Research article

Patient tissue-derived FGFR4-variant and wild-type colorectal cancer organoid development and anticancer drug sensitivity testing

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ABSTRACT

Objectives: FGFR4-variant and wild-type colorectal cancer (CRC) organoids were developed to investigate the effects of FGFR4-targeted drugs, including FGFR4-IN and erdafitinib, on CRC and their possible molecular mechanism.

Methods: Clinical CRC tissues were collected, seven CRC organoids were developed, and whole exome sequencing (WES) was performed. CRC organoids were cultured and organoid drug sensitivity studies were conducted. Finally, an FGFR4-variant (no wild-type) CRC patient-derived orthotopic xenograft mouse model was developed. Western blot measured ERK/AKT/STAT3 pathway-related protein levels.

Results: WES results revealed the presence of FGFR4-variants in 5 of the 7 CRC organoids. The structural organization and integrity of organoids were significantly altered under the influence of targeted drugs (FGFR4-IN-1 and erdafitinib). The effects of FGFR4 targeted drugs were not selective for FGFR4 genotypes. FGFR4-IN-1 and erdafitinib significantly reduced the growth, diameter, and Adenosine Triphosphate (ATP) activity of organoids. Furthermore, chemotherapeutic drugs, including 5-fluorouracil and cisplatin, inhibited FGFR4-variant and wild-type CRC organoid activity. Moreover, the tumor volume of mice was significantly reduced at week 6, and *p*-ERK1/2, *p*-AKT, and p-STAT3 levels were down-regulated following FGFR4-IN-1 and erdafitinib treatment.

Conclusions: FGFR4-targeted and chemotherapeutic drugs inhibited the activity of FGFR4-variant and wild-type CRC organoids, and targeted drugs were more effective than chemotherapeutic drugs at the same concentration. Additionally, FGFR4 inhibitors hindered tumorigenesis in FGFR4-variant CRC organoids through ERK1/2, AKT, and STAT3 pathways. However, no wild-type control was tested in this experiment, which need further confirmation in the next study.

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1. Introduction

Colorectal cancer (CRC) is the third most prevalent malignancy, and it ranked second in cancer-related deaths globally [1]. Early detection of CRC may help to reduce mortality and morbidity worldwide [2]. Currently, various treatment strategies are available for CRC, including surgery, chemotherapy, radiotherapy, and targeted therapy [3]. Drug resistance remains a major limitation in treating CRC despite significant advances in the past decades [4]. In general, various intrinsic mechanisms and adaptive responses of tumor cells to oncologic agents frequently cause treatment failure and tumor recurrence [5]. The most direct cause of drug resistance is the abnormal metabolism, transport, or targeting of antitumor drugs [6]. Therefore, investigating the relevant mechanisms of drug resistance is important for treating CRC.

Cancer organoids are a three-dimensional (3D) culture system of tumor cells derived from tumor tissue [7]. Organoids can self-renew and organize themselves so that they resemble the original organ or tumor in structure and function [8]. Organoids can be efficiently grown from patient-derived tumor tissue, which may enable patient-specific drug testing and personalized therapeutic regimen development [9]. In recent years, advances in 3D culture methods have facilitated the development of patient-derived organoids (PDOs) as an indispensable tool for studies related to CRC molecular biology [10]. Human CRC PDOs allow direct assessment of the effect of molecular alterations and treatments on tumor cell chemotherapy response, tumor-microenvironment interactions, and other CRC biology aspects [11]. Organoids are used as highly promising preclinical models for drug sensitivity testing. Studies have revealed that cancer organoids are highly predictive of drug response in metastatic CRC [12]. Therefore, we aimed to conduct drug sensitivity studies in CRC organoids.

As a heterogeneous disease, approximately 10 %–16 % of patients with CRC have pathogenic variants of cancer susceptibility genes [13]. Variants or abnormal expression of protein tyrosine kinases is one of the primary causes of cancer [14]. Fibroblast growth factor receptors (FGFRs) are a subfamily of tyrosine kinase receptors with four isoforms, including FGFR1, FGFR2, FGFR3, and FGFR4 [15]. Their aberrant expression is the primary cause of tumorigenesis and thus FGFR inhibition is considered an important target for cancer therapy [16]. The majority (66 %) of FGFR gene alterations involve gene amplification, followed by mutations (26 %) and rearrangements that produce fusion proteins (8 %) [17]. Elevated FGFR4 levels are associated with CRC development, advanced stage, and distal metastasis. Amino acid changes and FGFR4 expression level alterations due to genetic polymorphisms may affect CRC progression [18]. Additionally, studies targeting FGFR4 have revealed its applicability in CRC treatment [19]. The targeting drugs for FGFR4-variants have been developed and studied in various cancers, but their role in the FGFR4-variant CRC remains unknown.

Therefore, based on the above-mentioned studies, we developed CRC organoids and conducted whole exon sequencing (WES).
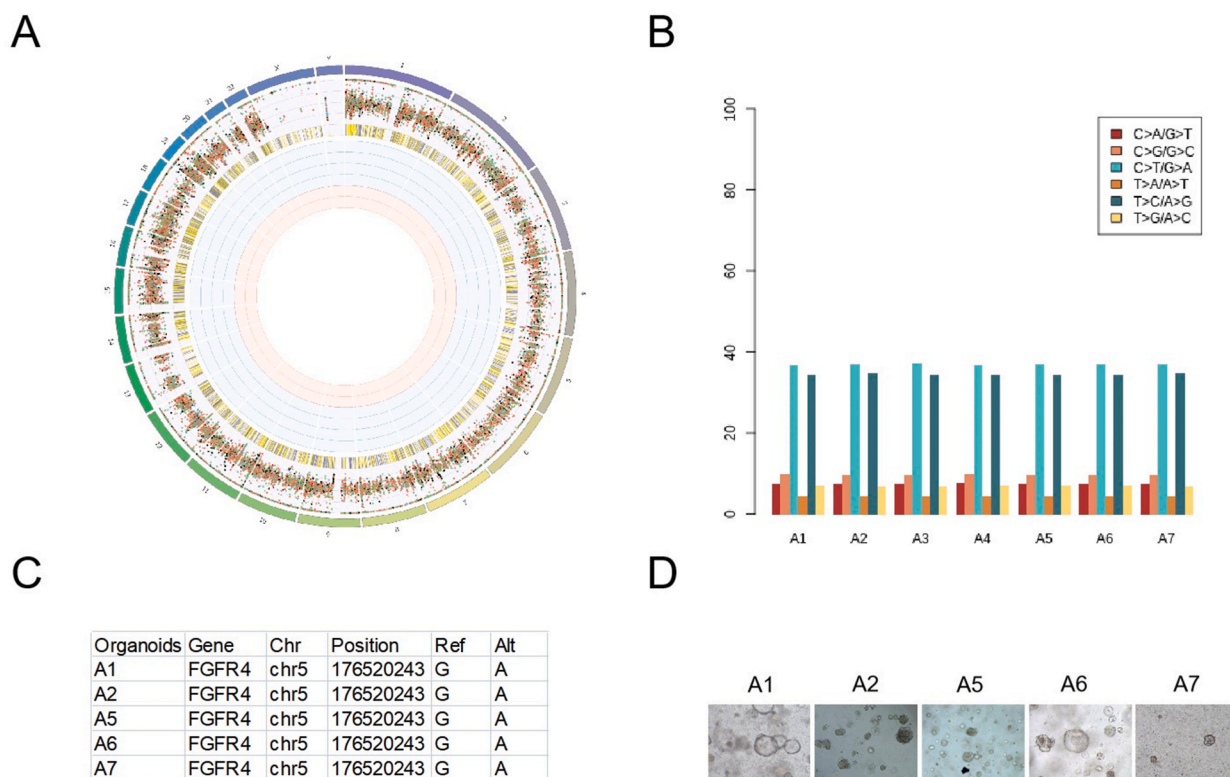

Organoids	Gene	Chr	Position	Ref	Alt
A1	FGFR4	chr5	176520243	G	A
A2	FGFR4	chr5	176520243	G	A
A5	FGFR4	chr5	176520243	G	A
A6	FGFR4	chr5	176520243	G	A
A7	FGFR4	chr5	176520243	G	A

Fig. 1. WES analysis of seven cases of CRC organoids variant. A. Mutation spectrum of various mutation types in seven CRC organoids. B. Histogram of point mutation distribution in seven CRC organoids. C. FGFR4-variant information in five CRC organoids (case A1, A2, A5, A6, and A7 organoids). D. Culture images of five CRC organoids (case A1, A2, A5, A6, and A7 organoids).

Additionally, we explored the drug sensitivity of FGFR4-variant and wild-type CRC organoids as well as their effect on tumor growth. Our study provides a reference of testing conditions for screening sensitive drugs using CRC organoids, which helps to develop precise and reliable individualized treatment plans and discover novel drugs for FGFR4-variant and wild-type CRC.

2. Results

2.1. WES analysis of seven cases of CRC organoid variants

Our study revealed that the tumor pathology of seven patients was moderately differentiated adenocarcinoma and moderate-to-poorly differentiated adenocarcinoma. We selected patients with FGFR4-variants for organoid as our point of interest considering that FGFR4-variants are uncommon. First, we constructed seven CRC organoids and performed WES. Fig. 1A shows the variant spectrum of various variant types in seven CRC organoids. Supplemental Table 1 shows WES driver gene variants of the seven organoids. Supplemental Table 2 presents the clinical and histopathological features of the seven cases. Fig. 1B shows the histogram of the distribution of seven CRC organoid point variants. Among them, C > T/G > A and T > C/A > G variants mainly occurred in seven CRC organoids. Additionally, we revealed FGFR4-variants in 5 out of 7 CRC organoids (cases A1, A2, A5, A6, and A7). Fig. 1C shows the FGFR4-variants in five CRC organoids. The FGFR4-variants occurred in chromosome 5 (chr5, a germline variation). The FGFR4-variants occur in the 176520243 genomic position. The bases and variant bases corresponding to the reference allele (Ref) and alternate allele (Alt) on the forward strand were G and A, respectively. Finally, Fig. 1D shows the culture of five CRC organoids (case A1, A2, A5, A6, and A7 organoids).

2.2. FGFR-targeted drugs (erdafitinib and FGFR4-IN-1) inhibit FGFR4-variant and wild-type CRC organoid activity

We conducted a CRC organoid drug sensitivity study by administering 0, 0.1, 1, and 10 μM of erdafitinib and FGFR4-IN-1 to treat case A2, A3, A4, and A5 organoids for 2 days. Fig. 2A shows the growth of A2 organoids (FGFR4-variant) after 2 days of FGFR4-IN-1
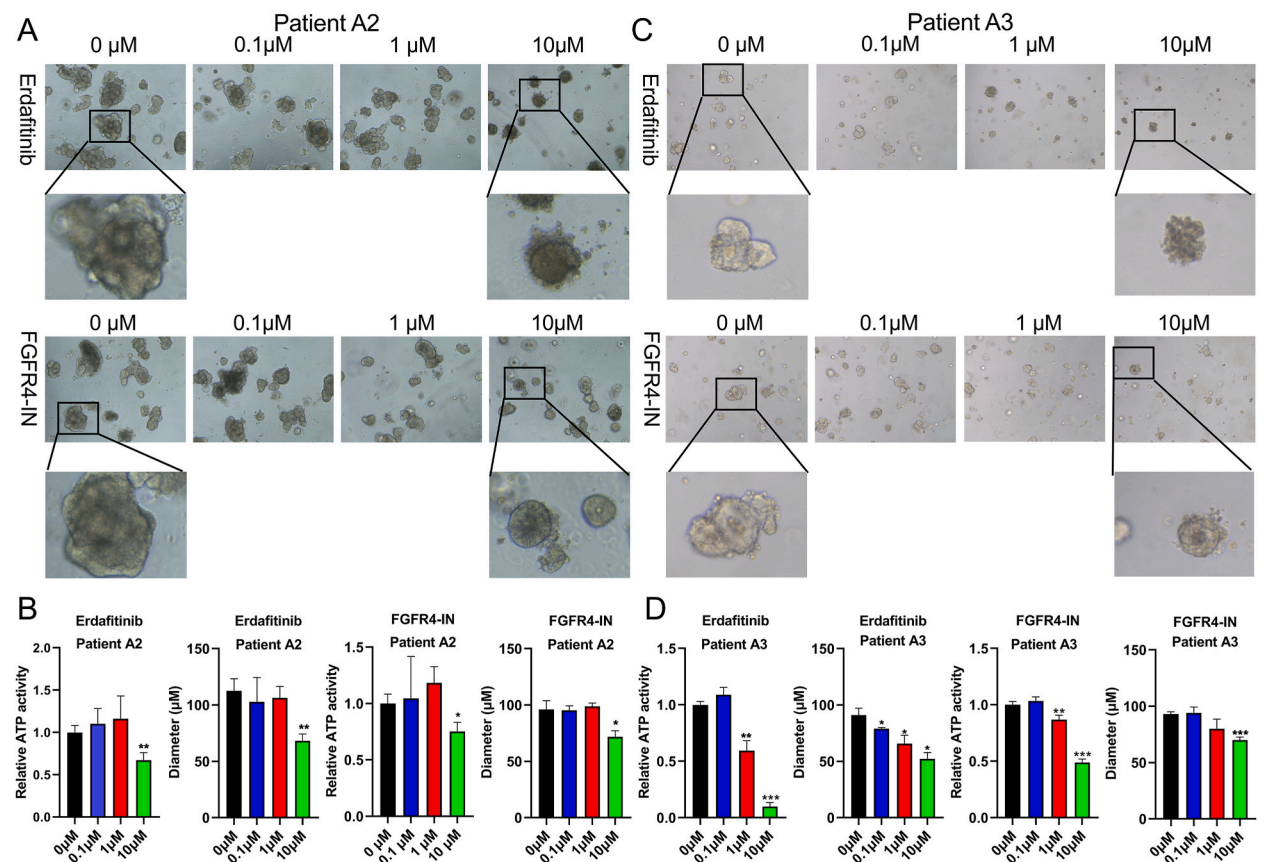

Fig. 2. FGFR4-targeted drugs inhibited the activity of FGFR4-variant and wild-type CRC organoids. A. Growth of case A2 organoids (FGFR4-variant organoids) after FGFR4-IN-1 and erdafitinib treatment for 2 days. B. ATP activity and diameter of case A2 organoids after FGFR4-IN-1 and erdafitinib treatment for 2 days. C. Growth of case A3 organoids after FGFR4-IN-1 and erdafitinib treatment for 2 days. D. ATP activity and diameter of case A3 organoids after FGFR4-IN-1 and erdafitinib treatment for 2 days. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

and erdafitinib treatment. The structural organization and integrity of A2 organoids were significantly changed under the influence of FGFR4-IN-1 and erdafitinib. The growth of A2 organoids was significantly blocked as the concentration of FGFR4-IN-1 and erdafitinib increased to 10 μM. Additionally, 10 μM of erdafitinib and 10 μM of FGFR4-IN-1 decreased the ATP activity and diameter of A2 organoids, which indicated that both erdafitinib and FGFR4-IN-1 inhibited organoid activity (Fig. 2B). Fig. 2C shows the growth of A3 organoids (wild-type) after 2 days of FGFR4-IN-1 and erdafitinib treatment. The data indicated that FGFR4-IN-1 and erdafitinib could change the structural organization and integrity of A3 organoids. Erdafitinib of 1 μM and 10 μM significantly reduced the ATP activity of A3 organoids, whereas erdafitinib of 0.1 μM, 1 μM, and 10 μM decreased the diameter of A3 organoids. FGFR4-IN-1 of 1 μM and 10
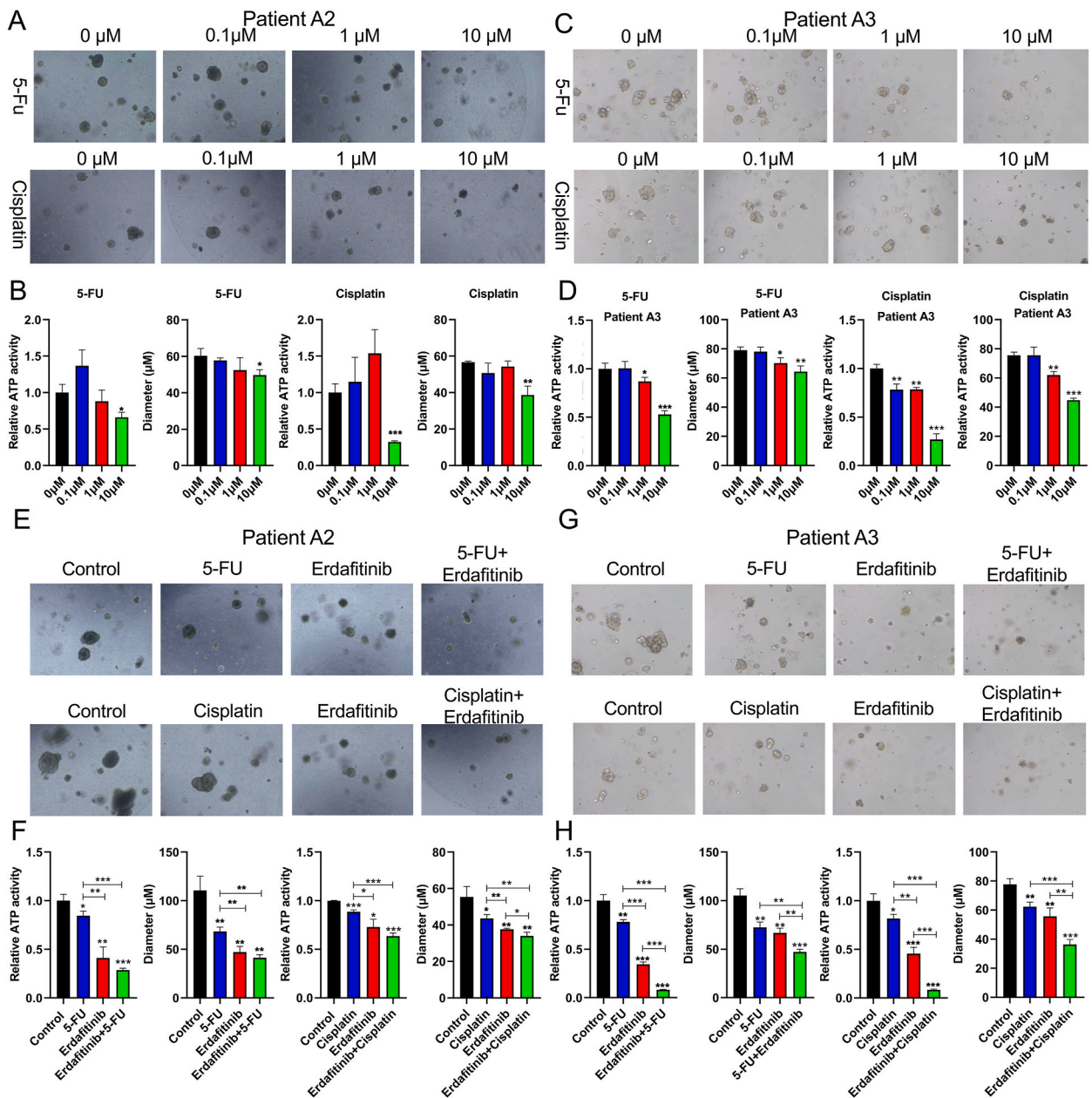

Fig. 3. FGFR-targeted drugs combined with chemotherapeutic drugs inhibited the activity of FGFR4-variant and wild-type CRC organoids.
A. Growth of case A2 organoids after 5-FU and cisplatin treatment for 2 days. B. ATP activity and diameter of case A2 organoids after 5-FU and cisplatin treatment for 2 days. C. Growth of case A3 organoids after 5-FU and cisplatin treatment for 2 days. D. ATP activity and diameter of case A3 organoids after 5-FU and cisplatin treatment for 2 days. E. Growth of A2 organoids (wild-type organoids) treated with erdafitinib in combination with 5-FU or cisplatin for 2 days. F. ATP activity and diameter in A2 organoids treated with erdafitinib in combination with 5-FU or cisplatin for 2 days. G. Growth of A3 organoids treated with erdafitinib in combination with 5-FU or cisplatin for 2 days. H. ATP activity and diameter in A3 organoids treated with erdafitinib in combination with 5-FU or cisplatin for 2 days. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

μM significantly reduced the ATP activity of A3 organoids, whereas FGFR4-IN-1 of 10 μM decreased the diameter of A3 organoids (Fig. 2D). Similarly, the data indicated that erdafitinib of 1 μM and 10 μM) reduced the ATP activity and diameter of A5 organoids, whereas FGFR4-IN-1 of 10 μM decreased the ATP activity of A5 organoids, FGFR4-IN-1 of 1 μM and 10 μM lowered the diameter of A5 organoids (Figs. S1A and S1B). Erdafitinib of 1 μM and 10 μM reduced the ATP activity and diameter of A4 organoids, whereas FGFR4-IN-1 of 1 μM and 10 μM lowered the ATP activity and diameter of A4 organoids (Figs. S1C and S1D). These results revealed that FGFR4-variant and wild-type organoids were sensitive to FGFR-targeted drugs.

2.3. Erdafitinib combined with 5-FU fluorouracil (5-FU) or cisplatin inhibits the activity of FGFR4-variant and wild-type CRC organoids

We treated FGFR4-variant (A2) and wild-type (A3) CRC organoids with 0, 0.1, 1, and 10 μM of chemotherapeutic drugs, including 5-FU and cisplatin, for 2 days. Fig. 3A shows the growth of A2 organoids after 2 days of 5-FU and cisplatin treatment. A2 organoid growth was significantly inhibited with an increase in 5-FU and cisplatin concentration. Additionally, ATP activity in A2 organoids decreased with an increase in 5-FU and cisplatin concentration, and the inhibitory effect of 5-FU and cisplatin started to appear at 10 μM, indicating that both 5-FU and cisplatin inhibited organoid activity (Fig. 3B). Fig. 3C shows the growth of A3 organoids after 2 days of 5-FU and cisplatin treatment. A3 organoid growth was significantly inhibited with an increase in 5-FU and cisplatin concentration, especially at 1 and 10 μM. Additionally, ATP activity in A3 organoids decreased with an increase in 5-FU and cisplatin concentration, and the inhibitory effect of 5-FU and cisplatin started to appear at 1 and 0.1 μM, respectively, indicating that both 5-FU and cisplatin inhibited A3 organoid activity (Fig. 3D). FGFR4-variant (A2) and wild-type (A3) CRC organoids were then processed with erdafitinib combined with chemotherapeutic drugs (5-FU and cisplatin) for 2 days. The data revealed that erdafitinib, 5-FU, and cisplatin treatment alone decreased the growth, ATP activity, and diameter of A2 and A3 CRC organoids; Erdafitinib demonstrated a stronger inhibitory effect on the growth, ATP activity, and diameter of A2 and A3 CRC organoids than that in 5-FU or cisplatin treatment groups.
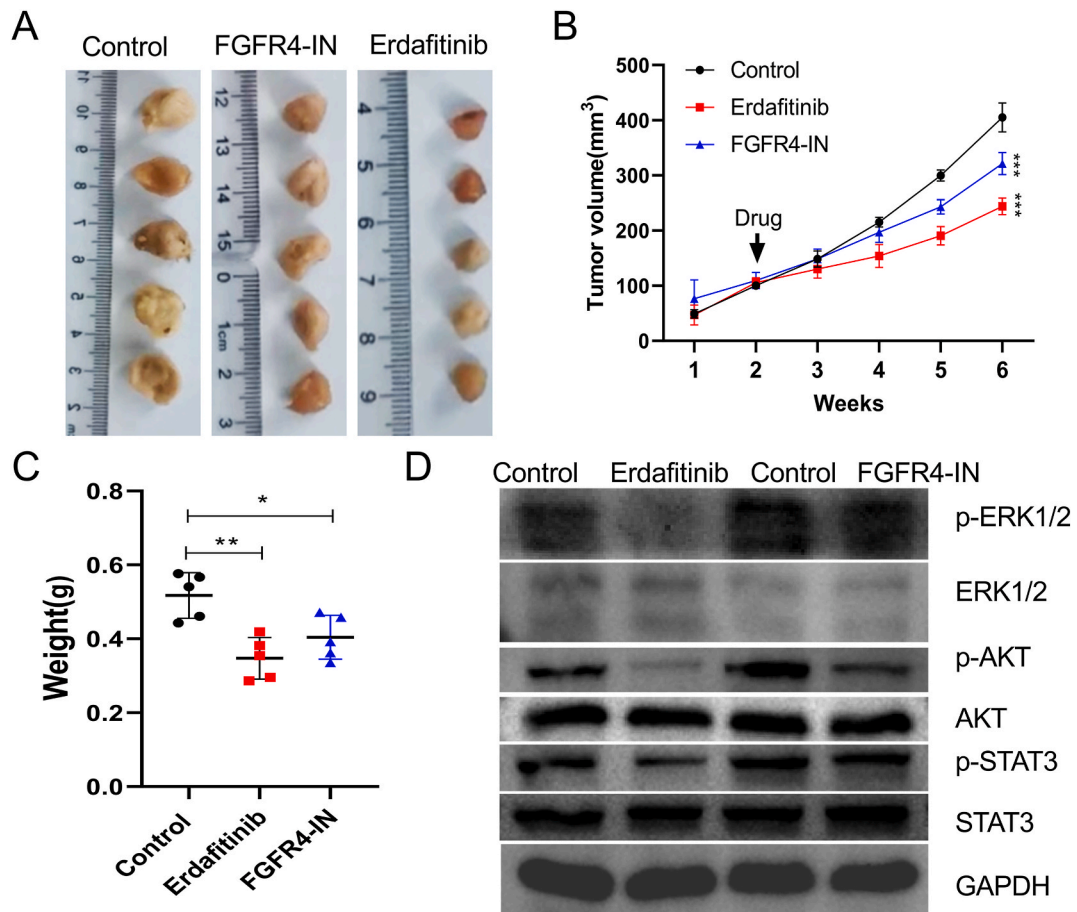

Fig. 4. FGFR4-targeted drugs inhibited tumorigenesis in FGFR4-variant CRC organoids. The PDOX model was developed by subcutaneous injection of FGFR4-variant CRC organoid cells into mice, and FGFR4-IN-1 and erdafitinib were injected intraperitoneally after 2 weeks. Doses of 12.5 mg/kg were administered once every other day for 4 weeks. A. At the end of the experiment, nude mice were sacrificed and tumors were obtained and displayed. B. Tumor volume was counted using tumor length, width, and height data. C. Western blot was used to measure ERK, AKT, and STAT3 pathway-related protein levels in each group of tumor tissue. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Additionally, the effect of erdafitinib combined with 5-FU on the growth, ATP activity, and diameter of A2 and A3 CRC organoids was stronger than that of erdafitinib or 5-FU alone. Moreover, the effect of erdafitinib combined with cisplatin on the growth, ATP activity, and diameter of A2 and A3 CRC organoids was also stronger than that of erdafitinib or cisplatin alone (Fig. 3E–H).

2.4. FGFR4-targeted drugs (erdafitinib and FGFR4-IN-1) inhibit tumorigenesis in FGFR4-variant CRC organoids

Finally, we developed the patient-derived orthotopic xenograft (PDOX) model using FGFR4-variant CRC organoids. Fig. 4A shows the tumor images of the control, FGFR4-IN-1, and erdafitinib groups. Compared with the control group, the tumor volume was significantly reduced at 6 weeks after FGFR4-IN-1 and erdafitinib treatment (Fig. 4B). Additionally, we investigated the expression of ERK/AKT pathway-associated proteins. Compared with the control group, p-ERK/1/2, p-AKT, and p-STAT3 expressions were down-regulated after FGFR4-IN-1 and erdafitinib treatment. However, no significant change in ERK/1/2, AKT, and STAT3 expressions was observed (Fig. 4C). These results indicated that FGFR4-targeted drugs inhibited tumorigenesis in FGFR4-variant CRC organoids, possibly involving the ERK/AKT/STAT3 pathway.

3. Discussion

CRC is one of the leading causes of cancer-related deaths globally, and it has become one of the most important public health problems worldwide [20,21]. Acquired drug resistance is a major clinical problem in CRC and one of the greatest limitations of chemotherapy regimens [22]. Herein, we developed seven CRC organoids and performed WES. The results revealed the presence of FGFR4-variant in 5 of the 7 CRC organoids. Based on this, we established the targeting and chemotherapeutic drug sensitivity of FGFR4-variant CRC. We revealed, through validation, that FGFR4-variant CRC organoids were sensitive to FGFR4-IN-1 and erdafitinib. Additionally, chemotherapeutic drugs inhibited FGFR4-variant CRC organoid activity, and targeted drugs were more effective than chemotherapeutic drugs at the same concentration. Furthermore, FGFR4 inhibitors hindered tumorigenesis in FGFR4-variant CRC organoids. Our study provides a new methodological reference for the screening of chemotherapeutic agents and the investigation of the mechanism of action.

Cancer organoids are 3D cultures of cancer cells that enable high success rates on an individual patient basis [23]. The ability of cancer organoids to retain original tumor characteristics makes them unique for cancer research at the individual patient level [24]. Organoid models of patient-specific tumors are revolutionizing our understanding of cancer heterogeneity and its implications for personalized medicine [25]. The development of 3D patient-specific organoids *in vitro* may significantly contribute to the establishment of personalized cancer medicine, considering the heterogeneity among patients with CRC. In 2009, Clevers et al. published groundbreaking results in *Nature*, where for the first time colorectal epithelial organoids were grown *ex vivo*, providing further possibilities for developing CRC organoids [26]. Organoids are the closest preclinical experimental platform to clinical practice and are used to guide the treatment of patients. However, no studies reported on cancer organoids related to FGFR4-variants. The present study first cultured five FGFR4-variant CRC organoids and investigated their drug sensitivity. Therefore, the innovation of this study is to construct CRC organoids containing FGFR4-variants and evaluate the sensitivity of anticancer drugs.

Organoid technology, as an emerging technology in the field of precision medicine, provides new models to determine the sensitivity of individual tumors to cancer drugs [27]. In 2018, Vlachogiannis G et al. reported in *Science* the first observational trial of the clinical efficacy of organoid drugs in intestinal cancer and obtained 88 % positive predictive value and 100 % negative predictive values, demonstrating, for the first time, the use of PDOs in predicting the effectiveness of anticancer drugs [28]. CRC, similar to many malignancies, is a heterogeneous disease with subtypes characterized by genetic alterations [29]. CRC develops from the sequential acquisition of genetic variants in the colonic epithelium, which is mainly through three pathways, including microsatellite instability, chromosomal instability, and sessile serrated pathway [30]. FGFR4 kinase domain activating variants have been found in rhabdomyosarcoma [31], lung adenocarcinoma [32], glioma [33], and other tumors [34]. Variants for sustained FGFR4 kinase activity activation are present in 7%–8% of patients with rhabdomyosarcoma, with mutation sites occurring mainly in K535 and E550 [35]. FGFR4-variants increase FGFR4 kinase activity and activate the downstream pathways through the STAT3 pathway, causing aggressive advanced tumors [36]. However, the variant sites of FGFR4 in tumor cells do not only occur in the kinase domain. The study revealed that FGFR4 Y367C variant in breast cancer cells caused spontaneous FGFR4 dimer formation, causing constitutive ERK activation, MAPK pathway enhancement, and tumor formation induction, and did not respond to stimulation with its ligands and antibodies [37]. Additionally, tumor cells, containing the FGFR4 allele Arg388 or a pure heterozygote, increase breast cancer invasion, decrease patient survival with squamous cell carcinoma and lung cancer, and increase prostate cancer malignancy by internalizing the FGFR4 receptor, which results in the sustained aberrant FGFR4 signaling activation [38,39]. A study revealed that FGFR4 provides an option for further mutational screening of tumors and is an attractive cancer target with therapeutic potential [40]. FGFR4-variants, overexpression, and amplification increase the incidence and progression of different cancer types, including breast cancer [41], hepatocellular carcinoma [42], non-small cell lung cancer [43], etc. Additionally, FGFR4 is a targeting regulator of chemoresistance in CRC [44]. However, the drug sensitivity of FGFR4-variant CRC remains unclear. Molecularly targeted therapies tend to induce specific and acquired resistance rapidly *in vitro* and *in vivo*. The present study administered different FGFR4-IN-1 and erdafitinib concentrations to treat case A2, A3, A4, and A5 organoids. We revealed that FGFR4-variant and wild-type CRC organoids were sensitive to FGFR4-IN-1 and erdafitinib. This kind of drug sensitivity study that uses an organoid model can better simulate the human response to drugs, more accurately evaluate the treatment effect, and is more in line with the developmental trend of individualized treatment, which has clinical application value.

Chemotherapy plays a crucial role in treating CRC, and cisplatin and 5-FU are prominently used chemotherapeutic agents [45,46].

After treatment, cancer cells may develop 5-FU and cisplatin resistance, resulting in chemotherapy failure [47]. Therefore, drug resistance remains a key problem causing poor prognosis in patients with CRC [48]. The present study administered different chemotherapeutic drug concentrations of 5-FU and cisplatin to treat organoids. We revealed that these chemotherapeutic drugs inhibited FGFR4-variant wild-type CRC organoid activity, and targeted drugs were more effective than chemotherapeutic drugs at the same concentrations. Additionally, our study revealed that the combined treatment of FGFR4-targeted drug (erdafitinib) and chemotherapeutic drugs (cisplatin and 5-FU) demonstrated a stronger effect on the growth of FGFR4-variant and wild-type CRC organoids than either treatment alone.

Preclinical data have indicated that small molecule inhibitors of FGFR demonstrated significant growth inhibitory effects on cancer cell lines or xenografts with FGFR gene amplification [49,50]. We revealed that FGFR4-targeted drugs inhibited tumorigenesis in FGFR4-variant CRC organoids by constructing an FGFR4-variant CRC PDOX mouse model administered with FGFR4-IN-1 and erdafitinib. Studies have revealed that the ERK/AKT pathway inhibition hindered CRC tumor growth [51]. Zhang Y et al. revealed that tanshinone IIA reversed CRC oxaliplatin resistance by inhibiting the ERK/AKT pathway [52]. Our study indicates that FGFR-targeted agents may hinder FGFR4-variant CRC organoid tumorigenesis by inhibiting the ERK/AKT pathway.

Organoids have become the closest preclinical experimental platform to clinical practice, and some articles have revealed that this model can be used to guide the treatment of clinical patients. However, no study has reported on organoids related to known FGFR4-variants. Therefore, the innovation of this study lies in the construction of CRC-like organs containing FGFR4-variants and their use in drug sensitivity studies. Such drug studies based on the PDOX model may further facilitate individualized CRC treatment. This project mainly aimed to develop a CRC organoid model with FGFR4-variant that can be applied for subsequent drug development. According to the organoid results, targeted drugs or targeted combined chemotherapy drugs demonstrated better effects. However, CRC organoids are currently mainly cultured in epithelial tissues, and a mature culture system that contains the stroma and immune system remains lacking, which needs to be explored in further studies. More clinical studies are required to determine the actual clinical effects due to the small sample size of this study, the differences in clinical dosing concentrations, and the complex human microenvironment. Additionally, whether FGFR4-variants had significance also need more clinical samples to verify.

4. Conclusion

Our study developed a 3D *in vitro* organoid model of FGFR4-variant CRC, demonstrating that FGFR4-IN-1 and erdafitinib, as FGFR4 inhibitors in CRC, may be new targeting agents for CRC, guiding its clinical treatment. Additionally, cancer organoids can be used as a model for drug screening and provide a reference for mining new drug screening models.

5. Materials and methods

5.1. Sample collection

We collected CRC tissues from seven patients diagnosed with CRC by imaging, serology, or histopathology in Foshan Fosun Chancheng hospital, named as case A1, A2, A3, A4, A5, A6, and A7 organoids. Exclusion criteria included: patients with pathologically confirmed non-CRC and those with incomplete clinical characteristics. Fresh tissue samples from patients with CRC were collected and immediately placed in a tissue protection solution (Biorgen, China) and stored at 4 °C for the next step of the study. Patients were not related to each other, but were all Han Chinese. All patients, who provided samples for this study, provided written informed consent, and the Ethics Committee of Foshan Fosun Chancheng Hospital approved this study (approval date: September 26, 2022; Ethical number: IRB-ATT-002.02-24). This study was performed in line with the principles of the Declaration of Helsinki.

5.2. Developing CRC organoids

CRC tissues were extracted and digested with the digestive solution (Biorgen, Cat. No. OGCP-04-03) for 1–2 h, and then suspended with CRC organoids medium (Biorgen, China) and mixed with Matrigel (Cat. no. 082755, Xiamen Mogengel, China) at 1:1 ratio. The CRC organoids medium was based on Advanced DMEM/F12 (Invitrogen) and supplemented with 10 % R-Spondin 1 (abs04398, Absin, Shanghai, China), 10 % Noggin (PrimeGene Bio-Tech Co., Ltd., Shanghai, China), 50 ng/ml of EGF (Invitrogen), 2 % B27 supplement (Invitrogen), 1.25 mM of N-acetylcysteine (Sigma-Aldrich), 10 μM of nicotinamide (Sigma-Aldrich), 500 nM of A83-01 (Tocris), 10 μM of SB202190 (ApexBio), 1 nmol/L of gastrinI (Tocris Bioscience, Bristol, United Kingdom), 5 μg/mL of prostaglandin E2 (Cayman Chemical, Ann Arbor, MI, USA), and 10 mmol/L of Y-27632 (Sigma-Aldrich). The 48-well plates placed 2 h in advance were taken out of the 37 °C incubator, and 50 μL of mixture was added to each well. The plates were placed in the 37 °C incubator for 5 min and then reversed and solidified for 20 min. After solidification, 200 μL of CRC organoids medium was added to a 48-well plate and cultured in a cell incubator. The growth of organoids was observed daily, and the culture medium was changed every 2–3 days.

5.3. WES

Genomic DNA was extracted using the Vazyme FastPure Blood/Cell/Tissue/Bacteria DNA isolation Mini Kit (DC112-02, Vazyme). NanoDrop™ One (ND-ONE-W, Thermo) was used to determine DNA purity, and Qubit™ 3 Fluorometer (Qubit3.0, Thermo) was utilized to identify DNA concentration. Agarose gel electrophoresis was used to investigate DNA integrity. Covaris (M220) was used to fragment DNA of 1 μg, the KAPA Hyper prep kit (07962363001, Roche) was utilized to construct the sequencing libraries, and the

SureSelect Target Enrichment Kit (5190–8864, Agilent) was employed for hybridization capture. Library concentration was identified using the Qubit™ dsDNA HS Assay Kit (Q32851, Invitrogen), followed by library fragment distribution using the D1000 Screen Tape (5067–5582, Agilent), and finally, library molar concentration was precisely determined using the KAPA Library Quant kit (Illumina) universal qPCR Mix (kk4824, KAPA). The NovaSeq 6000 instrument (Illumina) and NovaSeq S4 reagent kit (20012866, Illumina) were used to sequence libraries.

5.4. Determination of ATP activity in organoid

Case A2, A3, A4, and A5 organoids were inoculated into 96-well plates, and the medium was changed when the mean diameter was >50 µm. Organoids were treated with 0, 0.1, 1, and 10 µM of FGFR4 specific inhibitor (FGFR4-IN-1; HY-100631, MCE) and a general FGFR inhibitor (Erdafitinib; HY-18708, MCE) or/and 0, 0.1, 1, and 10 µM of chemotherapeutic drugs 5-FU (HY-90006, MCE), dissolved in dimethyl sulfoxide (DMSO; Sigma), and cisplatin (HY-17394, MCE), dissolved in DMSO (Sigma) for 2 days. Afterward, 96-well plates were taken out and placed for 30 min to balance the temperature of the plates to room temperature. CellCounting-Lite 3D (DD1102, Vazyme) with the same volume of 100 µL of cell culture was added and balanced to room temperature. The cells were fully lysed by vigorous oscillation for 5 min and left for 25 min to keep the luminescence signal stable. The activity of organoid ATP was then detected.

5.5. Western blot

Western blot was conducted to measure ERK/AKT pathway-related protein levels. Total protein was extracted through RIPA (P0013B, Beyotime), followed by protein quantification using a BCA protein assay kit (BL521A, Biosharp). Proteins (40 µg) were subjected to SDS-PAGE gel electrophoresis after the completion of quantification, transferred to PVDF membrane and closed for overnight incubation at 4 °C for primary antibody *p*-ERK/1/2 (1: 100; 80031-1-RR, Proteintech), *p*-AKT (1: 1000; 66444-1-Ig, Proteintech), p-STAT3 (1: 2000; #9145), ERK/1/2 (1: 1000; 11257-1-AP, Proteintech), AKT (1: 1000; 60203-2-Ig, Proteintech), STAT3 (1: 2000; 10253-2-AP, proteintech), and GAPDH (1: 1000; 11257-1-AP, Proteintech), and incubated for Peroxidase AffiniPure Goat Anti-Rabbit IgG (1:2000; 115-035-003, JACKSON). Finally, a supersensitive ECL chemiluminescent substrate (K-12045-D50, Advansta) was used for exposure.

5.6. Establishment of CRC PDOX mouse model

This study used athymic nu/nu nude mice (4–6 weeks). Mice were fed an autoclaved laboratory rodent diet and randomly categorized into the control, FGFR4-IN-1, and erdafitinib groups. FGFR4-variant CRC organoid cells of 2×10^5 were injected subcutaneously into mice to develop PDOX models, and then treated with intraperitoneal injections of FGFR4-IN-1 and erdafitinib after 2 weeks. Doses of 12.5 mg/kg were administered once every other day for 4 weeks. Tumor volumes were measured weekly. Mice were executed after 6 weeks of experimentation, and tumor samples were obtained and photographed. The Animal Research Ethics Board of Shantou University Medical College approved all animal experiments (date of approval: 01-06-2021, Ethical number: SUMC2021-498).

5.7. Statistical analysis

The GraphPad Prism version 8.0 software (GraphPad Software, Inc., San Diego, CA, USA) was used for statistical analysis. Measurement data were expressed as mean ± standard deviation. Experiments were repeated three times independently. One-way analysis of variance was used for between-group comparison. The difference was statistically significant at a *P*-value of <0.05.

5.8. Ethical declaration and written informed consent

All patients, who provided samples for this study, provided written informed consent, and the Ethics Committee of Foshan Fosun Chancheng Hospital approved this study (approval date: September 26, 2022; Ethical number: IRB-ATT-002.02-24). This study was performed in line with the principles of the Declaration of Helsinki.

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Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Hailing Lin: Writing – original draft, Methodology, Formal analysis, Data curation. **Hongbo Fu:** Resources, Data curation. **Shishen Sun:** Validation. **Hao Yin:** Software. **Jie Yuan:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Conceptualization. **Jilin Liao:** Writing – review & editing, Visualization, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e30985.

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