surgery. Eleven patients required NAC, and five organoids were successfully constructed from their tissue biopsies (Figure 1C). According to the BC subtype analysis, among the five successfully created BC organoids, two originated from TNBC, two from HER2+ BC, and one from hormone receptor-positive BC (Figure 1D). Hence, these five organoid lines were used for the clinical validation study.

Result 2. Long-term passage of organoids in vitro and maintenance of tumor heterogeneity.

Some organoids lose their viability during culture and passage; therefore, we consider organoids that can be successively passaged for more than three generations but not more than twenty generations as successful construction. In the early passages, the organoids mainly existed as single cells or small cell clusters, and cell clusters were more commonly observed in thin-walled cysts; in the late passages, the humanoid cell clusters were larger and more compact (Figure 2A). To explore whether the constructed organoids could be passaged stably for a long time, we observed the growth morphology of the organoids and detected the survival rate every two days using the CellTiter-Glo 3D cell viability assay. As the culture time increased, organoid growth persisted (Figure 2A-B). However, compared with cell lines, PDOs grow more slowly in the early stages.

Consistent with the heterogeneity of patient tumors, we observed that the morphology of individual BCOs was different, although normal organoids always demonstrated a cystic phenotype. The organoids derived from BCP21 manifested as solid organoids, while those derived from BCP22 were cystic, and those from BCB27 were “grape-shaped” (Figure 2C).

Next, we evaluated the response of different patients to NAC. Patients who received NAC received modified radical mastectomy after chemotherapy and color ultrasound evaluation. Surgical specimens were collected after chemotherapy, and the pathology department of the First Affiliated Hospital of Chongqing Medical University assessed whether the patient had achieved complete pathological response (pCR), which was defined as a primary tumor with no invasive cancer. The regional lymph nodes were negative (ypT0/Tis ypN0).

The surgical tissue evaluation revealed that, among the patients from whom the five successfully constructed organoids were derived, two patients achieved pCR after NAC. The bright-field images of the biopsy organoids and HE stain showed that the two specimens that responded well to chemotherapy showed “grape-like” cell clusters. HE stain of the postoperative tissues showed the tumors of patients who achieved pCR did not cross the myoepithelial cell layer. Cells and basement membranes entered the surrounding interstitial tissue. However, there was no infiltration of cancer cells into the adjacent tissues, or only a few cancer cells were seen (Figure 2D).

Overall, these results indicate that organoids from BC biopsy tissue can be passaged and survive for a long time in vitro; therefore, biopsy-derived organoids could be used to predict patient responses to NAC.

Result 3. Histological characterization of BC organoids

Most BCOs were determined to be malignant due to cell and nuclear atypia, such as typical cancerous features, enlarged nuclei and polymorphisms, high mitotic activity, apoptosis, and vacuole formation (Figure 3A-B).

In addition to the histological features visible by HE stain, representative BCOs should retain the expression of the most critical and common breast cancer biomarkers, such as estrogen receptor (ER), progesterone receptor (PR), antigen Ki-67, and human epidermal growth factor receptor (HER2). The ER and PR status could predict the outcome of hormone/endocrine therapy, while the level of HER2 could predict the outcome of targeted therapy [36, 37]. Our immunohistochemistry results showed that hormone receptor (HR) and HER2 status remained unchanged in most breast cancer tissues and PDOs. In this study, hormone receptor (HR)-negative tumors produced HR-negative organoids, and HR-positive tumors produced HR-positive tumor organoids (Figure 3A). HER2 status was preserved in BC tissues and organoids (Figure 3B). Due to the small sample size, 100% of breast cancer biomarkers in the tissue were retained.

In summary, we found that most BCOs matched the original tumor in terms of histopathology, hormone receptor levels, and HER2 receptor status.

Result 4. Response of human epidermal growth factor receptor-2 (HER-2)-positive PDOs to docetaxel and carboplatin.

Five patients from whom biopsy tissues were collected received NAC according to the BC clinical guidelines of the Chinese Society of Clinical Oncology. The clinical information and data regarding these five patients (BCP20-22, BCP27, BCP30) are summarized in Table 1. The biopsy results of BCP21 and BCP22 revealed HER-2 positivity, and they received TCb and TCbHP (T: paclitaxel, Cob: carboplatin, H: trastuzumab, P: epratuzumab) schemes, respectively. The specific treatment plans are shown in Table 3.

The organoids derived from the biopsy tissue of HER-2-positive patients were exposed to the same drugs administered to the patients. Since no HER-2 monoclonal antibody was used in this study, HER-2-positive

organoids were only exposed to docetaxel and carboplatin. To assess the response of organoids to docetaxel and carboplatin, we observed changes in the size of organoids and the growth of the spheroids after the dynamic administration of docetaxel and carboplatin (Figures 4A, 4C). Then, the number of surviving cells was quantified by the CellTiter-Glo 3D cell viability assay to verify the responsiveness of the organoids to the drugs (Figure 4B, 4D). As shown in Figure 4A-B, after treatment with different concentrations of docetaxel for six days, the size and morphology of BCb21 and BCb22 organoids did not change. The survival rate of the two organoids was subsequently observed to be greater than 50%. The responsiveness of carboplatin treatment was also assessed, as shown in Figure 4C-D, after six days of treatment with different concentrations of carboplatin, the size and morphology of BCb22 organoids was not significantly changed, while the cell clusters of BCb21 organoids decreased, the color darkened, and the cell mass lysed into individual cells. The CellTiter-Glo 3D cell viability assay showed that after treatment with different concentrations of carboplatin, the sensitivity of BCb22 organoids to carboplatin was significantly greater than that of BCb21 organoids. We compared the IC₅₀ values of the two organoids to carboplatin and found that the logIC₅₀ values of BCb21 and BCb22 were 16.90 μ M and -1.299 μ M, respectively (Figure 4D). The IC₅₀ of the former was more than five times larger than that of the latter.

We observed that HER2-positive organoids were insensitive to the paclitaxel drug docetaxel through in vitro experiments. BCb22 was sensitive to carboplatin, while BCb21 was resistant to carboplatin.

Next, we matched the organoid drug sensitivity data with the patient's clinical results. According to pathology, ypT0/Tis ypN0 is generally defined as pCR; ductal carcinoma in situ may remain in the breast after chemotherapy, but no residual cancer cells in the axillary lymph nodes can be present. According to the assessment of postoperative tissues by the Department of Pathology, the First Affiliated Hospital of Chongqing Medical University, BCP22 reached pCR after NAC, while BCP21 did not. Thus, among HER-2-positive patients, the response of the tissue-derived organoids to the drugs was consistent with the patients' clinical response.

Result 5. Response of organoids from patients receiving the TAC regimen to NAC.

According to the classification of molecular subtypes, the biopsy, and pathological results of BCP20 and BCP27 showed that their tumors were negative for HR and HER-2, indicating TNBC. The pathological results of BCP30's biopsy revealed ER positivity (60%), weak PR positivity (2%) and Ki67 positivity (40%). This patient's cancer axillary lymph node biopsy was also positive. All these patients received NAC with a TAC regimen (T: paclitaxel, E: anthracycline, C: cyclophosphamide). The specific treatment plans are shown in Table 3.

While the patients received TAC for 4-6 months, the organoids derived from these patients could not be assessed for cyclophosphamide responsiveness as this drug can only be metabolized in vivo; thus, our drug sensitivity test was limited to docetaxel and anthracyclines (epirubicin).

To observe the response of the organoids to docetaxel and epirubicin, we assessed the changes in organoid size and the growth of spheroids after dynamic administration of docetaxel and epirubicin (Figures 5A and 5C). CellTiter-Glo 3D cell viability measurements were directly compared to verify the organoid size measurement (Figure 5B, 5D). As shown in Figure 5A-B, after treatment with different concentrations of docetaxel for six days, the size and morphology of BCb20, BCb27, and BCb30 organoids did not change. The CellTiter-Glo 3D cell viability determination revealed that the survival rate of the two organoids was greater than 50%. As shown in Figure 5C-D, after six days of treatment with different concentrations of epirubicin, the size and morphology of BCb27 organoids did not change significantly, while the cell clusters of BCb20 organoids decreased, the color became darker, and the cell mass lysed into individual cells. The CellTiter-Glo 3D cell viability assay showed that after treatment with different concentrations of epirubicin, the sensitivity of BCb27 and BCb30 organoids to epirubicin was significantly higher than that of BCb20 organoids. The logIC₅₀ values of BCb20, BCb27, and BCb30 were -0.3728 μ M, 26.7300 μ M, and 0.1719 μ M, respectively (Figure 5D). The IC₅₀ of BCb27 and BCb30 was more than five times higher than that of BCb20.

The in vitro experiments suggest that organoids derived from triple-negative and luminal B-type BCs responded in the same manner as the parental tumors and the HR-positive organoids were not sensitive to docetaxel. BCb20 was sensitive to epirubicin, and BCb27 and BCb30 were resistant to epirubicin.

Next, we matched the organoid drug sensitivity data with the patient's clinical results. The pathological evaluation results of the Pathology Department of the First Affiliated Hospital of Chongqing Medical University showed that BCP20 achieved pCR after receiving TAC NAC, while BCP27 and BCP30 did not. In summary, the clinical response of the TNBC and HR+ patients was consistent with the response of tissue-derived organoids to drugs.

Result 6. Responsiveness of PDOs to combination medications

Breast cancer patients who meet the indications for NAC are typically administered multidrug combinations or NP sequential therapy when undergoing preoperative chemotherapy. Therefore, we also tried to match the clinical treatment plan for assessing BC organoid responses to drugs. TCbHP, TCBH, or THP regimens are often administered to HER2+ BC patients. Due to the lack of monoclonal antibodies, we used paclitaxel in combination with carboplatin to mimic the preoperative novelty of HER2-positive BC patients' NAC. TAC is often used clinically for BC patients who are negative for HER-2 to reduce tumor staging, rapidly reduce tumor volume, and increase breast-conserving opportunities. For HER2-negative breast cancer patients, preoperative adjuvant endocrine therapy and adjuvant immunotherapy are rarely performed clinically. Therefore, for organoids derived from HER2-negative breast cancer, we used anthracyclines combined with paclitaxel to treat simulated patients with neoadjuvant chemotherapy. For HER2-positive PDOs, as shown in Figure 6A-B, the changes in organoid size and the growth of spheroids after docetaxel combined with carboplatin were dynamically observed. When using 0.1 μ M docetaxel combined with different concentrations of carboplatin for six days, the size and morphology of BCb21

cells did not change. The CellTiter-Glo 3D cell viability assay showed that the LogIC₅₀ of BCb21 was 4.6860 μ M.

In contrast, cell spheres in BCb22 organoids treated with 0.1 μ M docetaxel combined with different concentrations of carboplatin for six days did not increase and in some cases, the cell clusters broke down into single cells. The color of the organoids darkened, and the CellTiter-Glo 3D cell viability assay indicated that the LogIC₅₀ of BCb21 was -0.3616 μ M. Thus, in agreement with the monotherapy results, BCb21 was resistant to docetaxel combined with carboplatin, while BCb22 was sensitive to the combined treatment. As shown in Figure 6C-D, we dynamically observed the changes in organoid size and the growth of spheres after treatment with anthracyclines combined with paclitaxel. When we treated the organoids with 0.1 μ M docetaxel combined with different concentrations of epirubicin, after six days, the size and morphology of BCb27 and BCb30 cells did not change. The CellTiter-Glo 3D cell viability assay showed that the logIC₅₀ values of the two were 2.2550 μ M and 1.8730 μ M, respectively.

In contrast, in the observation of BCb20, after using 0.1 μ M docetaxel combined with different concentrations of epirubicin to treat the organoids for six days, the organoid cell spheres did not increase, and in some cases, the cell clusters broke down into individual cells. The color of the cells darkened, and the CellTiter-Glo 3D cell viability assay indicated that the logIC₅₀ of BCb20 was 0.3203 μ M. Thus, consistent with the results observed with the monotherapy, BCb27 and BCb30 were resistant to anthracycline combined with docetaxel, while BCb20 was sensitive to the combined treatment.

Comparing the clinical responses with the results of the in vitro experiments, we concluded that our in vitro treatment data were consistent with the patient's clinical responsiveness (Figure 6E). In addition, our data showed that when the patient's tumor organoids were sensitive to at least one of the two therapeutic components, the patient achieved an excellent clinical response. Therefore, it may be possible to use organoids to test patient responses to monotherapy and predict the drug sensitivity of patients receiving NAC or adjuvant chemotherapy.

Discussion

In general, it is believed that patients who achieve pCR have a better outcome than those who do not [38, 39]. Thus, pCR is an established prognostic factor. However, whether BC patients undergoing NAC can achieve pCR is unknown. In clinical practice, the value of a single treatment cannot be evaluated in individual patients undergoing combined therapy. Therefore, it is impossible to individually screen the sensitivity of BC patients with different molecular subtypes to drugs to achieve individualized and precise treatment.

Patient-derived tumor organoids are promising tools for personalized cancer medicine approaches [40]. Importantly, PDOs may be superior models for drug screening [41, 42] and the prediction of cancer treatment responses [32, 41, 43].

We successfully constructed organoids from BC biopsies to simulate clinical treatment, demonstrating that viable tumoroid lines can be generated from a small sample of tissue. TCbH, TCbHP, or THP are clinically administered as NAC for patients with HER2-positive breast cancer. Therefore, we evaluated docetaxel combined with carboplatin therapy or as a monotherapy using PDOs derived from HER2-positive patients. TAC is administered as a NAC for patients with HER2-negative breast cancer. Therefore, we evaluated anthracycline combined with paclitaxel therapy or as a monotherapy using PDOs derived from HER2-negative patients. The histological verification of organoids and the results of the in vitro treatments showed that the organoids simulated the histological characteristics of the parental tumor and the clinical response of the individual patients to chemotherapy, demonstrating potential of PDOs in precision medicine.

However, our organoid construction success rate from puncture samples was insufficient. The main limitation of successfully establishing organoids from our biopsy tissues was the insufficient number of viable tumor cells in the biopsy material and the low mutation load of luminal BC. Furthermore, the duration required for organoid expansion in vitro varies from patient to patient and depends on the size of the biopsy tissue, the proportion of cancer cells in the tissue, and the cell proliferation rate. It took two weeks to successfully construct PDOs from BC biopsies, and approximately one month for the PDOs to achieve sufficient cell mass for in vitro treatment. It is unknown whether a patient's tumor will progress during the one-month in vitro drug screening of the organoids. Nevertheless, organoids were shown to be a viable in vitro model for predicting chemotherapy response and even radiotherapy response.

Since this study did not include monoclonal antibodies and cyclophosphamide, PDOs could not be used to assess the clinical responsiveness of patients without bias. A natural progression of this work is to construct a mouse model of patient-derived organoids and add monoclonal antibodies and cyclophosphamide to conduct in vivo experiments to further verify that organoids can be used as a tool for treatment response modeling.

We successfully constructed organoids from BC biopsy samples and proved the consistency of the organoids and tumor samples by histology. Our results indicate that organoid data can predict the clinical response of patients to NAC. The PDO model may identify BC patients who respond to NAC and thereby improve initial care. For patients with BC who are chemo-insensitive, PDOs could be used to select targeted or investigational agents, avoid overtreatment, and reduce treatment-induced side effects. In summary, our data imply that PDOs can predict BC patient responses in the clinic and could potentially be used as a companion tool for BC treatment selection.

Conclusions

The results of our analyses suggest that patient-derived organoids could predict the responses of BC patient in the clinic and may be possible to use organoids to test patient responses to monotherapy and predict the drug sensitivity of patients receiving NAC or adjuvant chemotherapy. In addition, PDOs could potentially be used as a companion tool for BC treatment selection.

Abbreviations

BC—Breast cancer—NAC—neoadjuvant chemotherapy —PDOs—patient-derived organoids—BCO—breast cancer-derived organoids—BCP—breast cancer patient—pCR—complete pathological response—ER—estrogen receptor—PR—progesterone receptor—HER-2—human epidermal growth factor receptor 2—TNBC—Triple-negative Breast cancer—LGR5—G protein-coupled receptor 5+ —BME—Cultrex Reduced Growth Factor Basement Membrane Extract —

Declarations

Ethics approval and consent to participate

Ethical approval This study was performed in line with the principles of the Declaration of Helsinki. The studies involving human participants were reviewed and approved by the Human Research Ethics Committee of the First Affiliated Hospital of Chongqing Medical University. This article does not contain any studies with animals performed by any of the authors.

Consent to participate Informed consent was obtained from all individual participants included in the study.

Consent for publication

Yes.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

All authors contributed to the study conception and design. SCL and ASJ designed this study. DS constructed BC organoids and finished this study. Material preparation, data collection and analysis were performed by DS. The first draft of the manuscript was written by DS. MYS, KL, XJH commented on

previous versions of the manuscript. HL, ZFT, YP, ZRT, CQ performed experiments. YW helped collect clinical specimens. All authors read and approved the final manuscript.

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References

1. **Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries.**
2. DeSantis CE, Ma J, Goding Sauer A, Newman LA, Jemal A: **Breast cancer statistics, 2017, racial disparity in mortality by state.** *CA Cancer J Clin* 2017, **67**(6):439-448.
3. McDonald ES, Clark AS, Tchou J, Zhang P, Freedman GM: **Clinical Diagnosis and Management of Breast Cancer.** *J Nucl Med* 2016, **57 Suppl 1**:9s-16s.
4. Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA *et al*: **Molecular portraits of human breast tumours.** *Nature* 2000, **406**(6797):747-752.
5. Sorlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, Deng S, Johnsen H, Pesich R, Geisler S *et al*: **Repeated observation of breast tumor subtypes in independent gene expression data sets.** *Proc Natl Acad Sci U S A* 2003, **100**(14):8418-8423.
6. Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS *et al*: **Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications.** *Proc Natl Acad Sci U S A* 2001, **98**(19):10869-10874.
7. Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, Lickley LA, Rawlinson E, Sun P, Narod SA: **Triple-negative breast cancer: clinical features and patterns of recurrence.** *Clin Cancer Res* 2007, **13**(15 Pt 1):4429-4434.
8. von Minckwitz G, Blohmer JU, Costa SD, Denkert C, Eidtmann H, Eiermann W, Gerber B, Hanusch C, Hilfrich J, Huober J *et al*: **Response-guided neoadjuvant chemotherapy for breast cancer.** *J Clin Oncol* 2013, **31**(29):3623-3630.
9. **Long-term outcomes for neoadjuvant versus adjuvant chemotherapy in early breast cancer: meta-analysis of individual patient data from ten randomised trials.** *Lancet Oncol* 2018, **19**(1):27-39.
10. Aronson SJ, Rehm HL: **Building the foundation for genomics in precision medicine.** *Nature* 2015, **526**(7573):336-342.

11. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A: **Global cancer statistics, 2012.** *CA Cancer J Clin* 2015, **65**(2):87-108.
12. Kamb A: **What's wrong with our cancer models?** *Nat Rev Drug Discov* 2005, **4**(2):161-165.
13. Caponigro G, Sellers WR: **Advances in the preclinical testing of cancer therapeutic hypotheses.** *Nat Rev Drug Discov* 2011, **10**(3):179-187.
14. Whittle JR, Lewis MT, Lindeman GJ, Visvader JE: **Patient-derived xenograft models of breast cancer and their predictive power.** *Breast Cancer Res* 2015, **17**(1):17.
15. Cheon DJ, Orsulic S: **Mouse models of cancer.** *Annu Rev Pathol* 2011, **6**:95-119.
16. Carmon KS, Gong X, Lin Q, Thomas A, Liu Q: **R-spondins function as ligands of the orphan receptors LGR4 and LGR5 to regulate Wnt/beta-catenin signaling.** *Proc Natl Acad Sci U S A* 2011, **108**(28):11452-11457.
17. Sato T, Vries RG, Snippert HJ, van de Wetering M, Barker N, Stange DE, van Es JH, Abo A, Kujala P, Peters PJ *et al*: **Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche.** *Nature* 2009, **459**(7244):262-265.
18. de Lau W, Barker N, Low TY, Koo BK, Li VS, Teunissen H, Kujala P, Haegebarth A, Peters PJ, van de Wetering M *et al*: **Lgr5 homologues associate with Wnt receptors and mediate R-spondin signalling.** *Nature* 2011, **476**(7360):293-297.
19. Jung P, Sato T, Merlos-Suárez A, Barriga FM, Iglesias M, Rossell D, Auer H, Gallardo M, Blasco MA, Sancho E *et al*: **Isolation and in vitro expansion of human colonic stem cells.** *Nat Med* 2011, **17**(10):1225-1227.
20. Sato T, Stange DE, Ferrante M, Vries RG, Van Es JH, Van den Brink S, Van Houdt WJ, Pronk A, Van Gorp J, Siersema PD *et al*: **Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium.** *Gastroenterology* 2011, **141**(5):1762-1772.
21. Boj SF, Hwang CI, Baker LA, Chio, II, Engle DD, Corbo V, Jager M, Ponz-Sarvise M, Tiriác H, Spector MS *et al*: **Organoid models of human and mouse ductal pancreatic cancer.** *Cell* 2015, **160**(1-2):324-338.
22. Chua CW, Shibata M, Lei M, Toivanen R, Barlow LJ, Bergren SK, Badani KK, McKiernan JM, Benson MC, Hibshoosh H *et al*: **Single luminal epithelial progenitors can generate prostate organoids in culture.** *Nat Cell Biol* 2014, **16**(10):951-961, 951-954.
23. Karthaus WR, Iaquinta PJ, Drost J, Gracanin A, van Boxtel R, Wongvipat J, Dowling CM, Gao D, Begthel H, Sachs N *et al*: **Identification of multipotent luminal progenitor cells in human prostate organoid cultures.** *Cell* 2014, **159**(1):163-175.

24. Bartfeld S, Bayram T, van de Wetering M, Huch M, Begthel H, Kujala P, Vries R, Peters PJ, Clevers H: **In vitro expansion of human gastric epithelial stem cells and their responses to bacterial infection.** *Gastroenterology* 2015, **148**(1):126-136.e126.
25. Kessler M, Hoffmann K, Brinkmann V, Thieck O, Jackisch S, Toelle B, Berger H, Mollenkopf HJ, Mangler M, Sehouli J *et al*: **The Notch and Wnt pathways regulate stemness and differentiation in human fallopian tube organoids.** *Nat Commun* 2015, **6**:8989.
26. Maimets M, Rocchi C, Bron R, Pringle S, Kuipers J, Giepmans BN, Vries RG, Clevers H, de Haan G, van Os R *et al*: **Long-Term In Vitro Expansion of Salivary Gland Stem Cells Driven by Wnt Signals.** *Stem Cell Reports* 2016, **6**(1):150-162.
27. DeWard AD, Cramer J, Lagasse E: **Cellular heterogeneity in the mouse esophagus implicates the presence of a nonquiescent epithelial stem cell population.** *Cell Rep* 2014, **9**(2):701-711.
28. Rock JR, Onaitis MW, Rawlins EL, Lu Y, Clark CP, Xue Y, Randell SH, Hogan BL: **Basal cells as stem cells of the mouse trachea and human airway epithelium.** *Proc Natl Acad Sci U S A* 2009, **106**(31):12771-12775.
29. Turco MY, Gardner L, Hughes J, Cindrova-Davies T, Gomez MJ, Farrell L, Hollinshead M, Marsh SGE, Brosens JJ, Critchley HO *et al*: **Long-term, hormone-responsive organoid cultures of human endometrium in a chemically defined medium.** *Nat Cell Biol* 2017, **19**(5):568-577.
30. Sachs N, de Ligt J, Kopper O, Gogola E, Bounova G, Weeber F, Balgobind AV, Wind K, Gracanin A, Begthel H *et al*: **A Living Biobank of Breast Cancer Organoids Captures Disease Heterogeneity.** *Cell* 2018, **172**(1-2):373-386.e310.
31. Drost J, Clevers H: **Organoids in cancer research.** *Nat Rev Cancer* 2018, **18**(7):407-418.
32. Vlachogiannis G, Hedayat S, Vatsiou A, Jamin Y, Fernández-Mateos J, Khan K, Lampis A, Eason K, Huntingford I, Burke R *et al*: **Patient-derived organoids model treatment response of metastatic gastrointestinal cancers.** *Science* 2018, **359**(6378):920-926.
33. van de Wetering M, Francies HE, Francis JM, Bounova G, Iorio F, Pronk A, van Houdt W, van Gorp J, Taylor-Weiner A, Kester L *et al*: **Prospective derivation of a living organoid biobank of colorectal cancer patients.** *Cell* 2015, **161**(4):933-945.
34. Ogston KN, Miller ID, Payne S, Hutcheon AW, Sarkar TK, Smith I, Schofield A, Heys SD: **A new histological grading system to assess response of breast cancers to primary chemotherapy: prognostic significance and survival.** *Breast* 2003, **12**(5):320-327.
35. Wang-Lopez Q, Chalabi N, Abrial C, Radosevic-Robin N, Durando X, Mouret-Reynier MA, Benmammar KE, Kullab S, Bahadoor M, Chollet P *et al*: **Can pathologic complete response (pCR) be used as a**

surrogate marker of survival after neoadjuvant therapy for breast cancer? *Crit Rev Oncol Hematol* 2015, **95**(1):88-104.

36. Brennan M, Lim B: **The Actual Role of Receptors as Cancer Markers, Biochemical and Clinical Aspects: Receptors in Breast Cancer.** *Adv Exp Med Biol* 2015, **867**:327-337.

37. Dai X, Xiang L, Li T, Bai Z: **Cancer Hallmarks, Biomarkers and Breast Cancer Molecular Subtypes.** *J Cancer* 2016, **7**(10):1281-1294.

38. Rastogi P, Anderson SJ, Bear HD, Geyer CE, Kahlenberg MS, Robidoux A, Margoless RG, Hoehn JL, Vogel VG, Dakhil SR *et al*: **Preoperative chemotherapy: updates of National Surgical Adjuvant Breast and Bowel Project Protocols B-18 and B-27.** *J Clin Oncol* 2008, **26**(5):778-785.

39. Kong X, Moran MS, Zhang N, Haffty B, Yang Q: **Meta-analysis confirms achieving pathological complete response after neoadjuvant chemotherapy predicts favourable prognosis for breast cancer patients.** *Eur J Cancer* 2011, **47**(14):2084-2090.

40. Pauli C, Hopkins BD, Prandi D, Shaw R, Fedrizzi T, Sboner A, Sailer V, Augello M, Puca L, Rosati R *et al*: **Personalized In Vitro and In Vivo Cancer Models to Guide Precision Medicine.** *Cancer Discov* 2017, **7**(5):462-477.

41. Tiriack H, Belleau P, Engle DD, Plenker D, Deschênes A, Somerville TDD, Froeling FEM, Burkhart RA, Denroche RE, Jang GH *et al*: **Organoid Profiling Identifies Common Responders to Chemotherapy in Pancreatic Cancer.** *Cancer Discov* 2018, **8**(9):1112-1129.

42. Broutier L, Mastrogiiovanni G, Verstegen MM, Francies HE, Gavarró LM, Bradshaw CR, Allen GE, Arnes-Benito R, Sidorova O, Gaspers MP *et al*: **Human primary liver cancer-derived organoid cultures for disease modeling and drug screening.** *Nat Med* 2017, **23**(12):1424-1435.

43. Schütte M, Risch T, Abdavi-Azar N, Boehnke K, Schumacher D, Keil M, Yildirim R, Jandrasits C, Borodina T, Amstislavskiy V *et al*: **Molecular dissection of colorectal cancer in pre-clinical models identifies biomarkers predicting sensitivity to EGFR inhibitors.** *Nat Commun* 2017, **8**:14262.

Figures

Figure 1

Study Design and construction of BC organoids. (A) Flow diagram of the study, including clinical neoadjuvant chemotherapy before surgery and drug tests on organoids. (B) The puncture tissue of the patient obtained by the core puncture under the guidance of color Doppler ultrasound is 1-2cm strip shape. (C) Neoadjuvant chemotherapy regimen of patients with different kind of subtype. (D) Successful cultivation of biopsy samples of different subtypes.

Figure 2

Long-term passage of organoids in vitro and maintain tumor heterogeneity (A) Bright field shows the morphology of organoids with different passage times and the proliferation ability of organoids. Scale bar, 250 mm. (B) The proliferation curve of organoids tested by CellTiter-Glo 3D cell viability assay proves that the cultured organoids can survive and inherit for a long time. (C) The morphology of individual BCOs with different subtype. Scale bar, 100 mm. (D) Morphological characteristics of tumor tissue and

organoids of patients who achieved pCR. The HE stain of puncture samples and surgery tissues of patients who reached pCR after neoadjuvant chemotherapy, Scale bar, 100 mm.

Figure 3

Histological Characterization of BC Organoids. A-B. Immunohistochemical comparison of tissues of luminal B and Her2+ breast cancer patients after neoadjuvant chemotherapy and organoids of biopsy specimens before neoadjuvant chemotherapy. Tissues generally present tumor epithelium surrounded by

mesenchymal and inflammatory cells, while organoids are exclusively epithelial with tumor cell organization being remarkably well conserved (HE). ER, PR, HER2 status and proliferation index of original BCs are similarly well retained in the derived organoid lines. Scale bar, 100 mm.

Figure 4

Sensitivity of PDOs derived from puncture tissues to docetaxel and carboplatin in patients with HER2-positive breast cancer. A□□The bright field shows the changes in the size and status of HER2-positive

organoids after treatment with different drug concentrations of docetaxel [A]and carboplatin[C](the upper row represents the organoids not treated with the drug, and the lower row represents the organoids treated with different drug concentrations for 6 days). Scale bar, 150 mm. B[D]The line graph shows the survival of HER2-positive organoids treated with different drug concentrations of docetaxel[B]and carboplatin[D].

Figure 5

Sensitivity of PDOs derived from HER2-negative breast cancer patients to docetaxel and anthracyclines. A–C The bright field shows the changes in the size and status of HER2-negative organoids after treatment with different drug concentrations of docetaxel and epirubicin (the upper row represents the organoids treated without the drugs, and the lower row represents the organoids treated with different drug concentrations for 6 days.) Scale bar, 150 μ m. B The line graph shows the survival rate of HER2-negative organoids treated with different drug concentrations of docetaxel and epirubicin. D Comparison of the sensitivity of HER2-negative organoids from the biopsy sample to epirubicin, and the logIC₅₀ of each type of organoid to epirubicin.

Figure 6

Patient-derived organoids show different sensitivities to the combination of epirubicin and docetaxel and the combination of carboplatin and docetaxel. A-C. The bright field shows the changes in the size and status of organoids treated with different drug concentrations of carboplatin and 0.1uM docetaxel (the upper row represents the organoids without drug treatment, and the lower row represents the organoids treated with different drug concentrations for six days.) Scale bar, 150 mm. B: The line graph shows the

survival rate and IC50 of carboplatin combined with docetaxel in the treatment of HER2-positive organoids. C: The bright field shows the changes in the size and status of organoids treated with different drug concentrations of epirubicin and 0.1uM docetaxel (the upper row represents the organoids without drug treatment, and the lower row represents the organoids treated with different drug concentrations for six days.) Scale bar, 150 mm. D: The line graph shows the survival rate and IC50 of the combined treatment of epirubicin and docetaxel in HER2-negative organoids E: The organoid response data matches the patient's clinical results (the first row: the sensitivity of HER2-positive organoids to epirubicin; the second row: the sensitivity of HER2-negative organoids to epirubicin; the third row: The bottom of the sensitivity of different types of organoids to docetaxel; the fourth row: the drug reactivity of the two-drug combination of different types of organoids; bottom: the heat map of the organoid response and the patient's clinical results, we can draw the conclusion that organoids can predict the prognosis of patients with neoadjuvant chemotherapy).