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Patient-derived bladder cancer organoid model to predict sensitivity and feasibility of tailored precision therapy

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Abstract

Background: Bladder cancer is a common and highly heterogeneous malignant tumor with a relatively poor prognosis. Thus, personalized treatment strategies for bladder cancer are essential for improving patient outcomes.

Materials and methods: We developed an efficient 3-dimensional in vitro organoid culture system for bladder cancer organoids (BCOs), which maintains the homology with the original patient tumors and the heterogeneity between different individuals. In addition, we constructed chimeric antigen receptor (CAR)-T cells targeting B7H3 and evaluated the antitumor function of CAR-T cells by coculturing them with BCOs.

Results: The BCOs closely resembled the characteristics of human tumors and were used to test individual sensitivity to platinum-based drugs and olaparib therapy. Coculture with CAR-T cells demonstrated specific antigen recognition and immune activation, indicating their potential in immunotherapy.

Conclusions: Our study highlights the potential of BCOs to facilitate the development of personalized medicine for bladder cancer and improve the efficiency of drug discovery for bladder cancer therapy.

Keywords: Bladder cancer; Chimeric antigen receptor T cells; Therapy response prediction; B7H3; Organoid

1. Introduction

Urinary bladder cancer is one of the most prevalent malignancies affecting the urinary system and can be classified as non–muscle-invasive bladder cancer or muscle-invasive bladder cancer.[1,2] Following transurethral resection of bladder tumors, additional intravesical chemotherapy and/or immunotherapy are required, in accordance with tumor grade, to prevent recurrence and open surgical resection.[3] Although transurethral resection of bladder tumors and neoadjuvant platinum combined chemotherapy are considered the standard treatment for muscle-invasive bladder cancer, posttreatment relapse rates remain high.[4,5] Through large-scale gene expression and sequencing efforts, researchers have come to understand that urothelial bladder cancer is a heterogeneous epithelial malignancy.[6,7] Various bladder cancer therapies are being developed using tumor cell lines grown and tested in traditional 2-dimensional (2D) cell culture containers to identify predictors of drug sensitivity.[8] However, numerous anticancer therapeutics developed from transformed or immortalized cell lines using conventional 2D culture have failed in clinical studies.[9,10] The high variability of driving mutations found in individual tumors and between patients poses a challenge to precise and personalized therapy, and new predictive models are urgently needed.[11–13]

Bladder cancer organoids (BCOs) are 3-dimensional in vitro tumors that retain tumor heterogeneity and a mutation spectrum that matches the primary cancer.[14–16] In addition, organoids exhibit phenotypic stability and plasticity.[17] Compared with a 2D cell culture system, BCOs also exhibit significant differences in drug sensitivity, which reveal significant similarities and differences between organoid lines, as well as partial correlation with their gene mutation spectra.[18,19]

Combination chemotherapy with cisplatin has been the standard treatment for bladder cancer since the late 1980s.[20] However, up to 50% of patients are not suitable candidates for cisplatin chemotherapy, some of whom may be suitable candidates for carboplatin therapy, whereas others may not be suitable candidates for any platinum chemotherapy.[21,22] The recent application of immune checkpoint inhibitors has shown promising results in the treatment of patients with bladder cancer.[23] Poly-ADP-ribose polymerase inhibitors, such as olaparib, target tumors with homologous recombination repair defects and are highly effective drugs that trigger synthetic lethality in patients with germline or somatic defects in DNA repair gene.[24,25] Various studies have reported that Poly-ADP-ribose polymerase inhibitors, as a single agent or in combination with platinum therapy, have great potential in the treatment of urothelial bladder cancer.[26]

Chimeric antigen receptor (CAR)-T therapy is a promising approach in tumor treatment. Traditional in vitro assessment methods involve incubating CAR-T cells with tumor cell lines that harbor targeted antigens. However, these methods are disadvantageous in accurately predicting the in vivo therapeutic potential as tumor cell lines are unable to mimic the complex tumor microenvironment. Fortunately, BCOs cocultured with CAR-T cells provide an effective means of evaluating immunotherapy in a variety of patient models.

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In this study, we developed a method to efficiently culture BCOs that closely resemble the characteristics of human tumors. We used these BCOs as a drug-screening platform to reveal characteristics of different drug sensitivities (Fig. 1). In addition, we prepared CAR-T cells targeting B7H3, which is highly expressed in most types of cancers but exhibits limited distribution in normal tissues (Supplementary Fig. S1, http://links.lww.com/CURRUROL/A43). Finally, we verified the functional toxicity of CAR-T cells by coculturing with the patient-derived BCOs expressing B7H3 (Fig. 1).

2. Materials and methods

2.1. Bladder cancer organoid culture

The samples were taken from 11 patients treated in the Urology Department of Shandong Provincial Hospital from March 9, 2022, to September 27, 2022, and were cultured separately as follows: 10 cases were derived from tumor tissues, numbered as BCO1T, BCO2T, BCO3T, BCO5T, BCO6T, BCO7T, BCO8T, BCO9T, BCO10T, and 1 case was obtained from ascites, numbered as BCO4A. After obtaining informed consent from patients and their immediate family members, intraoperative tumor samples were acquired and immediately preserved in DMEM/F12 (Gibco 11320033[Gibco, San Diego, CA]) containing 10 μM Y-27632 (Selleck S1049 [Selleck Chemicals, Houston, TX]), and the samples were transported to the laboratory at 4°C. Sterilized scissors were used to cut the tissue into 1-mm^3 samples, and the samples were placed on ice. The tissue samples were then digested in 3 mL of 5 mg/mL collagenase type II (Worthington LS004176 [Worthington Biochemical Corporation, Lakewood, NJ]) dissolved in Advance DMEM/F-12 (12634028l ThermoFisher [Thermo Fisher Scientific, Waltham, MA]) with 10 μM Y-27632 for 45 minutes at 37°C. The resulting cell suspension was filtered through a 100 μm nylon cell strainer (431752; Corning [Corning, New York, NY]) and centrifuged for 3 minutes at 1500 revolutions/min. If the resulting precipitate contained a lot of red blood cells, it was lysed with 1 mL 1× red blood cell lysis buffer (00-4333-57; ThermoFisher) at room temperature (20–25°C) for 3 minutes. The cells were collected by centrifugation and resuspended in 100 μL Advance DMEM/F12 and 150 μL Matrigel (356231; Corning), and then seeded into 4 individual wells of a 24-well plate. Bladder cancer organoids were cultured in Advanced DMEM/F12 medium containing HEPES (10 mM of Gibco 15630080), 1× penicillin/streptomycin, GlutaMAX Supplement (2 mM of Gibco 35050079), epidermal growth factor (EGF) (10 ng/mL of Novoprotein [Novoprotein, Summit, NJ]), FGF-10 (100 ng/mL of Novoprotein), FGF-7 (25 ng/mL of Novoprotein), FGF-2 (12.5 ng/mL of Novoprotein), B-27 supplement Minus Vitamin A (2% of Gibco 12587010), A83-01 (5 μM of Abmole M5037 [Abmole, Houston, TX]), *N*-acetylcysteine (1.25 mM of MedChem Express, Monmouth Junction, NJ), and nicotinamide (10 mM of MedChem Express). For samples taken from ascites, tissue cutting was omitted, and the remainder of the procedure was unchanged. The organoids were passaged at 1:2 or 1:3 every 3 to 4 days, and 10 μM Y-27632 was added to the media after passaging. The organoids were frozen in 90% fetal bovine serum and 10% dimethyl sulfoxide.

2.2. Immunohistochemistry and immunofluorescence assay of cancer tissue and bladder cancer organoids

The cancer tissues were immobilized in 4% paraformaldehyde for 1 to 6 hours and dehydrated, and 4-μm paraffin sections were stained according to a standard protocol using the following antibodies: CK20 (CST, 13063, 1:200), Ki-67 (14-5698-82, 1:200; eBioscience [eBioscience, San Diego, CA]) and B7H3 (14058S, 1:200; CST [Cell Signaling Technology, Danvers, MA]).

For immunofluorescence, the organoids were fixed in 4% formaldehyde solution for at least 45 minutes. The organoids were blocked and permeated with 0.5% Triton in phosphate-buffered saline (PBS) for 20 minutes. Next, the organoids were sealed with 2% bovine serum albumin V (BSA; Solarbio, Beijing, China). The primary antibody was incubated in 0.1% Triton in PBS at 4°C overnight. The antibodies used in this experiment were CK20 (13063, 1:200; CST), Ki-67 (14-5698-82, 1:200; eBioscience) and B7H3 (14058S, 1:200; CST). After incubation with the primary antibody, the organoids were washed and then incubated with fluorophore-conjugated secondary antibodies (1:200; Invitrogen, Carlsbad, CA) at room temperature for 2 hours. Finally, DNA was stained with 4′,6-diamidino-2-phenylindole (Yeasen, Shanghai, China).[27] The resulting images were acquired on a Mantra fluorescence microscope (CLS140089/B; PerkinElmer Inc, Hopkinton, MA).

2.3. Drug treatment organoid viability assays

The BCOs were released from the Matrigel by incubating in TrypLE (12605036; ThermoFisher) for 4 minutes at 37°C, after which they were resuspended in Advance DMEM/F12 medium. After diluting the mixture with a 1:1.5 ratio of Advance DMEM/F12 medium to Matrigel, it was separated into a 384-well plate (Corning) at 10 μL per well (3000 organoids per well). This procedure was performed on ice, and the 384-well plate was precooled in advance. A blank group with 10 μL per well was created by mixing the same ratio of Advance DMEM/F12 medium and Matrigel. The plate was then solidified in a 5% CO_2 cell culture incubator at 37°C for 30 minutes, and 54 μL BCO medium was added to each well.

In this experiment, we tested 4 drugs: cisplatin, carboplatin, oxaliplatin (Sigma-Aldrich, St Louis, MO) and olaparib (MedChem Express). The maximum concentration of drugs in vitro was set

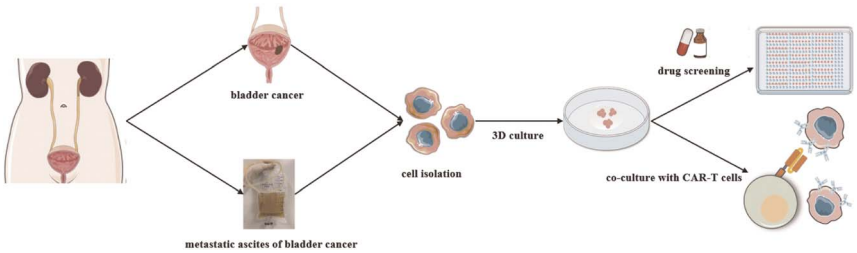

Figure 1. Bladder cancer organoids demonstrate high-throughput drug screening and evaluate sensitivity to CAR-T cell–mediated killing, indicating accurate treatment potential. CAR = chimeric antigen receptor.

according to the clinical peak plasma concentration of each drug, followed by 4-fold dilution, with 6 concentration gradients for each drug. Three days later, half of the medium was changed, and 6 μL of drug-containing medium was added to each well. During the treatment period, the organoids were viewed under a microscope and photographed. After 4 days, cell viability was assayed using CellTiter-Glo Luminescent Cell Viability Assay (Promega Corporation, Madison, WI), following the protocol. Contents were mixed vigorously for 2 minutes to induce cell lysis and then incubated at room temperature for 8 minutes to stabilize the luminescent signal. Chemiluminescence detection was performed by recording luminescence using a microplate chemiluminescence reader. Data analysis to calculate the half-maximal inhibitory concentration (IC_{50}) was performed using GraphPad Prism 9.0 software (GraphPad, San Diego, CA).

2.4. Plasmid construction

The construction of CD19 CARs follows the method referred to in a previous publication,[28] with the single-chain fragment variable of CD19 CAR being replaced by B7H3 single-chain fragment variable to construct the B7H3 CAR.[29]

2.5. Lentivirus preparation and construction of chimeric antigen receptor T cells

HEK293FT cells (laboratory stocked) were infected with Lipofectamine 3000 (Invitrogen). After 72 hours' transfection, the virus supernatant was harvested, filtered through a 0.45-μm filter, and stored at −80°C. Human primary T cells were isolated from peripheral blood mononuclear cells using anti-CD3 microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany). The isolation, activation, culture, and transduction of human primary CD3 T cells were performed as previously described.[30]

2.6. Flow cytometry

Chimeric antigen receptor-T cell transfection rate detection: On the fifth day after T cell transfection with PGK-Enhanced Green Fluorescent Protein (EGFP)-CD19-CAR and PGK-EGFP- B7H3-CAR, luminescence was observed under fluorescence microscopy. Then, 10^5 cells were collected and suspended with PBS and analyzed via the fluorescein isothiocyanate channel (Agilent, Santa Clara, CA). *Detection of B7H3 expression:* B7H3 expression on the cell surface was detected using PerCP/cyanine5.5 anti-human CD276 (B7H3) antibody (BioLegend, San Diego, CA). An appropriate amount of BCOs and cells were collected and resuspended in PBS. Flow cytometry antibodies were added to the suspension and incubated for 15 minutes in the dark at room temperature and then washed twice for flow cytometry analysis. Unstained cells were used as controls to normalize the staining intensity. All data were processed using FlowJo v10 software (FlowJo LLC, Ashland, OR).

2.7. Quantification of cell viability and luciferase activity

T24-Luc cells, human bladder cancer cell lines that have been transfected and screened in our laboratory, were cultured in complete medium containing McCoy's 5A (Procell, Wuhan, China), 10% fetal bovine serum, and 1% penicillin/streptomycin. The T24-Luc cells were used as target cells and seeded into 96-well plates. Next, 200 μL of X-VIVO 15 medium (Lonza, Basel, Switzerland) containing 100 IU/mL of interleukin 2 (IL-2) (Dakewe Biotech Co, Ltd, Shenzhen, China) was added to each

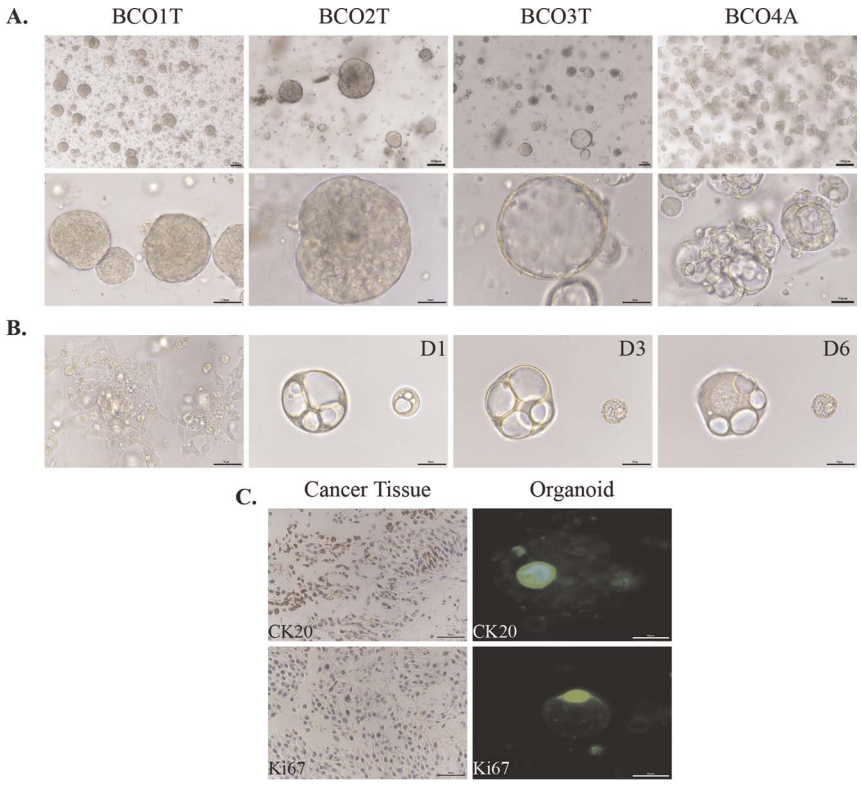

Figure 2. Bladder cancer organoids formed rapidly (<7 days) and maintained biological characteristics of primary tumor tissue when embedded in basement membrane matrix in vitro. (A) Organoids derived from bladder cancer tissues: BCO1T, BCO2T, and BCO3T; organoids derived from metastatic ascites: BCO4A. (B) Adherent morphology and 3-dimensional shape of BCOs after prolonged culture. (C) Immunofluorescence of BCOs for CK20 (green) and Ki-67 (green); immunohistochemistry of cancer tissue for CK20 (brown) and Ki-67 (brown). BCOs = bladder cancer organoids.

well. Effector viable T cells were then added to the target cells in a ratio determined by the designed effector-target ratio until the degree of cell convergence reached between 75% and 85%.

After coculturing the cells for 24 hours, they were harvested by centrifugation at 500 g for 8 minutes and lysed in 1× lysis buffer (Promega) for 30 minutes. The resulting cell suspensions were collected and mixed with an equal volume of luciferase assay substrate (Promega) to quantify luciferase activity, which was measured in relative light units using a TriStar 2 multimode reader (Berthold Technologies, Bad Wildbad, Germany).

2.8. Coculture of bladder cancer organoids with chimeric antigen receptor T cells

Bladder cancer organoids were harvested using 2 mL of TrypLE at 37°C for 4 minutes and centrifuged at 300 g for 3 minutes. Digestion was terminated by diluting with 4 mL of Hanks balanced salt solution, followed by centrifugation at 300 g for 3 minutes, after which the supernatant was removed. The dissociated organoids were resuspended in the BCO medium and inoculated in the middle of a precoated 96-well plate containing 50% Matrigel. After being incubated overnight, the BCO medium was discarded, and 200 μL of X-VIVO 15 was used to resuspend control T cells and B7H3 CAR-T cells.

2.9. Cytokine secretion assay

B7H3 CAR-T cells and BCOs were coincubated for 24 hours at a 5:1 ratio in cytokine-free medium. After coincubation, supernatants were collected to measure the levels of IL-2 and interferon γ (IFN-γ) using a human IL-2 enzyme-linked immunosorbent assay kit and an IFN-γ enzyme-linked immunosorbent assay kit (Dakewe Biotech Co, Ltd), respectively, following the manufacturer's protocol. The optical density was measured at 450 nm using the TriStar 2 multimode reader (Berthold Technologies).

2.10. Statistical analysis

All experiments in this study were conducted at least 3 times, and the results were expressed as the mean ± standard error. Data analysis was performed using GraphPad Prism 9.0 software, and statistical significance was determined using the Student *t* test with a significance level set at $p < 0.05$.

3. Results

3.1. Cultured bladder cancer organoids resembling human disease

Bladder cancer organoids were successfully established from 9 of 11 fresh transurethral resection of bladder tumor samples (Supplementary Table 1, http://links.lww.com/CURRUROL/A43), with a success rate of approximately 82%. Initially, organoid formation was observed in DMEM/F12 medium with freshly cut bladder cancer samples (Fig. 2A). However, after 2 days, organoid growth stagnated, and the form gradually disintegrated. We found that BCOs displayed adhesive growth in the early stages of culture, hindering the formation of a 3-dimensional structure and potentially affecting follow-up experiments. However, increasing the proportion of Matrigel to 60% and prolonging the inversion time to 45 minutes improved the issue of

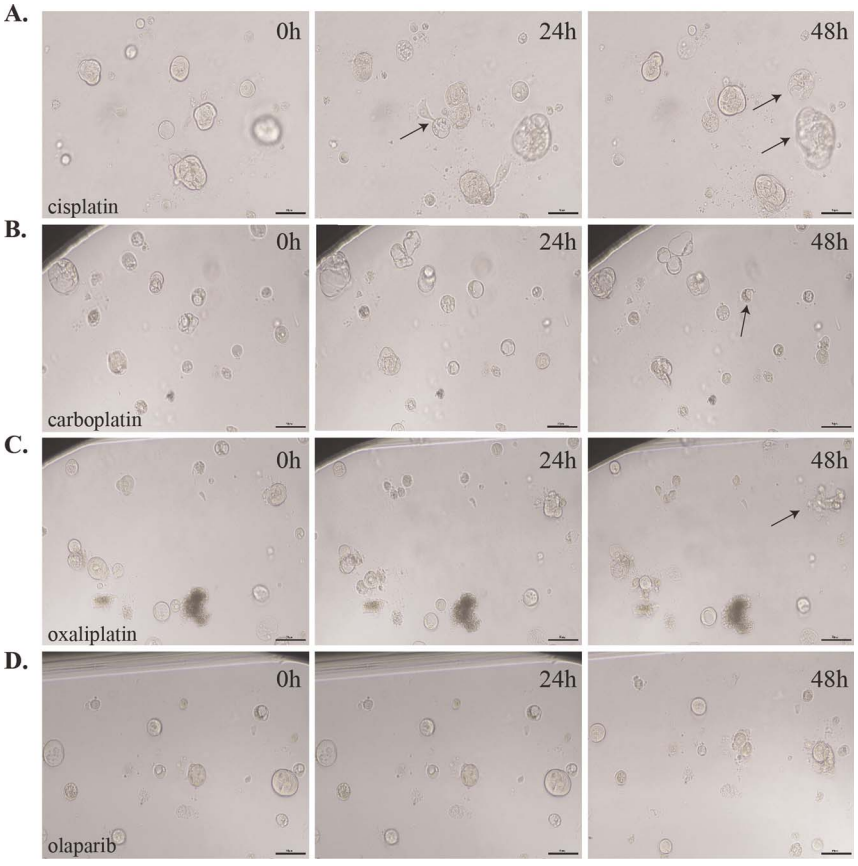

Figure 3. Bladder cancer organoids show varying sensitivities when subjected to the chemotherapy drugs. (A–D) Bright-field images depict changes in the size and status of BCOs treated with 2.5 μM cisplatin, carboplatin, oxaliplatin, and olaparib at 0, 24 and 48 hours, as indicated by the black arrows. BCOs = bladder cancer organoids.

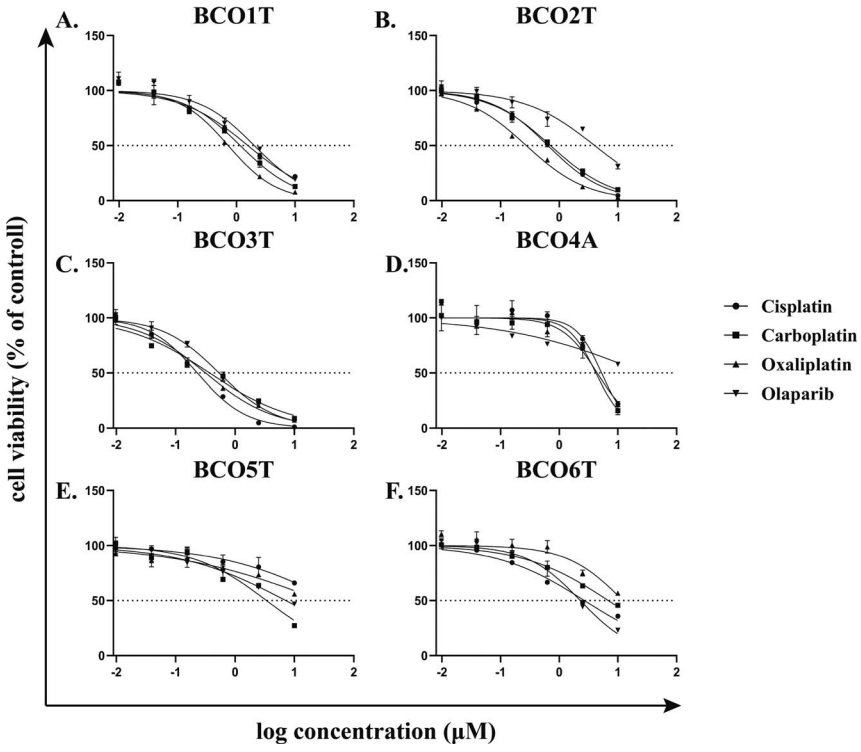

Figure 4. Organoids grew over 7 days and were treated with varying concentrations of cisplatin, carboplatin, oxaliplatin, and olaparib for 72 hours, and IC_{50} was calculated. (A–F) Standardized IC_{50} values of BCOs were analyzed using paired *t* test to compare sensitivity to the 4 types of drugs. BCOs = bladder cancer organoids; IC_{50} = half-maximal inhibitory concentration.

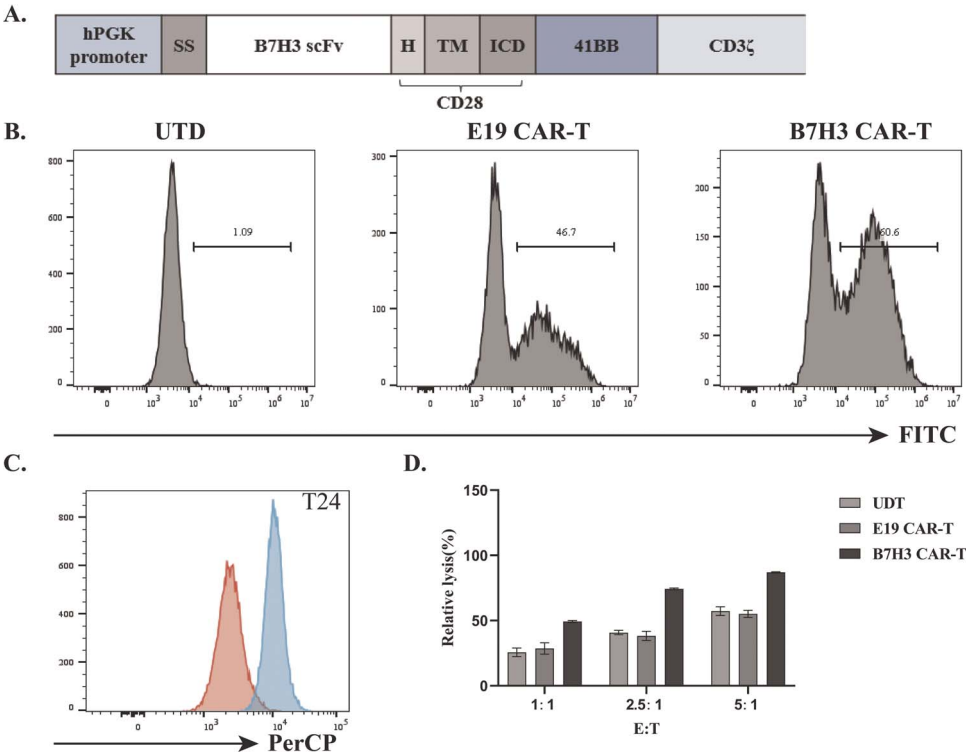

Figure 5. Antitumor responses in bladder cancer cell lines by targeting B7H3 CAR-T cells. (A) Diagram showing the construction of B7H3 CAR-T. (B) Transduction efficiency of E19 CAR and B7H3 CAR on T cells was detected by flow cytometry. (C) Analysis of B7H3 expression on T24 cells by flow cytometry. (D) Cytotoxicity of the specified CAR-T cells upon varying E:T ratio was measured by the luciferase-based killing assay. CAR = chimeric antigen receptor; E:T = effector-target ratio.

adhesion. Bladder cancer organoids were usually solid spherical or hollow cystic structures with less smooth edges (Figs. 2A, B).

To further characterize the BCOs, we performed immunohistochemical or immunofluorescence analysis on the expression of markers in organoids and their corresponding parental tumor tissue. Our results showed that the expression profiles of markers were consistent in tumor tissues and their derived organoids. Bladder cancer organoids and parent tumors expressed Ki67 and CK20 (a luminal marker), confirming the epithelial origin and high proliferative activity of the BCOs (Fig. 2C). In summary, BCOs retained the histopathological structure of parental tumors, thus maintaining homology with the original individual patients and heterogeneity between different individuals.

Bladder cancer cells were also isolated from malignant metastatic ascites and implanted in Matrigel. We observed that the isolated cells mostly formed spheroid clusters with a wide range of sizes (Fig. 2A). Wright-Giemsa staining revealed a large number of tumor cells from ascites-derived cells. The cells appeared piled with less cell mass, blue color, large nuclei, high nucleus-to-cytoplasm ratio, dense chromatin, and obvious nucleoli (Supplemental Fig. S2, http://links.lww.com/CURRUROL/A43). Cell morphology analysis was consistent with urothelial carcinoma.

3.2. Individual sensitivity of bladder cancer organoids to platinum-based drugs and olaparib therapy

The established BCOs exhibited heterogeneous responses to chemotherapy. Because none of the patients had ever received chemotherapy, there was no acquired resistance, enabling us to study the heterogeneity of responses and individual differences of the organoids. We treated 5 BCOs derived from tumor tissues and 1 organoid derived from metastatic ascites in vitro with cisplatin, carboplatin, oxaliplatin, and olaparib, respectively. Microscopic observation showed changes in platinum-sensitive BCOs over time after drug treatment, with black arrows indicating some examples of organoids disintegrated into debris piles (Figs. 3A–C).

There were differences in the in vitro chemosensitivity of the BCOs to platinum-based drugs and olaparib. BCO1T, BCO2T, BCO3T, and BCO6T demonstrated relatively high sensitivity to platinum chemotherapeutic drugs and olaparib (Figs. 4A–C and F). BCO4A showed high sensitivity to platinum chemotherapeutic drugs but a

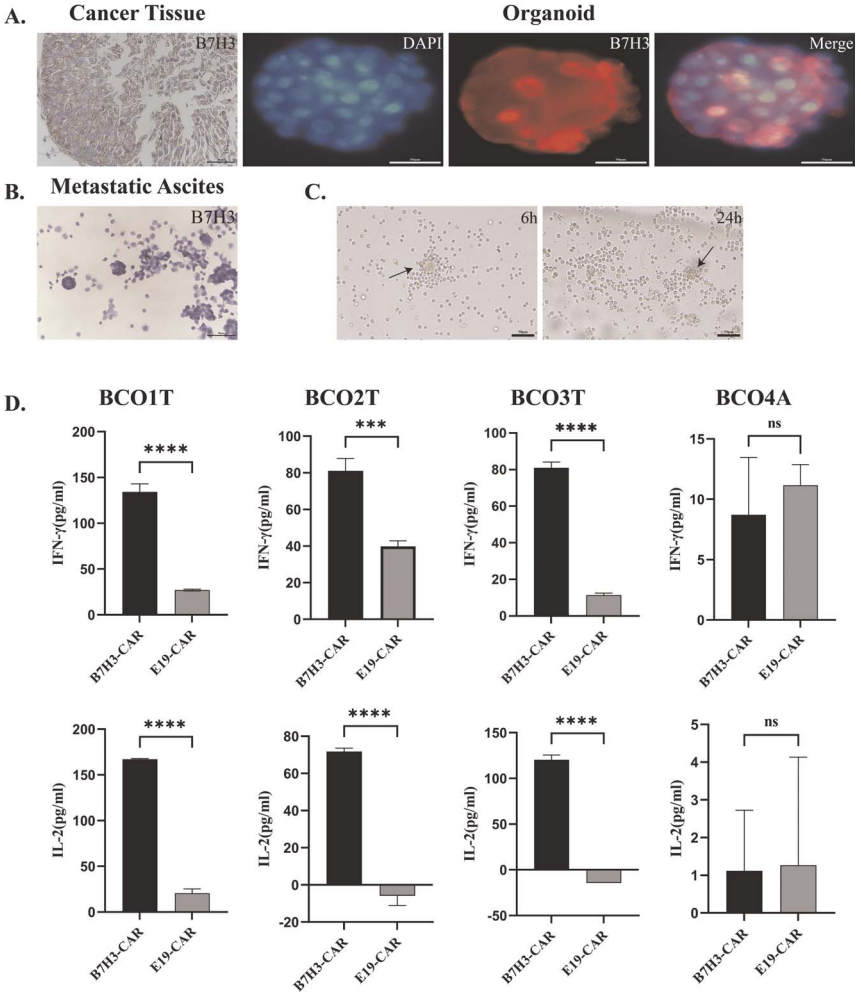

Figure 6. Antitumor responses in BCOs by targeting B7H3 CAR-T cells. (A) Immunohistochemical staining shows bladder cancer tissue stained with B7H3 (brown), and organoids were stained for B7H3 (red) using immunofluorescence staining. Nuclei counterstained with DAPI (blue). (B) Immunohistochemical staining shows metastatic ascites not stained with B7H3. (C) Images of coculture of BCOs with B7H3 CAR-T cells at 24 hours. (D) ELISA of effector cytokines (IL-2 and IFN-γ) from B7H3 CAR-T cells or CD19 CAR-T cells with BCOs. Data represent mean ± SD, $^{*}p \le 0.05$, $^{**}p \le 0.01$, $^{***}p \le 0.001$, $^{****}p \le 0.0001$. BCO = bladder cancer organoids; CAR = chimeric antigen receptor; DAPI = 4′,6-diamidino-2-phenylindole; ELISA = enzyme-linked immunosorbent assay; IFN-γ = interferon γ; IL-2 = interleukin 2; ns = not significant.

weaker response to olaparib. However, BCO5T displayed less sensitivity to cisplatin (IC_{50} = 44.4 μM) and oxaliplatin (IC_{50} = 27.65 μM). Interestingly, BCO5T showed relatively high sensitivity to olaparib (IC_{50} = 7.148 μM). This result suggests that olaparib could also be effective in patients who are not sensitive to platinum drugs. On the contrary, the most notable finding was that bladder cancer cells from ascitic fluid were sensitive to platinum drugs but resistant to olaparib (IC_{50} = 27.57 μM) (Fig. 4D). Thus, the BCOs showed heterogeneity in their chemoresponse to cisplatin, carboplatin, oxaliplatin, and olaparib doses.

3.3. Quantitative analysis and verification of B7H3 chimeric antigen receptor–mediated cytotoxicity

We constructed a third-generation CAR molecule targeting B7H3 (Fig. 5A). The expression of EGFP on T cells after lentivirus transduction was confirmed by flow cytometry (Fig. 5B). Chimeric antigen receptor-T cells targeting CD19 were used as a control. Using a luciferase assay, we selected bladder cancer cell line T24-luc cells expressing B7H3 (Fig. 5C) to further verify the toxicities of B7H3 CAR-T cells. Compared with untransduced T cells and E19 CAR-T cells group, B7H3 CAR-T cells showed specific and significant toxicities with T24-luc cells (Fig. 5D).

3.4. Functional test of B7H3 chimeric antigen receptor T cells in bladder cancer organoids

To assess the expression of B7H3 in bladder tumor tissue and BCOs by immunohistochemistry and immunofluorescence, we selected 3 BCOs expressing B7H3 and ascites cells that did not express B7H3 (Figs. 6A, B). To evaluate the effectiveness of BCOs in testing the immunotherapeutic response to bladder cancer, we cocultured B7H3 BCOs with B7H3 targeting CAR-T cells. After coculture for 24 hours, culturing on a Matrigel-coated layer resulted in marked CAR-induced lysis (Fig. 6C). Compared with CD19 CAR-T cells, when B7H3 CAR-T cells were cocultured with B7H3 BCOs, the secretion of cytokines IFN-γ and IL-2 was significantly increased. However, there was no significant difference in the results of ascites-derived organoids without B7H3 expression (Fig. 6D). These results suggest that specific antigen recognition and immune activation occur during coculture.

4. Discussion

Bladder cancer is a common malignant tumor worldwide, with approximately 80,000 to 90,000 new cases diagnosed annually in China. The incidence rate is higher in males than in females, and the elderly population has a higher incidence and mortality rate than younger people. The histological subtypes of bladder cancer are related to local invasion, metastasis, and poor response to treatment, and personalized management should be applied to these patients. Although progress has been made in cancer initiation in recent years, the commonly used cancer models cannot fully reproduce the histological complexity and genetic heterogeneity of human tumors. Therefore, using organoid models derived from patient tumors may be a better model for identifying and testing new anticancer drugs. Organoids can be derived from adult stem cells or pluripotent stem cells and exhibit self-organizing abilities that allow for disease modeling, potentially leading to the development of patient-specific drug testing and personalized treatment plans.

While precision medicine holds promise for improving cancer treatment, the majority of paired drug regimens used in oncology clinical trials have never been approved by the US Food and Drug Administration.[31] High-throughput sequencing has been widely used in precision medicine research to identify somatic mutations that could be used in cancer treatment and drug development,[32] but the role of genome analysis is limited in predicting responses to targeted therapy.[33] Therefore, precision targeted therapy could be a major breakthrough in clinical cancer therapy. The combination of gene detection and corresponding experiments using organoids could not only improve the efficiency of treatment but also provide clear and accurate therapeutic choices for patients.

Organoids have the potential to simulate human physiology and disease, but there are limitations to their application to cancer research, such as their inability to simulate complex multicellular systems and the intricate ecosystem and interactions of tumors. Therefore, future research should continue to explore and develop more realistic and comprehensive cancer models. Further research will be required, including trials involving the continuous treatment of clinical patients, to confirm and expand upon the findings of this study.

5. Conclusions

The BCO model has been established to determine drug sensitivity and immune-specific responses in vitro. This strategy has the potential to overcome the limitations of personalized immunotherapy for bladder cancer and other solid tumors and to accelerate clinical translation.

Acknowledgments

None.

Statement of ethics

This study was approved by by the Ethics Committee of the Second Hospital of Shandong University (Approval No. KYLL-2023LW052). Informed consent was obtained from all patients before the experiments were conducted. All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Conflicts of interest statement

DT is the managing editor of *Current Urology*. This article was accepted after a normal external review. No conflict of interest has been declared by the other authors.

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Author contributions

YJ, DT, WZ: Conceptualization and design of the experiments;
YJ, X. Sun, X. Song, ZL: Acquisition of the data;
YJ, PZ: Analysis and interpretation of the data;
YJ, WZ: Writing and review of the manuscript.

Data availability

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

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