

Establishment of gastric cancer organoid and its application in individualized therapy

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Abstract. The application of cancer organoids is of great value in individualized therapy as an embodiment of the tumor of a patient, a gastric cancer organoid model was established and its application in individualized drug screening was explored. The primary tumor tissues of 3 patients with gastric cancer who underwent primary surgery at the Fourth Department of General Surgery of the First Hospital of Lanzhou University (Lanzhou, China) between July and August 2021 were selected and digested with mixed enzymes to prepare cell suspensions. Of these, two were cultured by mixing with Matrigel, while the cells from the third patient were placed in a 24-well ultra-low adhesion plate for suspension organoid culture. After intensive organoid growth, they were digested, passaged, cryopreserved and thawed for further analyses. The formation of gastric cancer organoids was observed under an inverted microscope. One case was selected, and organoids were compared with the original tumor tissue via H&E and immunohistochemical staining to evaluate the consistency of the two. Finally, paclitaxel, oxaliplatin and fluorouracil were administered to the organoids to verify the value of screening individualized drugs. It was indicated that the passage and cryopreservation of gastric cancer organoids were successfully established in all three cases. The H&E and immunohistochemical staining results suggested that the structure and protein expression of

the organoids were highly similar to those of the source tumor tissue. The use of established gastric cancer organoids for individualized chemotherapy drug screening is of high clinical value. Gastric cancer organoids with high similarity to the original tissue may be successfully constructed by the suspension growth culture method. The established organoids may serve as an effective model for individualized drug screening.

Introduction

Gastric cancer is the fifth most common cancer type worldwide, following lung, breast, colorectal and prostate cancers. Its incidence has declined since the mid-20th century, but it remains the third leading cause of cancer-associated mortality, with a 5-year survival rate of only 29% (1-4). Multiple studies have demonstrated that surgery alone for gastric cancer decreases the survival and increases the recurrence rate compared with multimodal therapy (5-8). Therefore, there is an urgent need for novel methods to predict the efficacy of individual treatments (6-9).

The development of organoids is an important challenge to overcome for establishing *in vivo* and *in vitro* patient-derived personalized medicine platforms. While cancer cell lines have been valuable in basic cancer research, these models have the significant disadvantage of bearing little resemblance to the patient tumor (7-11). The development of high-throughput analytical methods now allows addressing the clinical relevance of these human cancer-derived cell lines. At the genomic level, driver mutations may be retained in cancer cell lines. However, certain studies have revealed shifts at the transcriptome level, suggesting that cancer cell lines are more similar to each other than the clinical samples from which they were originally derived. These shortcomings may be resolved by establishing organoids (10-14).

The application of cancer organoids is of great value in individualized therapy as an embodiment of the tumor of a patient (15). Individual cancer organoids may be used to predict the therapeutic response to certain drugs, and

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the establishment of large patient-derived organoid (PDO) biobanks with combined drug screening may help to describe new therapeutic strategies for gastric cancer (16). Previously, research teams have successively reported the establishment of gastric-related organoids for research purposes (17-19).

In the present study, the establishment of gastric cancer organoid models was explored using a new organoid culture system to prolong the culture time of gastric cancer primary cells. Furthermore, organoids were cultivated under three-dimensional (3D) conditions to provide an experimental model for follow-up studies of individualized treatment of patients.

Materials and methods

Tissue source. Gastric cancer tissues were collected from 3 patients with gastric cancer, who underwent primary surgery at The First Hospital of Lanzhou University (Lanzhou, China) from July to August 2021.

Inclusion criteria were preoperative pathologically confirmed gastric cancer in patients over 18 years of age and informed consent. The exclusion criteria were as follows: Patients who received radiotherapy and/or chemotherapy prior to surgery or did not agree to participate in the experiment.

The clinical and pathological data are as follows: Patient 1 was a 75-year-old male, cardia cancer, moderately differentiated; Patient 2 was a 58-year-old male, gastric antrum cancer, poorly differentiated; Patient 3 was a 74-year-old male, gastric antrum cancer, moderately-poorly differentiated. After surgery, this patient received six cycles of systemic chemotherapy (XELOX).

The present study was approved by the Ethics Committee of the First Hospital of Lanzhou University (Lanzhou, China; approval no. LDYYLL2022-292) and written informed consent was obtained from the patients.

Main reagents and instruments. The 24-well culture plates were purchased from Corning, Inc. DMEM/F12 medium, fetal bovine serum and cryopreservation solution were purchased from Biological Industries. GlutaMax, TrypLE, collagenase II and dispase II were from Gibco (Thermo Fisher Scientific, Inc.). The light microscope was from Nikon Corporation.

Preparation of the media. The complete medium consisted of DMEM/F12 supplemented with 1X GlutaMax, 10% fetal bovine serum and 1% penicillin and streptomycin. The tissue transport medium comprised DMEM/F12 with 1% penicillin and streptomycin. The mixed digestive enzymes medium comprised DMEM/F12 with 1 mg/ml collagenase II and 1.5 mg/ml dispase II.

Organoid establishment. The principle of aseptic operation was strictly followed. First, an appropriate amount of fresh tumor tissue (~5 mm²) was cut according to the size of the tumor and stored in the transport solution. The retrieved cancer tissue samples were placed in a 60-mm petri dish and washed with PBS to remove blood clots, as well as necrotic and fibrous components. A sterile surgical blade was then used to excise a part of the tumor tissue and a portion of the sample was frozen in a -80°C refrigerator for subsequent experiments. The

remaining tissue (~0.3 cm³) was minced as much as possible, added to a 15-ml centrifuge tube, mixed with 10 ml digestive enzymes solution and placed in a shaker to be digested for ~40 min at 37°C. During this period, the digestion of the tissue block was closely observed. Once the tissue volume was reduced by half, the digestion solution was filtered through a 100-mesh cell sieve, collected into a 15-ml centrifuge tube and centrifuged at 400 x g for 4 min at 4°C, after which the supernatant was discarded. The centrifugation step was repeated twice, the cell pellet was collected and the cells were resuspended in 300 μ l complete culture medium. The cell suspensions of 2 patients were individually mixed with 300 μ l Matrigel® (BD Pharmingen) at a ratio of 1:1 and then inoculated into 24-well culture plates (cat. no. 3527; Corning, Inc.) at 100 μ l/well for organoid culture for 3 weeks. For the third case, the cell suspension of 6 ml was directly inoculated into a 24-well ultra-low adhesion plate (cat. no. 3473; Corning, Inc.) at 1 ml/well and organoids were formed by suspension culture at 37°C in a humidified atmosphere containing 5% CO₂.

Passaging, cryopreservation and recovery of organoids. The organoids were passaged when they reached a size of 100 μ m under the microscope. The organoids and complete medium to be passaged were transferred to a 15-ml centrifuge tube, an appropriate amount of PBS was added, and the suspension was gently mixed with a pipette tip and then centrifuged at 400 x g for 4 min at 4°C, before the supernatant was removed and the pellet collected. Subsequently, ~2 ml TrypLE was added to the pellet and the suspension was gently mixed with a pipette to disperse the clumps, followed by digestion at 37°C while maintaining microscopic observation until the organoids were enzymatically digested to a small cell clump state of 3-5 cells. When terminating the digestion, 5 ml complete medium was added to the cell pellet, which was gently mixed with a pipette tip and centrifuged at 400 x g for 4 min at 4°C before removing the supernatant and collecting the pellet. The passage ratio was 1:2-1:3. A part of the organoids was added to the freezing medium and frozen at a density of ~1x10⁶ cells/ml; they were placed in a gradient freezing box at -80°C overnight and then transferred to liquid nitrogen for long-term storage. Prior to subsequent experiments, the cryopreservation tube was placed in a 37°C water bath. After thawing, the sample was quickly transferred to a 15-ml centrifuge tube, 5 ml complete medium was added, and the cell suspension was mixed by passing it through a pipette three times.

Preparation of embedded organoid sections. The organoids cultured in suspension were separated by centrifugation at 400 x g for 4 min at 4°C, immersed in 4% paraformaldehyde solution at 37°C for 24 h and then dehydrated with an ethanol gradient (50, 70, 80, 95, 100% x2; 30 min for each gradient step). Subsequently, the dehydrated organoids were placed into 50% xylene and 50% ethanol for 30 min and immersed in pure xylene for 1 h twice at 37°C. A total of 200 organoids were agar-embedded, cut into thin slices with a thickness of ~5 μ m, placed onto glass slides and dried.

Hematoxylin and eosin (H&E) staining. The slices of the aforementioned steps were placed in xylene I for 10 min, xylene II for 10 min, anhydrous ethanol I for 5 min, anhydrous

Table I. Primary antibodies used.

Antibody	Supplier	Catalogue number	Dilution
P53	Wuhan Servicebio Technology Co., Ltd.	GB111740	1:200
CK18	Wuhan Servicebio Technology Co., Ltd.	GB11232	1:600
Ki-67	Wuhan Servicebio Technology Co., Ltd.	GB111499	1:400
CKL	Fuzhou Maixin Biotech Co., Ltd.	MAB-0051	1:300

CK18, cytokeratin 18; CKL, cytokeratin low molecular weight.

ethanol II for 5 min, 95% ethanol for 5 min, 90% ethanol for 5 min, 80% ethanol for 5 min, 70% ethanol for 5 min and then distilled water for washing at 37°C. The sections were then stained with Harris hematoxylin for 3-8 min at 37°C, washed with tap water, differentiated with 1% hydrochloric acid ethanol for several seconds, rinsed with tap water, incubated in 0.6% ammonia water for nuclear staining, and rinsed with running water. The sections were then stained in eosin staining solution at 37°C for 1-3 min. Thereafter, the slices were placed for 5 min each into 95% ethanol I, 95% ethanol II, absolute ethanol I, absolute ethanol II, xylene I and xylene II to dehydrate and generate transparent slices at 37°C. After the last step, the slices were removed from xylene and dried before being sealed with neutral gum. Finally, the slides were examined under a light microscope (S40-Slider; Leica Microsystems) and images were acquired.

Immunohistochemistry. A total of 500-1,000 PDO 3D spheres were centrifuged at 500 x g for 5 min at 4°C, the supernatant was discarded, 4% paraformaldehyde was added, and spheres were fixed overnight at 25°C. Subsequently, they were dehydrated with a graded ethanol series, cleared with xylene for 1 h and soaked in paraffin (temperature, <50°C) twice, each for 1 h. After embedding, serial sections were made with a thickness of 3 μm. Another part of the fresh gastric cancer tissue removed by surgery was immersed in formaldehyde solution and then routinely fixed, dehydrated, embedded and sliced. The PDO sections and primary tumor sections were placed in a 65°C oven for 30 min, dewaxed with xylene, hydrated with a gradient of ethanol, processed with 0.5 mol/l sodium citrate antigen retrieval solution under high pressure at 98°C for 5 min and naturally cooled to 25°C. The sections were then blocked with 3% bovine serum albumin (Wuhan Servicebio Technology Co., Ltd.) at 25°C for 15 min and then incubated with primary antibodies against human P53, Ki-67, cytokeratin low molecular weight (CKL) and cytokeratin (CK)18 (Table I) at 4°C overnight. Subsequently, the sections were incubated with 3% hydrogen peroxide at 25°C for 15 min, horseradish peroxidase-conjugated goat anti-mouse IgG (cat. no. GB23303; 1:200 dilution; Wuhan Servicebio Technology Co., Ltd.) at 25°C for 15 min and horseradish peroxidase-labeled streptavidin (cat. no. A0303; 1:500 dilution; Beyotime Institute of Biotechnology) at 25°C for 15 min, and the samples were then washed three times with PBS for 3 min. After color development with 3,3'-diaminobenzidine, the nuclei were stained with hematoxylin at 37°C for 150 sec, then dehydrated with a gradient

concentration of ethanol, made transparent with xylene and observed under a light microscope (IX73+DP74; Olympus Corporation).

Chemotherapy drug screening. The organoids cultured for 1 week were centrifuged and the pellet was obtained and resuspended in complete medium as previously mentioned. Subsequently, the cell suspension was seeded into 24-well ultra-low adhesion plate (cat. no. 3473; Corning, Inc.) at 0.5 ml/well (~500 organoids), and 0.5 ml complete medium was added to top up the total liquid volume to 1 ml per well. The experiment consisted of one control group and three experimental groups. In the control group, the same concentration of solvent was used. In the experimental groups, the commonly used chemotherapy drugs for gastric cancer, paclitaxel (Jiangsu Osaikang Pharmaceutical Co., Ltd.), oxaliplatin (Jiangsu Hengrui Pharmaceutical Co., Ltd.) and fluorouracil (Tianjin Jinyao Pharmaceutical Co., Ltd.), were used. At 24 h after inoculation, chemotherapy drugs were added to each experimental group. According to the relevant literature data (20,21), the final concentrations were 20 ng/ml for paclitaxel, 20 μM for oxaliplatin and 384 μM for fluorouracil. The morphological changes of the organoids (morphology, structure, size and quantity) were then observed under a light microscope (S40-Slider; Leica Microsystems) and compared every 24 h for 96 h.

Results

Establishment of gastric cancer organoids. After the gastric cancer tissues from 3 patients were individually digested with mixed enzymes, the organoids were successfully established by gradually increasing from a small granular type (Fig. 1Aa) to single spherical or multiple spore-like structures of different sizes (Fig. 1B). This culturing process took between 7 and 21 days when organoids reached a size of 100 μm under the microscope, depending on the characteristics of the tumor itself.

Under 2D culture conditions, gastric cancer cells exhibited a typical epithelioid cell morphology and cobblestone-like adherent growth, whereas the cell morphology was mainly of the short-spindle and round type (Fig. 2A and B). Under suspension culture conditions, early tumor cells first formed spherical sac-like structures (Fig. 2C), and with time, the spherical sac-like structures continued to develop into irregular clumps, and finally, gastric cancer organoids formed typical irregular lobulated lobes (Fig. 2D).

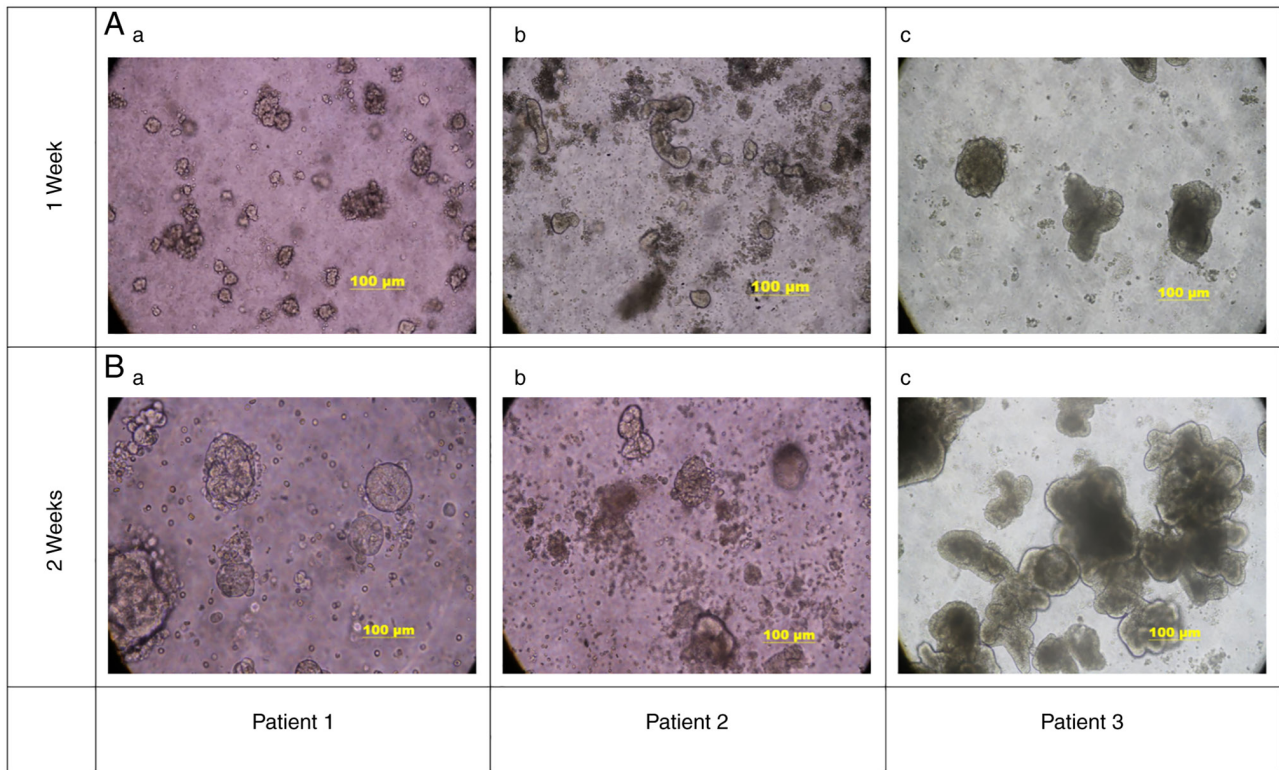


Figure 1. Morphology of patient-derived gastric cancer organoids at (A) 1 week and (B) 2 weeks. When tumor cells are inoculated in Matrigel, they may form (Aa) spherical or (Ab) microspore-like gastric cancer organoids, or (Ac) microspore-like gastric cancer organoids after suspension culture for 1 week. With increasing culture time, the organoids gradually grew into (Ba) large spheres or (Bb) small glandular tubular-like gastric cancer organoids and appeared with (Bc) varying numbers of glandular duct structures. These features were dependent on the characteristics of the tumor (scale bars, 100 μm).

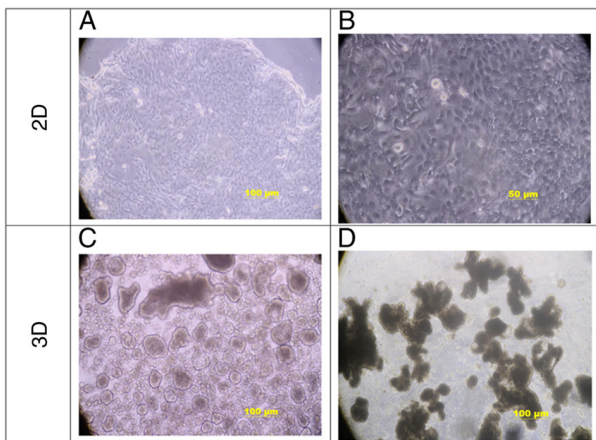


Figure 2. Morphological comparison of gastric cancer cells in 2D and 3D. (A and B) Under 2D culture conditions, gastric cancer cells exhibited a typical epithelioid cell morphology. The cell shape was mainly short, spindle-like and round, with large nuclei and a small amount of cytoplasm. Tumor cells grew in layers and lost contact inhibition, while they did not detach [scale bars, (A) 100 μm ; (B) 50 μm]. (C and D) 3D morphology of gastric cancer organoids; when suspended in ultra-low adsorption plates, (C) tumor cells first formed spherical sac-like structures, and (D) over time the spherical sac-like structures continued to develop into irregular clumps, and finally, gastric cancer organoids formed typical irregular lobes (scale bars, 100 μm). D, dimensional.

H&E staining of gastric cancer organoids and original tumor tissue. After 2 weeks of culture, gastric cancer organoids were embedded, sliced and analyzed by H&E staining.

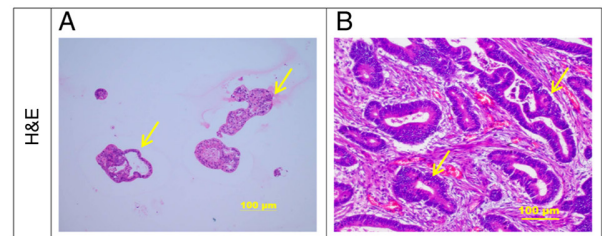


Figure 3. H&E staining of gastric cancer organoids and original tumor tissue. (A) H&E staining showed that the gastric cancer organoids had cystic or irregular glandular duct-like structures, irregular cell arrangement, large and hyperchromatic nuclei and irregular ring-shaped glandular structures (arrow). (B) The biological characteristics of the organoids were highly similar to those of the original gastric cancer tissues (arrow) (scale bars, 100 μm).

It was observed that the gastric cancer organoids had cystic or irregular glandular duct-like structures, an irregular cell arrangement, large and hyperchromatic nuclei and irregular ring-shaped glandular structures (Fig. 3A). This was highly similar to the biological characteristics of the original gastric cancer tissues (Fig. 3B).

Immunohistochemistry of gastric cancer organoids and original tumor tissue. Immunohistochemistry was used to detect the protein levels of P53, Ki-67, cytokeratin low molecular weight (CKL) and cytokeratin (CK)18 in gastric cancer organoids and original tumor tissues (Fig. 4). The results indicated that P53 (Fig. 4Aa), Ki-67 (Fig. 4Ba) and CKL (Fig. 4Ca)

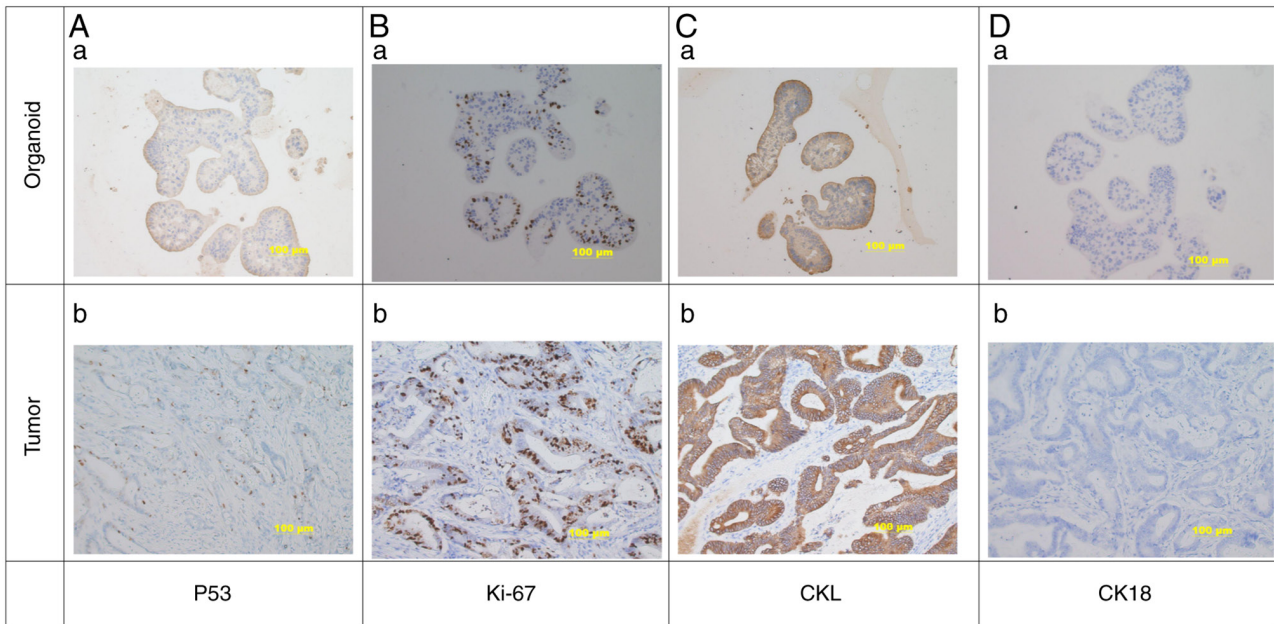


Figure 4. The expression of P53, Ki-67, CKL and CK18 in (A) gastric cancer organoids and (B) original tumor tissue of patient 3. Immunohistochemistry indicated that (Aa) P53, (Ba) Ki-67 and (Ca) CKL were all positively expressed (brown stain) in cultured gastric cancer organoids, while (Da) CK18 was negatively expressed. The results were consistent with the original gastric cancer tissue in terms of expression of (Ab) P53, (Bb) Ki-67, (Cb) CKL and (Db) CK18 (scale bars, 100 μ m). CK18, cytokeratin 18; CKL, cytokeratin low molecular weight.

were all expressed in the cultured gastric cancer organoids, while staining for CK18 was negative (Fig. 4Da). The results were consistent with those for the original gastric cancer tissue (Fig. 4Ab, Bb, Cb and Db).

Chemotherapy drug screening. Paclitaxel, oxaliplatin and fluorouracil were added to the gastric cancer organoids cultured to the 8th day. The morphological changes of the organoids were observed every 24 h. Using light microscopy, it was indicated that, compared with that in the control group (Fig. 5A), the morphology of the experimental group was more disordered than that of the control group with certain organoids exhibiting cell death, among which the paclitaxel and oxaliplatin groups exhibited the most obvious changes. Organoid death gradually increased as the culture time progressed. After 96 h, it was observed that most of the organoids in the paclitaxel and oxaliplatin groups had disintegrated appearance and lost their morphological structure, while only a few organoids in the fluorouracil group had disintegrated (Fig. 5B-D). Patient 3 was treated with oxaliplatin and capecitabine combination chemotherapy for six cycles after surgery; carcinoembryonic antigen (Fig. 6A) and carbohydrate antigen 19-9 (Fig. 6B) have been stable, and no signs of tumor recurrence were found for 7 months after the operation. This verifies the feasibility and effectiveness of organoid drug screening to a certain extent.

Discussion

In the present study, gastric cancer organoids were successfully established by enzymatic digestion of surgically resected gastric cancer tissue to obtain gastric cancer primary cells for subsequent organoid culture. Histochemical staining proved that the original tissue and the cultured PDOs had a high degree of similarity. Finally, the efficacy of a variety of traditional

tumor chemotherapy drugs on the obtained patient tumor tissue organoids was examined to test the relationship between organoid drug screening and clinical treatment. In accordance, the similarity of drug sensitivity between organoids and the patients verified that the construction of the gastric cancer organoid model and its application in individualized drug screening has clinical translational significance.

In China, gastric cancer ranks third in terms of incidence and mortality, accounting for >40% of global cases of gastric cancer (22). Endoscopic or surgical resection is the only potential cure; however, since most patients are in the middle and late stages of the disease, comprehensive treatments, such as surgery, radiotherapy and chemotherapy, targeted therapy, immunotherapy and traditional Chinese medicine, are required to achieve a therapeutic effect (16,23,24).

Individualized chemotherapy is a method of using specific and optimal chemotherapeutic drug regimens according to the pharmacogenetic characteristics of patients with cancers. Individualized chemotherapy may help patients receive appropriate chemotherapy drugs, improve the pertinence of treatment and prolong the overall survival to the greatest extent. In recent years, with the development and maturation of tumor organoid models for screening and selecting appropriate chemotherapy drugs, individualized chemotherapy has become a reasonable choice to improve efficacy and reduce ineffective treatments (16,25-27).

In the present study, gastric cancer organoids were successfully cultured by inoculation in Matrigel and suspension culture. Differing from the organoid culture method including inoculation in Matrigel (28-30), the establishment of gastric cancer organoids by suspension culture has the following advantages: i) In the absence of Matrigel, the cost of the organoid culture may be markedly reduced; ii) in terms of collection, passage, cryopreservation and drug screening, the model is simpler and more

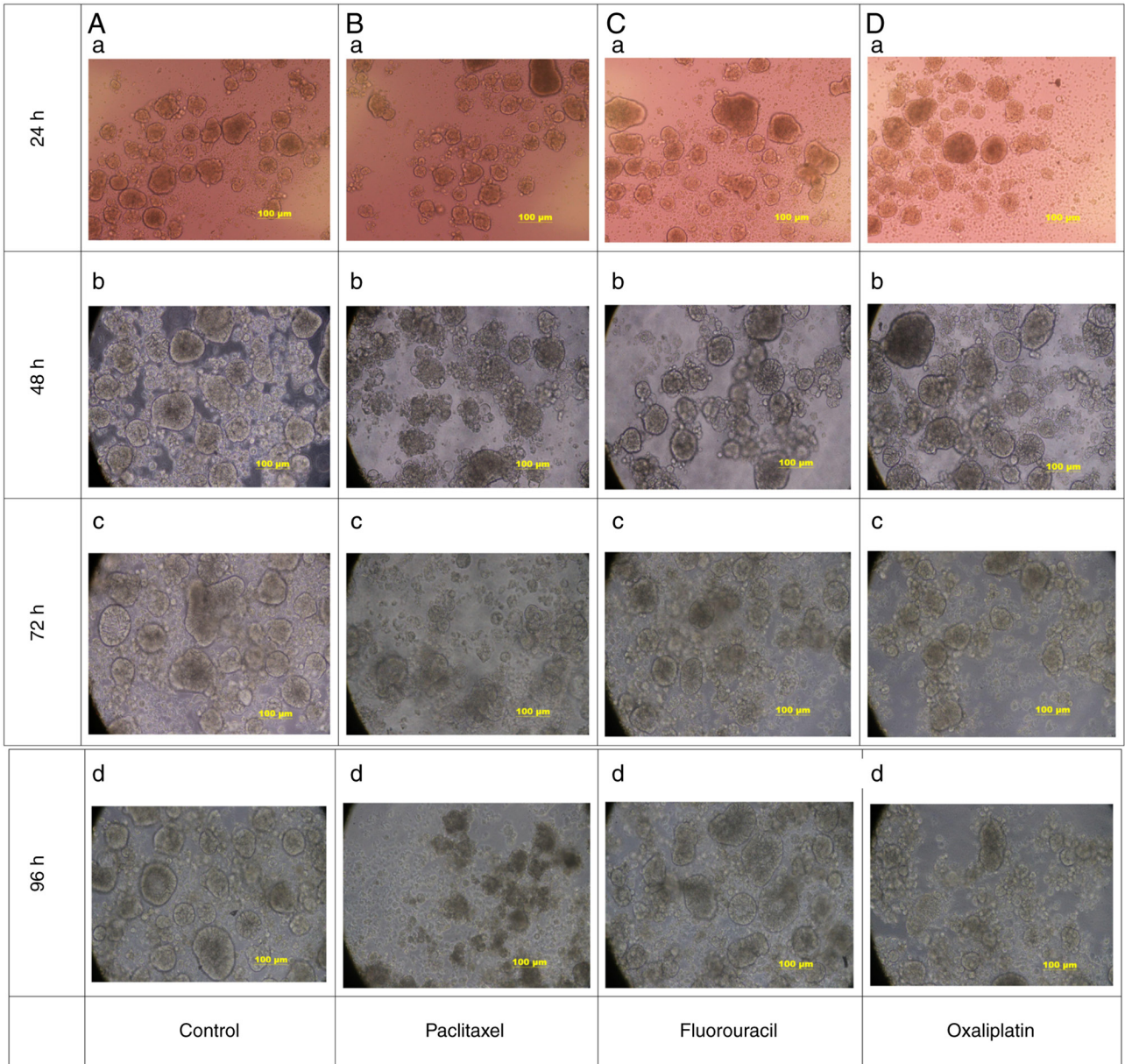


Figure 5. Screening of sensitive chemotherapeutic drugs. Morphology in the (A) control, (B) paclitaxel (C) fluorouracil and (D) oxaliplatin groups. The morphology of the experimental group was more disordered than that of the control group; certain organoids had died, among which the paclitaxel and oxaliplatin groups exhibited the most obvious changes. After 96 h, most of the organoids in the paclitaxel and oxaliplatin groups had disintegrated and lost their morphological structure, while in the fluorouracil group, only a small number of organoids had disintegrated (scale bars, 100 μm).

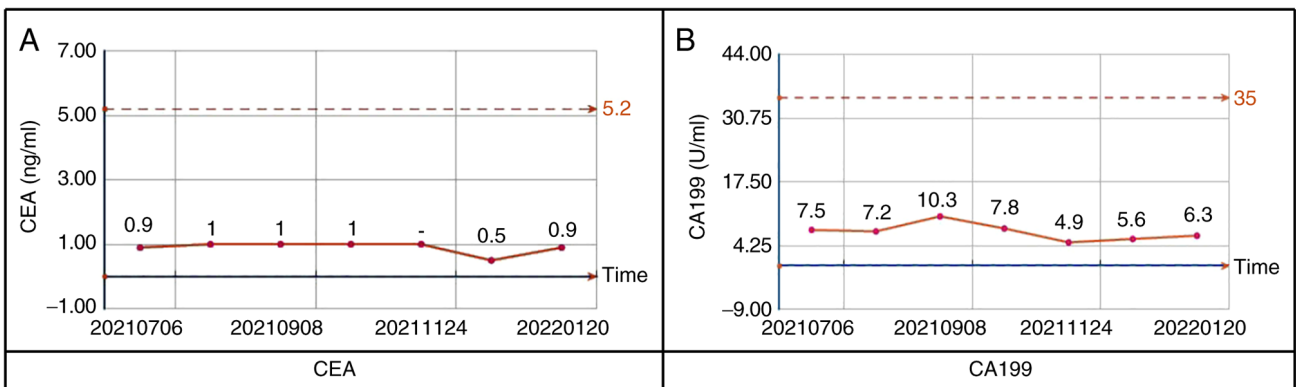


Figure 6. Changes of tumor markers in patient 3 at different time-points. Patient 3 was treated with oxaliplatin and capecitabine combination chemotherapy for six cycles after surgery. (A) CEA and (B) CA199 were stable, and no signs of tumor recurrence were found. Dotted lines indicate the normal reference value. CEA, carcinoembryonic antigen; CA199, and carbohydrate antigen 19-9.

convenient to handle; and iii) without Matrigel in the culture process, the impact of the interaction between mouse-derived components and human-derived tumors is reduced. Regarding the acquisition of primary cells, based on the literature and previous cell culture experience (28-32), it is recommended that during the enzymatic digestion process, tissue digestion is diligently observed and duly terminated in time. In this regard, cell mass-like structures rather than single cells may markedly improve the success rate of cell lines or organoids.

In the present study, an ordinary complete medium was used rather than a protocol of serum-free medium and high concentrations of various growth factors recommended in the literature (17,19,33). The success of this model indicates that for the culture of tumor organoids, the characteristics of the cell itself are most important. Even in a simplified culture system, tumor organoids may be successfully established and maintain the phenotypical and molecular biological characteristics of the primary tumor, which met the needs of subsequent experimental applications, such as drug screening and functional verification.

Previous studies by our group have indicated how fibroblast contamination may be reduced during organoid culture (31,32). First, the epithelial layer should be sampled as much as possible when the tumor tissue is obtained. Furthermore, the digestion time in the process of tumor tissue digestion should be controlled to avoid excessive digestion of the tissue into single cells. In addition, a 100-mesh sieve should be used to remove large pieces of undigested tissue. The rotation speed of the centrifuge should be adjusted to settle the tumor cell clusters and sub-duct structures. Through the above measures, most of the fibroblasts may be removed. Of course, a small number of fibroblasts may remain in the gastric cancer organoid culture. However, this does not obviously conflict with the experimental purpose of organoid culture. As fibroblasts actually exist in gastric cancer tissue, in theory, the presence of fibroblasts in organoids is closer to the actual situation in the human body and the drug sensitivity results obtained are more accurate in terms of the clinical application, which is also the reason for exploring organoid co-culture at present.

The present study further validates the feasibility and effectiveness of tumor organoids for personalized drug screening. However, unlike other tumors, the success rate of gastric cancer organoids is relatively low and the 50% success rate reported in the literature for gastric cancer organoids is already high (19,34). Therefore, the gastric cancer organoid culture system of the present study requires further optimization to continuously improve the success rate of gastric cancer organoid culture. This model may serve as a guide for individualized treatment of gastric cancer, which is important for improving the long-term survival rate of patients with an advanced stage of cancer. As a limitation of the present study, the number of gastric cancer samples used was relatively small and not all differentiated types of gastric cancer were included. In this experiment, the organoids were not quantified. Instead, the organoids were centrifuged and resuspended with culture medium, and the suspension of the same volume was inoculated to each well. The present experiments aim to avoid ineffective treatment through rapid and economical drug selection methods for clinical individualized treatment. The results of the present study are qualitative, not quantitative

results. The present experiments only verified the feasibility of organoid screening for chemotherapeutic drugs. In the next step, the number of samples and types of differentiation will be increased, and it will be endeavored to pursue the quantification of experimental results and refinement of the models, including the co-culture of tumor organoids with patient-derived stromal cells and immune cells for individualized screening of targeted or immunotherapeutic drugs.

In summary, gastric cancer organoids with high similarity to the original tissue may be successfully constructed by the suspension growth culture method. The established organoids may serve as an effective model for individualized drug screening.

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Availability of data and materials

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

Authors' contributions

Conceptualization, HX and WCZ; methodology design, HX, XM, CMW and KXZ; validation, HX, XM, CMW, CPC, HT; formal analysis, HT and JJH; investigation, ZJZ and HZ; resources, HX, CPC, HT, KXZ; collection of clinical data in the study, and the collection and backup of records and photos generated during the experiment, WL and KXZ; writing-original draft preparation, XM, CMW and KXZ; writing-review and editing, HX and WCZ; visualization, XM, CPC, HT, WL and KXZ; supervision, HX, KXZ and WCZ; project administration, HX, WCZ; funding acquisition, HX, CPC, ZJZ and WL. XM, CMW, KXZ, WCZ and HX checked and confirmed the authenticity of the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the institutional Ethics Committee of the First Hospital of Lanzhou University (Lanzhou, China; no. LDYLL2022-292). The patients provided written informed consent for the use of their tissues.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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