Cabazitaxel suppresses colorectal cancer cell growth via enhancing the p53 antitumor pathway

Wen Zhang1,2, Ruiqian Sun3, Yongjun Zhang4, Rong Hu1,2, Qian Li1,2, Weili Wu1,2, Xinyu Cao5, Jiajian Zhou4, Jianfeng Pei6 and Ping Yuan1,2

1 Guangdong Provincial Key Laboratory of Colorectal and Pelvic Floor Disease, The Sixth Affiliated Hospital of Sun Yat-sen University, Guangzhou, China
2 Guangdong Institute of Gastroenterology, Guangzhou, China
3 Guangdong Country Garden School, Foshan City, China
4 Dermatology Hospital, Southern Medical University, Guangzhou, China
5 Institute of Clinical Medical Sciences, Center of Respiratory Medicine, China-Japan Friendship Hospital, Beijing, China
6 Center for Quantitative Biology, Academy for Advanced Interdisciplinary Studies, Peking University, Beijing, China

Keywords
cabazitaxel; colorectal cancer cell; HCT116; RNA-sequencing; xenograft

Correspondence
P. Yuan, Guangdong Provincial Key Laboratory of Colorectal and Pelvic Floor Disease, The Sixth Affiliated Hospital of Sun Yat-sen University, Guangzhou 510655, China
Tel: +86 18819239657
E-mail: yuanp8@mail.sysu.edu.cn
and
J. Pei, Center for Quantitative Biology, Academy for Advanced Interdisciplinary Studies, Peking University, Beijing 100871, China
Tel: +86 13501063672
E-mail: jfpei@pku.edu.cn

There were approximately 1.93 million new cases and 940 000 deaths from colorectal cancer in 2020. The first-line chemotherapeutic drugs for colorectal cancer are mainly based on 5-fluorouracil, although the use of these drugs is limited by the development of drug resistance. Consequently, there is a need for novel chemotherapeutic drugs for the efficient treatment of colorectal cancer patients. In the present study, we screened 160 drugs approved by the Food and Drug Administration and identified that cabazitaxel (CBT), a microtubule inhibitor, can suppress colony formation and cell migration of colorectal cancer cells in vitro. CBT also induces G2/M phase arrest and apoptosis of colorectal cancer cells. Most importantly, it inhibits the growth of colorectal cancer cell xenograft tumors in vivo. Transcriptome analysis by RNA-sequencing revealed that Tub family genes are abnormally expressed in CBT-treated colorectal cancer cells. The expression of several p53 downstream genes that are associated with cell cycle arrest, apoptosis, and inhibition of angiogenesis and metastasis is induced by CBT in colorectal cancer cells. Overall, our results suggests that CBT suppresses colorectal cancer by upregulating the p53 pathway, and thus CBT may have potential as an alternative chemotherapeutic drug for colorectal cancer.

Abbreviations
5-FU, 5-fluorouracil; CBT, cabazitaxel; CI, confidence interval; CRC, colorectal cancer cell; GFP, green fluorescent protein; GO, Gene Ontology; GSEA, geneset enrichment analysis; IC50, half-maximal inhibitory concentration; KEGG, Kyoto Encyclopedia of Genes and Genomes; KO, knockout; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide; RNA-seq, RNA-sequencing.
Colorectal cancer is the third most diagnosed cancer and leads to the second greatest mortality among cancers worldwide. There were approximately 1.93 million new cases and 940,000 deaths of colorectal cancer in 2020 according to the World Health Organization. Multiform therapeutic strategies, such as surgery, chemotherapy, radiotherapy, and recent immunotherapy, have been developed and applied to colorectal cancer patients. Surgical resection plus chemotherapy is the most common treatment for early stage of primary colorectal cancer. The first-line chemotherapeutic drugs of colorectal cancer are mainly based on 5-fluorouracil (5-FU). However, these drugs exhibit compromised efficacy as a result of significant toxicity, drug resistance, or patient inconvenience. The high mortality of colorectal cancer indicates that the current therapy is far from ideal. Novel chemotherapeutic drugs for the efficient treatment of colorectal cancer patients are urgently needed.

As the safety of Food and Drug Administration (FDA)-approved drugs is demonstrated, the exploration of their therapeutic application to colorectal cancer can greatly reduce the cost and time for drug application. Cabazitaxel (CAS183133-96-2; RPR 116258; XRP6258; TXD258; Jevtana; CBT) is an FDA-approved drug for the treatment of patients who are diagnosed with metastatic castration-resistant prostate cancer that is resistant to paclitaxel and docetaxel treatment. Cabazitaxel is a semi-synthetic taxane derivative. It promotes the polymerization of tubulin and stabilizes microtubules. It inhibits prostate cancer cells by inhibiting androgen receptor and heat shock protein and shows antitumor activity in docetaxel-refractory metastatic prostate cancer and breast cancer. It is also reported to induce autophagy via the phosphoinositide 3-kinase/Akt/mechanistic target of rapamycin pathway in lung adenocarcinoma cancer cell line A549. However, it is not clear whether CBT is effective in inhibiting colorectal cancer, nor what the underlying mechanism comprises.

HCT116 cell is a commonly used colorectal cancer cell line. It can be cultured without growth factors in vitro. HCT116 cells are highly motile and invasive and showed high efficiency with respect to forming tumors in a subcutaneous xenograft experiment. Using this cell line, we screened 160 FDA-approved drugs and found that CBT can efficiently inhibit HCT116 cells. Employing a series of in vitro assays, we found that CBT can suppress HCT116 cell proliferation and migration. CBT induces G2/M phase cell cycle arrest and apoptosis of HCT116 cells. Most interestingly, CBT can efficiently inhibit tumor growth in the HCT116 cell xenograft mouse model. By comparing the transcriptome of CBT-treated and control HCT116 cells, we found that CBT treatment leads to upregulation of genes involved in the p53 signaling pathway. Further knockout (KO) of p53 in HCT116 cells confirms the key role of p53 signaling for the CBT inhibitory effect in colorectal cancer cells.

Overall, the present study reports a novel anti-colorectal cancer role for CBT, which may be used as an alternative chemotherapeutic drug for the efficient treatment of colorectal cancer patients.

Materials and methods

Cell culture

HCT116 cells were cultured in Dulbecco’s modified Eagle’s medium basic media supplemented with 10% fetal bovine serum at 37 °C in an incubator with 5% CO₂. The cells were passaged by 0.25% trypsin at a ratio of 1:3 after confluence. Cabazitaxel was purchased from Topscince (Shanghai, China). For the in vitro experiment, 5 mg of CBT was dissolved in 0.598 mL of dimethylsulfoxide (10 mM) and further diluted with PBS to different concentrations. For the in vivo experiment, based on the formulation of Jevtana (Sanofi-Aventis Groupe, Paris, France), 10 mg of CBT was dissolved in 0.26 g of polysorbate 80 (Tween 80) and mixed with 0.95 mL of 13% ethanol (w/w) in ddH₂O before injection, with 0.9% sodium chloride solution being used in the final dilution.

Cabazitaxel solution preparation

Cabazitaxel was purchased from Topscience (Shanghai, China). For the in vitro experiment, 5 mg of CBT was dissolved in 0.598 mL of dimethylsulfoxide (10 mM) and further diluted with PBS to different concentrations. For the in vivo experiment, based on the formulation of Jevtana (Sanofi-Aventis Groupe, Paris, France), 10 mg of CBT was dissolved in 0.26 g of polysorbate 80 (Tween 80) and mixed with 0.95 mL of 13% ethanol (w/w) in ddH₂O before injection, with 0.9% sodium chloride solution being used in the final dilution.

Half-maximal inhibitory concentration (IC₅₀) measurement

The indicated colorectal cancer cells and prostate cancer cells were treated with a series of diluted CBT for 48 h.
The cell viability was measured by the MTT colorimetric assay. The values of CBT-treated samples were normalized with untreated samples in EXCEL (Microsoft Corp., Redmond, WA, USA) and then input in a nonlinear sigmoidal curve of PRISM, version 7 (GraphPad Software Inc., San Diego, CA, USA) to calculate the IC\(_{50}\). Three independent biological experiments were performed for each assay.

### RNA extraction and RT cDNA synthesis

Total RNA was extracted from the control or CBT-treated cells using TRizol Reagent (Invitrogen, Waltham, MA, USA). The concentration and purity of RNA was measured by spectrophotometry (Nanodrop Technologies, Inc., Wilmington, DE, USA). cDNA was synthesized from 2 \(\mu\)g of RNA using 5 \(\times\) PrimeScript RT Master Mix (Takara, Shiga, Japan) in accordance with the manufacturer's instructions.

### Western blotting

Total proteins of the cells were harvested with RIPA buffer and separated via SDS/PAGE. Subsequently, the proteins were transferred to a poly(vinylidene difluoride) membrane and blocked by 5% skim milk in TBS plus 0.1% Tween-20. The membrane was then blotted with \(\beta\)-actin antibody (dilution 1:1000; SC47778; Santa Cruz Biotechnology, Santa Cruz, CA, USA), Phospho-Histone H2A.X antibody (dilution 1:1000; catalogue no. 2577; Cell Signaling Technology, Danvers, MA, USA) or p21/Waf1/Cip1 antibody (dilution 1:1000; catalogue no. 2947; Cell Signaling Technology), respectively, overnight at 4°C. The membrane was washed with TBS plus 0.1% Tween-20 solution and then blotted with proper horseradish peroxidase-conjugated secondary antibodies. After washing, CHEMDOC (Bio-Rad, Hercules, CA, USA) was used to detect the signals.

### Real-time PCR

The relative expression of mRNA was examined by real-time PCR using SYBR Green Master Mix (Takara) on an ABI QuantStudioTM 7 real-time PCR system (Thermo Fisher Scientific, Waltham, MA, USA). The thermal cycling conditions included an initial hold period at 95°C for 30 s followed by a two-step PCR program, comprising 95°C for 5 s and 60°C for 30 s with 40 cycle repeats. To evaluate the relative expression, the Ct value of the examined sample gene was first normalized with the Ct value of endogenous Gapdh and then with the Ct values of the respective control sample gene. All experiments were performed with three biological repeats and three technique repeats. Student’s \(t\)-test was used for statistical analysis. The primer sequences for real-time PCR are provided in Table 1.

### Apoptosis assay

The apoptosis assay was performed using an Annexin V-FITC/PI Apoptosis Kit (MultiSciences Biotech, Hangzhou, China). Cancer cells were seeded at 1 \(\times\) 10^5 cells per well in a six-well plate for overnight culture. Then, the cells were treated with PBS and cabazitaxel at the IC\(_{50}\) of the respective cells for 48 h. Next, cells were collected for the apoptosis assay in accordance with the manufacturer's instructions. Flow cytometry analysis was performed using a FACSCanto II (BD Biosciences, Franklin Lakes, NJ, USA) flow cytometer. The percentage of cells at different cell cycle phases was analyzed using FLOWJO (https://www.flowjo.com). Three biological repeats were employed for each experiment. Student’s \(t\)-test was used for the statistical analysis.

### Cell cycle assay

Cancer cells were seeded at 1 \(\times\) 10^5 cells per well in a six-well plate. After overnight culture, the cells were treated with cabazitaxel at the IC\(_{50}\) of the respective cell line for 48 h. The cells were gently lifted with 0.25% Trypsin (Gibco, Waltham, MA, USA) at 37°C for 1 min. Next, the cells were washed once with PBS and fixed with 75% alcohol at −20°C overnight. Subsequently, the cells were centrifuged at 395 g for 5 min and suspended in propidium iodide solution (50 \(\mu\)g/mL, 1 propidium iodide, 0.1 mg/mL RNase A and 0.05% Triton X-100 in PBS) and incubated at 37°C for 40 min. After centrifugation, the supernatant was removed and the cells were resuspended in 500 \(\mu\)L of PBS for flow cytometry analysis using a FACSCanto II (BD Biosciences) flow cytometer. The percentage of cells at different cell cycle phases was analyzed using FLOWJO. Three biological repeats were tested for each experiment. Student’s \(t\)-test was used for statistical analysis.

### Colony formation assay

Agarose mixture containing 0.5 mL of growth media plus 20% fetal bovine serum and 0.5 mL of 0.8% agarose gel was used to coat each well of a six-well plate. The plates were subsequently cooled at 4°C for 5 min to solidify the agarose and then transferred to the tissue culture hood and warmed to 37°C. 5 \(\times\) 10^5 HCT116 cells thoroughly mixed with low density agarose mixture containing 0.5 mL of growth media plus 20% fetal bovine serum and 0.5 mL of 0.4% agarose gel were added to each well of the agarose-coated plates. After solidification for another 20 min, complete media (1 mL) plus cabazitaxel at different concentrations was added to the wells. After 72 h, the media containing cabazitaxel was removed and 1 mL of fresh media was used for replenishment. The medium was changed every 3 days up to day 14. Colonies were stained with...
0.05% crystal violet for 1 h and then washed with PBS. The images of the colonies were captured using a microscope (Olympus, Tokyo, Japan) with a 4x objective lens. The number of colonies was counted manually. The area of colonies was quantified using IMAGEJ (NIH, Bethesda, MD, USA). The experiments were performed with three biological repeats and three technical repeats.

**Wound healing assay**

3 x 10^4 HCT116 cells were seeded in each well in 12-well plates with Culture-Insert 4 Well silicone inserts (Ibidi, Gräfelfing, Germany). The cells were incubated at 37 °C and 5% CO2 for 24 h for attachment. The Culture-Insert 4 Well was then removed with sterile tweezers. Growth medium with or without 0.03 μM cabazitaxel was added to the culture. All experiments included three biological repeats. The culture images were captured at different time points using a microscope (Leica, Wetzlar, Germany). The gap of the culture was measured using IMAGEJ.

**In vivo antitumor assay**

All animal experiments were approved by the Institutional Animal Care and Use Committee of the Sixth Affiliated Hospital of Sun Yat-sen University (Guangzhou, China). Five-week-old female BALB/c nude mice were purchased from Charles River Laboratories (Beijing, China) and maintained under specific pathogen-free condition under a 12:12-h dark/light photocycle. A maximum of five mice were kept in one microisolator cage with ad libitum feeding of autoclaved food and water. One hundred microliters of green fluorescent protein (GFP)-labeled HCT116 cells in PBS at a concentration of 5 x 10^4 cells·μL⁻¹ were subcutaneously injected into the left flank of 6-week female mice anesthetized using inhaled isoflurane. Seven days later, the xenografted tumors grew to approximately 30–200 mm³ in size. The mice were randomly assigned to five groups (n = 3 per group) for the administration of different reagents. Intraperitoneal injections with 8 and 16 mg·kg⁻¹ CBT, 8 and 16 mg·kg⁻¹ 5-FU and PBS were performed, respectively, at days 0, 5 and 10 after group assignment. The growth of tumor was monitored with an in vivo imaging system (IVIS Spectrum; Xenogen, Alameda, CA, USA) after the mice were anesthetized using inhaled isoflurane. Tumor volume was measured every 3 days and calculated as V = (length x width x height)/2. The mice were weighed every 3 days and their general physical status was recorded daily. The experiment was terminated before the tumor size reached 2000 mm³. The mice were killed with CO2 and the tumors were dissected out for the subsequent experiments.

**Gene expression analysis**

RNA was extracted from the indicated cells. The RNA-sequencing (RNA-seq) libraries were constructed and sequenced with NovaSeq 6000 sequencer by Berry Genomics Co Ltd (Beijing, China). Raw sequencing reads were subjected to quality filtering and adapter removal. The remain reads were then aligned to the reference human genome (hg19) using STAR2 (v2.7.3a) [14]. The gene expression was quantified as FPKM (i.e. fragments per kilobase of gene per million mapped read) using CUFFLINKS, version 2.2.1 [15]. Differential expression genes were determined using |log2(fold change)| ≥ 0.58 in CBT-treated HCT116 cells versus control HCT116 cells. The |log2(fold change)| prerank gene list was used for the subsequent enrichment analyses. Geneset enrichment analysis (GSEA) was used to assess the enrichment from the Hallmark geneset collection provided by the v4.0 MsigDB [16] and Kyoto Encyclopedia of Genes and Genomes (KEGG) [17-19].
### Table 2. FDA-approved drugs for colorectal cancer screen.

| Drug name                          | Drug name                          | Drug name                          | Drug name                          | Drug name                          | Drug name                          | Drug name                          |
|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|
| Nitisinone                         | Methacholine chloride              | Dutasteride                        | Edoxaban tosylate monohydrate      | Ipragliflozin                      | AP24534                           | Montelukast sodium                 |
| Pomalidomide                       | Ciclesonide (RPR251526)            | WY-14643                           | Brexiprazole                       | Trelagliptin succinate             | Afatinib (BIBW2992)               | Indacaterol                        |
| Nandrolone                         | Flupenthixol dihydrochloride       | Guanethidine monosulfate           | Nandrolone decanoate               | TAK-438 (Vonoprazan fumarate)      | Edoxaban                          | Felbamate                          |
| Dexmedetomidine                    | Ivabradine hydrochloride           | Difenestramide                     | Estradiol                          | Dalasetron mesylate hydrate        | Peramivir trihydrate              | Bazedoxifene hydrochloride monohydrate |
| Lercanidipine                      | Fluoxymesterone                   | Lacosamide                         | Prucalopride                       | Tirofiban hydrochloride monohydrate | Allylestrenol                     | Acldinium bromide                  |
| Alizapride hydrochloride           | Tapentadol hydrochloride           | Cabazitaxel                        | Alcaftadine                        | Levosimendan                      | Pazopanib                         | Entecavir                          |
| Carbidopa hydrate                  | Benactyzine hydrochloride          | Levomilnacipran mesylate           | Ifosfamide                         | Daclatasvir dihydrochloride        | Doxycorticosterone acetate        | Fimasartan (BR-A-657)             |
| Avasanil                           | Acotiamide hydrochloride           | Ebastine                           | Tolvaptan                          | Efinacozole                        | Radotinib                         | Menatetrenone                      |
| Nicotinic acid hydrizide           | Hesperetin                         | Drofenine hydrochloride            | Luteolin                           | Formononetin                       | Fulvestrant                       | Oxfendazole                        |
| Lisinopril                         | Cyclophosphamide monohydrate       | Pimaricin                          | L-Cycloserine                      | Naftopidil                         | Broxiquinoline                    | Gallamine triethiodide             |
| Ganciclovir                        | c-Phenylalanine                    | Quinine                            | Allopurinol                        | Niflumic acid                      | Pranlukast                         | Gabapentin                         |
| Ribavirin                          | Orlistat                           | Hydroxyurine                       | Hydroxyurea                        | Chloral hydrate                    | Tilmicosin                        | Cytarabine                         |
| Capanthrol                         | Carbadox                           | Piroxycam                          | Omeprazole                         | Salicylanilide                     | Sulfaazotol                        | Mephaline                          |
| Pyrithioxin                        | Pindolol                           | L-Ornithine                        | Urea                               | Dimetridiazole                     | Acetylcysteine                     | Pimelode                            |
| Behenic alcohol                    | Sulfamethoxazole                   | 0(+)-Glucose                       | Mefenamic acid                     | Thiame hydrochloride               | Oxytetraacyline (Terramycin)       | Ofloxacin                           |
| Adrenosterone                      | Cepharanthine                      | Guaiifenesin                       | 0-5-Hydroxytryptophan              | Doxycycline hyclate                | Salicylic acid                    | Oxytocin                           |

Cabazitaxel suppresses colorectal cancer

W. Zhang et al.
Establishment of TP53 KO HCT116 cells by CRISPR/Cas9

TP53 KO HCT116 cells were generated by CRISPR/Cas9 using gRNAs as described previously [20]. Two hTP53 gRNA KO plasmids (YKO-RP003-hTP53, YKO-RP003-hTP53, Ubige) were obtained from Ubige Company (Guangzhou, China). gRNA oligos are listed in Table 1. The hTP53 gRNA KO plasmids were transfected into HCT116 cells using Lipofectamine 3000 (Invitrogen). The cells were selected using a concentration of 0.8 μg·mL⁻¹ of purimycin at 24 h after transfection to eliminate the non-transfected cells. The survived cells were subcultured and checked for expression of GFP. Knockout of TP53 was confirmed by western blotting.

Results

Cabazitaxel can efficiently inhibit the proliferation and migration of colorectal cancer cells

To identify drugs that have potential to treat colorectal cancer, we utilized HCT116 cells as a colorectal cancer cell model and screened 160 FDA-approved drugs (Table 2). Our initial trial revealed that CBT could efficiently reduce the number of viable HCT116 cells after 48 h of drug treatment (Fig. 1A). A concentration of 0.03 μM CBT reduced the viable HCT116 cells to 50%, whereas 0.24 μM CBT reduced the cell viability to 30% (Fig. 1B). To determine whether CBT plays a broad inhibitory role for different colorectal cancer cells, we next investigated its cytotoxicity to HCT116, LoVo, HCT8 and DLD1 cells. Because CBT is an FDA-approved drug for prostate cancer, we also included prostate cancer cell DU145 and PC3 in the experiment as positive controls. We examined cell viability at 48 h after CBT treatment at different concentrations by the MTT colorimetric assay and calculated the IC₅₀. IC₅₀ values of CBT to HCT116, LoVo, HCT8 and DLD1-cells were 0.029 μM [0.023–0.036 μM, 95% confidence interval (CI)], 0.063 μM (0.047–0.087 μM, 95% CI), 0.255 μM (0.198–0.328 μM, 95% CI) and 0.532 μM (0.438–0.646 μM, 95% CI), respectively. Meanwhile, IC₅₀ values of CBT to prostate cancer cell DU145 and PC3 cells were 0.054 μM (0.033–0.090 μM, 95% CI) and 0.066 μM (0.030–0.148 μM, 95% CI) (Fig. 1C). These results suggest that CBT inhibits colorectal cancer cell HCT116 and LoVo cells as efficiently as prostate cancer cell DU145 and PC3 cells. However, a much higher dose of CBT is required to inhibit colorectal cancer cell HCT8 cells and DLD1 cells.

Next, we examined the effect on colony formation. The number of HCT116 colonies decreased gradually with an increase in CBT concentration (Fig. 1D–F). To investigate whether CBT can inhibit the migration of colorectal cancer cells, we then examined the effect of CBT on cell motility by the wound-healing assay using HCT116 cells. An Ibidi culture insert was used to generate the wound gap and serum-free culture medium was added to the cells after the insert was removed to reduce the effect of cell proliferation. Obviously, the Ibidi culture insert generated gap demonstrated much slower closing for CBT pretreated colorectal cancer cells than for control cells at 48 h after insert removal (Fig. 1G, H). Immunostaining of tubulin revealed that CBT-treated HCT116 cells showed cytoskeleton disorder and morphological malformation with a reduced pseudopod, which is line with the reduced motility of CBT-treated cells. (Fig. 1I). Taken together, CBT can efficiently inhibit the growth and migration of colorectal cancer cells.

CBT induces G2/M phase cell cycle arrest and apoptosis in colorectal cancer cells

To determine how CBT suppresses colorectal cancer cell proliferation, we performed flow cytometry assay to examine the effect of CBT on the cell cycle distribution of colorectal cancer cells and prostate cancer cells. The CBT concentration at IC₅₀ to the respective cell lines was adopted for the assay. As expected, CBT treatment led to G2/M cell cycle arrest in all tested cell lines (Fig. 2A). There were approximately 3-fold more cells at G2/M phase in CBT-treated cells than in the control cells (Fig. 2B). This observation is consistent with previous studies reporting that CBT causes G2/M cell cycle arrest in cancer cells [21,22].

Because CBT induced G2/M phase arrest, we next investigated whether CBT treatment triggers apoptosis of colorectal cancer cells. Similarly, the CBT concentration at IC₅₀ to the respective cell lines was adopted for the assay. Forty-eight hours after CBT treatment, the control and CBT-treated cells were stained with annexin V-fluorescein isothiocyanate and propidium iodide to analyze the apoptosis rate of these cells via flow cytometry. Compared to the control cells, CBT treatment led to an approximately 2-fold or more increase in cell apoptosis (Fig. 2C,D). Western blotting revealed that the apoptosis marker-cleaved caspase-3 was also greatly increased in CBT-treated HCT116 cells (Fig. 2E).

Cabazitaxel inhibits tumor growth in colorectal cancer xenograft model

To evaluate the antitumor effect of cabazitaxel against colorectal cancer, we subcutaneously injected GFP-labeled HCT116 cells into nude mice to derive a...
Cabazitaxel suppresses colorectal cancer

(A) Control CBT (0.03 μM) CBT (0.24 μM)

(B) Relative cell viability (%)

(C) HCT116

(D) Control CBT (0.03 μM) CBT (0.06 μM) CBT (0.12 μM)

(E) Numbers of colony

(F) Area of colony (%)

(G) 0 h 24 h 48 h

(H) % Wound Gap

(I) Tubulin+DAPI
Fig. 1. CBT can efficiently inhibit colorectal cancer cells. (A) Cell morphology of control and 0.03 and 0.24 μM CBT-treated HCT116 cells at high magnification (Top: scale bar = 100 μm) and log magnification (Bottom: scale bar = 150 μm). (B) Relative cell viability measured by the MTT assay. Error bars indicate the SD. Student’s t-test was used for statistical analysis. Data are mean ± SD (n = 6). ***P < 0.001. (C) In vitro cytotoxicity of CBT at a different concentration to human colorectal cancer cells HCT116, LOVO, HCT8 and DLD1, as well as Du145 and PC3 prostate cancer cells. Data are the mean ± SD (n = 6). Red lines indicate the nonlinear fit sigmoidal curve. The cell viability rate was obtained by normalizing the MTT assay output of CBT-treated cells with corresponding dimethylsulfoxide-treated cells. (D) Colony morphology of control HCT116 cells and CBT (at the indicated concentration)-treated HCT116 cells. (E) The number of colonies formed by HCT116 cells after treatment with control or CBT of the indicated concentration. Error bars indicate the SD. Student’s t-test was used for statistical analysis. Data are the mean ± SD (n = 3). ***P < 0.001. (F) Percentage of control or CBT-treated HCT116 cell formed colony area in the total cell culture plate area. The area was measured using IMAGEJ. Error bars indicate the SD. Student’s t-test was used for statistical analysis. Data are the mean ± SD (n = 3). ***P < 0.001. (G) Microscopic images of the wound-healing assay with control HCT116 cell culture and 0.03 μM CBT-treated cell culture at the indicated time. Scale bar = 200 μm. (H) Quantification of wound gap in control HCT116 cell culture and 0.03 μM CBT-treated cell culture at different time points compared to the wound gap at 0 h. Data are the mean ± SD (n = 3). **P < 0.01. (I) Representative immunofluorescence images of control and CBT-treated cells blotted with antibody against tubulin. Nuclear DNA was counterstained with DAPI. Scale bar in the 20-fold magnified image = 50 μm, whereas the scale bar in the 40-fold magnified image = 20 μm.

xenograft model of colorectal cancer. Based on the dose of Jevtana (cabazitaxel) used for the patient, the dose of CBT utilized for the mouse experiments was derived according to the body surface area [23-25]. Accordingly, 8 and 16 mg·kg⁻¹ CBT were tested for the efficacy. Because 10-40 mg·kg⁻¹ 5-FU was reported to be effective in inhibiting tumor growth [26,27], we utilized 8 mg·kg⁻¹ 5-FU as a negative drug control and 16 mg·kg⁻¹ 5-FU as a positive drug control for the assay. Mice injected with PBS were also used as a negative control. All experimental mice had xenograft tumors at day 7 after subcutaneous injection of HCT116 cells. We then randomized the mice and treated them with CBT and 5-FU, respectively. Using IVIS Spectrum to monitor tumor growth, we found that mice treated with 8 mg·kg⁻¹ CBT, 16 mg·kg⁻¹ CBT and 16 mg·kg⁻¹ 5-FU showed relatively smaller xenograft tumors than those treated with 8 mg·kg⁻¹ 5-FU and PBS (i.e. negative control groups) (Fig. 3A,B). After further analyses of the drug efficacy by normalizing the tumor with tumor at the injection starting point, we concluded that 8 and 16 mg·kg⁻¹ CBT can inhibit HCT116 cell formed tumors in nude mice as efficiently as 16 mg·kg⁻¹ 5-FU (Fig. 3C,D).

Cabazitaxel treatment induces the abnormal expression of Tubb family gene expression in colorectal cancer cells

To further investigate why CBT can efficiently inhibit colorectal cancer, we performed RNA-seq assays to determine the transcriptomic changes between the control and CBT-treated HCT116 cells. Compared to the control, 421 genes were upregulated and 340 genes were downregulated in CBT-treated HCT116 cells (Fig. 4A and Table 3).

Gene Ontology (GO) analysis on molecular function term enrichment revealed that CBT treatment led to the upregulation of genes involved in a variety of binding events, such as protein binding, protein dimerization, DNA binding and organic cyclic compound binding, etc. (Fig. 4B). The abnormal binding events indicate the disruption of normal dynamics of the microtube lattice inside the cells. Indeed, GO analysis revealed that multiple TUBB and TUBA family genes were upregulated in CBT-treated HCT116 cells (Fig. 4C). This might be a result of the inhibition of the disassembly of the microtube by CBT forcing the cells to complementarily express microtube assembly-related genes. A function chord diagram further revealed that Tubb3, Tubb6, Tubb2a, Tubb4a and Tubb2b are linked to the microtubule-based process, the response to an external stimulus, and the mitotic cell cycle process (Fig. 4D), suggesting a disruptive role of CBT on these processes.

Cabazitaxel inhibits colorectal cancer cell growth via activating the p53 signaling pathway

In addition to a number of Tubb family genes being upregulated in CBT-treated cells, KEGG pathway analysis revealed that CBT treatment-induced genes were enriched in the well-known antitumor p53 signaling pathway (Fig. 5A). Meanwhile, CBT treatment indicated that downregulated genes were related to multiple metabolism processes, such as carbon metabolism and glycine, serine and threonine metabolism, as well as glycolysis (Fig. 5B). Furthermore, GSEA revealed a positive correlation between p53 pathway genes and CBT upregulated genes in HCT116 cells, indicating that CBT indeed enhances the expression of p53 pathway genes (Fig. 5C). Rending the genes to the
Cabazitaxel suppresses colorectal cancer

W. Zhang et al.
p53 pathway clearly showed that multiple cell cycle arrest-related genes, such as p21, 14-3-3-σ and Gaff45, were increased in CBT-treated cells (Fig. 5D). p53 downstream genes Fas, Noxa, PUMA and PIGs, which induce apoptosis, and PAI, TSP1 and Maspin, which inhibit angiogenesis and metastasis, were also upregulated in CBT-treated cells (Fig. 5D). Furthermore real-time PCR assays also confirmed that the mRNA levels of p53 downstream genes such as Tp53i3, Gadd5a, Pmaip1, Cdkn1a and Fas were significantly higher in CBT-treated HCT 116 cells than in control cells (Fig. 5E). In addition, the expression of p53 major downstream protein p21(Waf1/Cip1) that links DNA damage to cell cycle arrest was enhanced.
Fig. 4. Cabazitaxel inhibits colorectal cancer cell growth via activating the P53 signaling pathway. (A) Scatter plot showing the upregulated genes (red points) and downregulated genes (blue points) in CBT-treated HCT116 cells compared to control cells. The cut-off for expression difference is 0.58. (B) GO enrichment analysis showing the enriched molecular of upregulated genes in CBT-treated HCT116 cells compared to control HCT116 cells. GO terms of upregulated and downregulated differentially expressed genes were assessed separately for enrichment using Fisher’s exact test. (C) DAVID analysis of the enriched TUBB and TUBA family related genes. (D) Function chord plot of CBT induced upregulated TUBB family genes.
| Gene name | CBT vs Control upregulated genes |
|-----------|----------------------------------|
| CDC37L1-AS1 | GADD45B CDC20 CEACAM1 ACTA2 MIR4435-2HG CCDC86 TCTA RYBP |
| HIST2H2BE | HIST2H2BC DUSP1 CSF2 RSRC2 EPC1 GEM DEFB1 NSDHL |
| CXCL3 | HIST1H2BE RIPK4 TNNC1 HIST1H2BN HSPB8 SBDS PRMT5 ZNF821 |
| HIST1H2BO | IFIT2 CKLF-CMTM1 LOC105373383 TAF3 FAS LATS2 HIST1H2AE MIR31HG |
| HIST2H2AA4 | TUBB2A LIN28A HIST1H4J MYL9 JOSD2 SFPQ SCAF4 SLC25A25 |
| HIST2H2AA3 | TFAP2A-AS2 RND3 HIST1H2AC DNAJC2 LSMEM1 NR2F2 MUC2 |
| KRTAP3-1 | GADD45G LOC101928841 GPR87 LYAR AURKAPS1 MYL2 NOV |
| CXCL1 | GPR3 NR4A1 H19 IGFBP6 ATAD3B CLTB AKR1B10 ARRDC3 SNAI2 HES1 C6orf226 SESN1 NXF1 RBM38 DAG110 ARP1B10 DAB11 MIR259 |
| SMCI1 | GADD45A H19 IGFBP6 ATAD3B CLTB AKR1B10 ARRDC3 SNAI2 HES1 C6orf226 SESN1 NXF1 RBM38 DAG110 ARP1B10 DAB11 MIR259 |
### Table 3. (Continued).

| Gene name           | CBT vs Control upregulated genes | CBT vs Control downregulated genes |
|---------------------|----------------------------------|-----------------------------------|
| TNFAIP3             | PDE6G                            | NUAK2                             |
| GAST                | EGR1                             | MOBP                              |
| HBA1                | HIST1H4E                         | VHL                               |
| NFKBIA              | EGR3                             | LOC101927765                      |
| HIST1H2BD           | ADM                              | SRTT                              |
| AQP3                | NOCT                             | ARC                               |
| HIST2H2F            | TUBB6                            | HIST1H2AM                         |
| HIST3H2B            | ACTA1                            | MAP2K3                            |
| CCL26               | S100A6                           | ZNF79                             |
| LINCO0115           | BIRC3                            | TRIM29                            |
| CDKN1A              | MAGEA2B                          | HBA2                              |
| JUNB                | ACH2                             | CSRNP1                            |
|                     |                                  |                                   |
| TNFAIP3             | PDE6G                            | NUAK2                             |
| GAST                | EGR1                             | MOBP                              |
| HBA1                | HIST1H4E                         | VHL                               |
| NFKBIA              | EGR3                             | LOC101927765                      |
| HIST1H2BD           | ADM                              | SRTT                              |
| AQP3                | NOCT                             | ARC                               |
| HIST2H2F            | TUBB6                            | HIST1H2AM                         |
| HIST3H2B            | ACTA1                            | MAP2K3                            |
| CCL26               | S100A6                           | ZNF79                             |
| LINCO0115           | BIRC3                            | TRIM29                            |
| CDKN1A              | MAGEA2B                          | HBA2                              |
| JUNB                | ACH2                             | CSRNP1                            |

**CBT vs Control downregulated genes**

| Gene name | CBT vs Control downregulated genes |
|-----------|-----------------------------------|
| SNORA4    | CA9                               |
| SNORA81   | PL2G4B                            |
| SNORA23   | AMT                               |
| BCYRN1    | MOCOS                             |
| RMRP      | PHGDH                             |
| SNORA53   | RHBD1                             |
| SCARNA5   | SLPI                              |
| RPPH1     | LOC155060                         |
| FAM129A   | LHPP                              |
| LOC692247 | CDK5RAP3                          |
| PABPC1L   | SLFN5                             |
| SCARNA10  | THSD4                             |
| RNA5-8SN4 | KCNQ1                             |
| PCK2      | EPOR                              |
| ABCB3     | ANGPT4                            |
| SNORA73B  | TRIM4                             |
| KLHDC7B   | PAQ2R6                            |
| RBCK1     | MLX1PL                            |
| GTPBP2    | GTPM2                            |
| LOC109864269 | HISTH41                         |
| LCN2      | R3HDM2                            |
| LOC102725254 | CR1L                          |
| TRIB3     | MUSTN1                            |
| CCPG1     | RPS10P7                           |
| Gene   | Gene   | Gene   | Gene   | Gene   | Gene   | Gene  |
|--------|--------|--------|--------|--------|--------|-------|
| CBR2   | KF1B   | OR2A7  | DTD1   | SLC45A1| JDP2   | UCN   |
| HIST2H2AC| SLC22A31| MKNK2  | PET100 | PRSS53 | SCARNA9| GSEC  |
| HIST1H4A| GALNT12| SLC7A1 | TOX2   | LHB    | TMEM268| CACNA2D2|
| CHAC1  | FIBCD1 | DHR51  | LINC01133| GSTO2  | LONP1  | GFPT1 |
| XLOC_007697 | TLCD2 | ARHGEF2 | P4HA1 | TSPAN18 | ENKD1  | C1QTNF6|
| NBR2   | H6PD   | SERPINA5| CAMK1D | FOXC1  | RNF144A| PAN2  |
| LAMP3  | PTPRC  | LOC642846| CPQ    | GNAZ   | PKD1   | PTP4A3|
| IL20R8 | FUT3   | CALB2  | GOLGA8A| PTK6   | FLJ23867|       |
| PTPDC1 | NRBP2  | LINC01775| GOLGA8A| PTK6   | FLJ23867|       |
| MAPK15 | BRICD5 | FBXO36 | NTSR1  | HLCS   | SYNP0  |       |
| PIP5KL1| SYTL1  | C15orf65| NREP   | SGSM2  | NOO2   |       |
| SCARNA6| RNA18S3| RADA9  | DMGDH  | TMEM200B| KIAA0895L|       |
| LEMD1  | CRYL1  | ERRF11 | ACCS   | IL11RA | MAFA-AS1|       |
| LURAP1L| SLOC6A9| BNIP3L | MPP25-AS1| HDAC6  | LRC75B |       |
| OR2A20P| MLPH   | BCKDHA | WDR27  | SKAP1  | ZNF395 |       |
| HKDC1  | ENO2   | IZUMO4 | PLAUR  | EML2   | SLC9A3-AS1|       |
| GAS5   | KLK10  | ARSG   | ANO9   | TRIM66 | CCDC146|       |
| SLC7A11| ATP2C2 | TROBP  | NUP210 | SLC2A3 | SPRY4-AS1|       |
| MEGF6  | RGL3   | NUDT8  | CBLC   | HIGD1B | HIST1H4B|       |
| PRPH   | GRAMD1B| ANKRD19P| SLC16A5| VEGFA  | GRB7  |       |
| LOC103021295| PYGB | KCTD15 | SEC31B | LINC01503| OCEL1 |       |
| ASNS   | CLGN   | TMEM254| FAM86B1| LARP6  | GOLGA8B|       |
| TMC4   | FBXO48 | LRC56  | EC11   | TNFRSF25| LOCT2724404|       |
| AKNA   | PAQR4  | EMID1  | LOC100505585| TNRC6C-AS1| ARHGEF19|       |
| SLC1A4 | ADAMS1T10| MYO15B| NRNS2-AS1| KLF2  | ROBO3 |       |
| SLC22A18| TEMEM91 | ITGA3  | ALDOC  | R3HDM4 | JMJD7 |       |
| KCNG1  | NRP1   | ASI1   | TAZ    | DDIT4  | ZFAS1 |       |
| CTBS   | KLF9   | PHYKPL | HIST2H2AB| SLC43A1|       |       |
in CBT-treated HCT116 cells compared to the control cells (Fig. 5F). Meanwhile, CBT treatment led to obvious DNA damage, as manifested by the expression of p-H2A.X, p-Chk1 and p-Chk2 (Fig. 5G).

To examine whether activation of p53 pathway plays a key role for CBT efficacy, we used the CRISPR/Cas9 system to knock out TP53, a p53 encoding gene, in HCT116 cells by two different gRNAs and generated TP53 KO1 cells and TP53 KO2 cells. Western blotting revealed that p53 was completely depleted in the TP53 KO cells (Fig. 5H). The MTT assay revealed that the IC_{50} values of CBT to TP53 KO1 cells and TP53 KO2 cells were 0.175 and 0.096 μM, respectively, which were approximately at least 3-fold higher than the IC_{50} of CBT in HCT116 cells. The enhanced resistance to CBT of TP53 KO cells indicates that the inhibitory effect of CBT to HCT116 cells relies on the TP53 pathway (Fig. 5I). All of these results substantiate our conclusion that CBT inhibits HCT116 cells mainly by activating the p53 pathway.

**Discussion**

As a result of the resistance of colorectal cancer to current drug therapies, there is an urgent need to develop new antitumor drugs. In the present study, we found that FDA-approved drug CBT exhibits potent antitumor efficacy to colorectal cancer. CBT is a microtubule inhibitor [28] that has been reported to bypass some cancer resistance mechanism toward chemotherapeutic agents and shows good efficacy to metastatic prostate cancer, breast cancer and ovarian cancer [4,29,30]. In the present study, we demonstrated that CBT also has potent antitumor function with respect to colorectal cancer.

Tubulins are the primary targets of CBT. Tubulin is the basic block of microtubes that contributes to the cytoskeleton and cell mobile elements. Hence, polymerization and depolymerization of tubulin are essential in mitosis, intracellular transport and cell movement, etc. CBT binds to tubulin and promotes microtube assembly and inhibits its disassembly. Hence, CBT seriously interferes with the recycling of tubulin and the normal dynamics of microtube networks in cells that are required for biological processes. We observed significant upregulation of Tub family gene expression, which manifests as the compensative expression of these genes by cells in response to the microtube assembly-related units after CBT treatment. Consistent with this, we observed a series of microtube inhibition-related cell biology abnormalities, such as cell cycle arrest, cell proliferation, and migration inhibition and apoptosis. In the end, we found that CBT efficiently inhibits the growth of HCT116 xenograft tumor. Unlike inhibition of androgen receptor and heat shock proteins in prostate cancer cells or targeting the phosphoinositide 3-kinase/Akt/mechanistic target of rapamycin pathway in lung adenocarcinoma cells [5], CBT enhances the antitumor pathway-p53 signaling pathway in colorectal cancer cells. p53 and its downstream genes are well characterized with respect to inducing apoptosis and senescence of cancer cells and inhibiting tumor growth and angiogenesis in cancers [31]. The p53 signaling pathway is frequently dysregulated in colorectal cancer. Approximately 40-50% of sporadic colorectal cancer harbor a p53 mutation [32]. Reactivation or restoration of the p53 pathway downstream effectors can efficiently improve the prognosis of colorectal cancer. In line with the apoptotic phenotype triggered by CBT, we found that CBT treatment leads to activation of multiple p53 downstream target genes, such as apoptosis activating genes including Gadd45a [33], Tp53Ii3 [34] and Pmaip1 [35]. The marker for DNA damage, p-HA2.X was also elevated in CBT-treated HCT116 cells. p-HA2.X not only recruits...
(A) CBT upregulated genes

(B) CBT downregulated genes

(C) Enrichment Plot: HALLMARK_P53_Pathway

(D) Enrichment Plot: KEGG_P53_Pathway

(E) (F) (G) (H) (I)

β-Actin 42 KD

P21 20 KD

CBT

Control

TP53KO1

TP53KO2

Control

p53

β-Actin

42 KD

53 KD

Control

CBT

TP53KO2

TP53KO1

HCT116

p-H2A.X

p-Chk1

p-Chk2

β-Actin

42 KD

56 KD

62 KD

15 KD

Cabazitaxel suppresses colorectal cancer

W. Zhang et al.

FEBS Open Bio 11 (2021) 3032–3050 © 2021 The Authors. FEBS Open Bio published by John Wiley & Sons Ltd on behalf of Federation of European Biochemical Societies
proteins involved in DNA repair, but also correlates with apoptosis. As a consequence of apoptosis, DNAs are fragmented and trigger the phosphorylation of H2A.X. Hence, p-H2A.X levels can be used to monitor the anticancer therapy effect as well. An increase in p-H2A.X in CBT-treated HCT116 cells demonstrates the efficacy of CBT with respect to anti-colorectal cancer at the molecular level. DNA damage generally activates p53 and its major downstream target p21 and leads to cell cycle arrest. An increase in p21 in CBT-treated HCT116 cells confirms the activation of the p53-p21 pathway. To support our conclusion, we also generated TP53<sup>-/-</sup> HCT116 cells. Compared to HCT116 cells, TP53<sup>-/-</sup> HCT116 cells are more resistant to CBT treatment, suggesting that CBT inhibitory effect to HCT116 cells relies on the P53 signaling pathway. Furthermore, p53 mutated HCT8 cells and DLD1 cells are more resistant to CBT treatment than HCT116 cells and LoVo cells also demonstrate the need for p53 signaling so that CBT can exert its function in colorectal cancer cells. We also noted that multiple metabolism processes of HCT116 were also disturbed by CBT. A well known characteristic of cancer cell is that they adopt special metabolic features. The disturbance of these features would affect cancer cell survival, proliferation and migration. Detailed mechanistic studies of the effect of CBT on the metabolism of colorectal cancer are needed in the future.

In the present study, we have shown that CBT can efficiently inhibit colorectal cancer proliferation and migration. It suppresses colorectal cancer via enhancing the expression of multiple p53 downstream effector genes and promoting cell cycle arrest, apoptosis and inhibition of angiogenesis. Hence, CBT may serve as an alternative option for colorectal cancer treatment in the future.

Acknowledgements

This work was partly supported by National Natural Science Foundation of China (grant numbers 81773156 and 31970674).

Conflict of interest

The authors declare no conflict of interest.

Data accessibility

The RNA-seq data that support the findings of the present study are openly available in the Sequence Read Archive (SRA) (https://www.ncbi.nlm.nih.gov/sra) under accession number PRJNA687151.

Author contributions

PY and JP designed the study. WZ and RS performed the key experiments. RH, QL, WW and XC carried out the supportive experiments. YZ and JZ performed the bioinformatics analysis. WZ and PY analyzed the data and wrote the manuscript. All authors read and approved the final version of the manuscript submitted for publication.

References

1 Ferlay J, Ervik M, Lam F, Colombet M, Mery L, Piñeros M, Znaor A, Soerjomataram Iand Bray F (2020). Global Cancer Observatory: Cancer Today. Lyon, France: International Agency for Research on Cancer. Available from: https://gco.iarc.fr/today. Accessed 1 October 2021.

2 Benson AB, Venook AP, Al-Hawary MM, Cederquist L, Chen YJ, Ciombor KK, Cohen S, Cooper HS, Deming D, Engstrom PF et al. (2018) Rectal cancer, version 2.2018, NCCN clinical practice guidelines in oncology. J Natl Compr Canc Netw 16, 874–901.

3 Kopetz S, Chang GJ, Overman MJ, Eng C, Sargent DJ, Larson DW, Grothey A, Vauthey JN, Nagorney DM and McWilliams RR (2009) Improved survival in metastatic colorectal cancer is associated with adoption of hepatic resection and improved chemotherapy. J Clin Oncol 27, 3677–3683.

4 Vrignaud P, Semiond D, Lejeune P, Bouchard H, Calvet L, Combeau C, Riou JF, Commercon A, Lavelle F and Bissyery MC (2013) Preclinical antitumor activity of cabazitaxel, a semisynthetic taxane active in taxane-resistant tumors. Clin Cancer Res 19, 2973–2983.

5 Rottach AM, Ahrend H, Martin B, Walther R, Zimmermann U, Burchardt M and Stope MB (2019) Cabazitaxel inhibits prostate cancer cell growth by inhibition of androgen receptor and heat shock protein expression. World J Urol 37, 2137–2145.

6 Miia AC, Denis LJ, Rowinsky EK, Debono JS, Goetz AD, Ochoa L, Forouzesh B, Beeram M, Patniak A, Molpus K et al. (2009) Phase I and pharmacokinetic study of XRP6258 (RPR 116258A), a novel taxane, administered as a 1-hour infusion every 3 weeks in patients with advanced solid tumors. Clin Cancer Res 15, 723–730.

7 Pivot X, Koralewski P, Hidalgo JL, Chan A, Goncalves A, Schwartzmann G, Assadourian S and Lotz JP (2008) A multicenter phase II study of XRP6258 administered as a 1-h i.v. infusion every 3 weeks in taxane-resistant metastatic breast cancer patients. Ann Oncol 19, 1547–1552.

8 Huo R, Wang L, Liu P, Zhao Y, Zhang C, Bai B, Liu X, Shi C, Wei S and Zhang H (2016) Cabazitaxel-induced autophagy via the PI3K/Akt/mTOR pathway.
contributes to A549 cell death. *Mol Med Rep* **14**, 3013–3020.

9 Awwad RA, Sergina N, Yang H, Ziober B, Willson JK, Zborowska E, Humphrey LE, Fan R, Ko TC, Brattain MG *et al*. (2003) The role of transforming growth factor alpha in determining growth factor independence. *Cancer Res* **63**, 4731–4738.

10 Howell GM, Humphrey LE, Awwad RA, Wang D, Koterba A, Periyasamy B, Yang J, Li W, Willson JK, Ziober BL *et al*. (1998) Aberrant regulation of transforming growth factor-alpha during the establishment of growth arrest and quiescence of growth factor independent cells. *J Biol Chem* **273**, 9214–9223.

11 Jiang D, Yang H, Willson JK, Liang J, Humphrey LE, Zborowska E, Wang D, Foster J, Fan R and Brattain MG (1998) Autocrine transforming growth factor alpha provides a growth advantage to malignant cells by facilitating re-entry into the cell cycle from suboptimal growth states. *J Biol Chem* **273**, 31471–31479.

12 Sawhney RS, Zhou GH, Humphrey LE, Ghosh P, Kreisberg JI and Brattain MG (2002) Differences in sensitivity of biological functions mediated by epidermal growth factor receptor activation with respect to endogenous and exogenous ligands. *J Biol Chem* **277**, 75–86.

13 Wang J, Sun L, Myeroff L, Wang X, Gentry LE, Yang J, Liang J, Zborowska E, Markowitz S, Willson JK *et al*. (1995) Demonstration that mutation of the type II transforming growth factor beta receptor inactivates its tumor suppressor activity in replication error-positive colon carcinoma cells. *J Biol Chem* **270**, 22044–22049.

14 Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M and Gingeras TR (2013) STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* **29**, 15–21.

15 Roberts A, Trapnell C, Donaghey J, Rinn JL and Pachter L (2011) Improving RNA-Seq expression estimates by correcting for fragment bias. *Genome Biol* **12**, R22.

16 Liberzon A, Birger C, Thorvaldsdottir H, Ghandi M, Mesirov JP and Tamayo P (2015) The Molecular Signatures Database (MSigDB) hallmark gene set collection. *Cell Syst* **1**, 417–425.

17 Kanekisa M and Goto S (2000) KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res* **28**, 27–30.

18 Kanekisa M (2019) Toward understanding the origin and evolution of cellular organisms. *Protein Sci* **28**, 1947–1951.

19 Kanekisa M, Furumichi M, Sato Y, Ishiguro-Watanabe M and Tanabe M (2021) KEGG: integrating viruses and cellular organisms. *Nucleic Acids Res* **49**, D545–D551.

20 Doench JG, Fusi N, Sullender M, Hegde M, Vainberg EW, Donovan KF, Smith I, Tothova Z, Wilen C, Orchard R *et al*. (2016) Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9. *Nat Biotechnol* **34**, 184–191.

21 Neshasteh-Riz A, Zeinizada E, Safa M and Mousavizadeh K (2018) Cabazitaxel inhibits proliferation and potentiates the radiation response of U87MG glioblastoma cells. *Cell Biol Int* **42**, 815–822.

22 Chen R, Cheng Q, Owusu-Ansah KG, Chen J, Song G, Xie H, Zhou L, Xu X, Jiang D and Zheng S (2018) Cabazitaxel, a novel chemotherapeutic alternative for drug-resistant hepatocellular carcinoma. *Am J Cancer Res* **8**, 1297–1306.

23 Corn PG, Heath EI, Zurita A, Ramesh N, Xiao L, Sei E, Li-Ning-Tapia E, Tu SM, Subudhi SK, Wang J *et al*. (2019) Cabazitaxel plus carboplatin for the treatment of men with metastatic castration-resistant prostate cancers: a randomised, open-label, phase 1–2 trial. *Lancet Oncol* **20**, 1432–1443.

24 Reagan-Shaw S, Nihal M and Ahmad N (2008) Dose translation from animal to human studies revisited. *FASEB J* **22**, 659–661.

25 Vallome G, Cattrini C, Messina C, Cerbone L, Boccardo F and Zanardi E (2019) Reduced dose of cabazitaxel in metastatic castration-resistant prostate cancer: from PROSELICA trial to the real life: a single institution experience. *Anticancer Drugs* **30**, 854–858.

26 Tao L, Yang JK, Gu Y, Zhou X, Zhao AG, Zheng J and Zhu YJ (2015) Weichang’an and 5-fluorouracil suppresses colorectal cancer in a mouse model. *World J Gastroenterol* **21**, 1125–1139.

27 Zhu P, Zhao N, Sheng D, Hou J, Hao C, Yang X, Zhu B, Zhang S, Han Z, Wei L *et al*. (2016) Inhibition of growth and metastasis of colon cancer by delivering 5-fluorouracil-loaded pluronic P85 copolymer micelles. *Sci Rep* **6**, 20896.

28 Villanueva C, Bazan F, Kim S, Demarchi M, Chaigneau L, Thiery-Vuillemin A, Nguyen T, Cals L, Dobi E and Pivot X (2011) Cabazitaxel: a novel microtubule inhibitor. *Drugs* **71**, 1251–1258.

29 Koutras A, Zagouri F, Koliou GA, Psoma E, Chryssogonidis I, Lazaridis G, Tryfonopoulos D, Kotsakis A, Res E, Kentepozidis NK *et al*. (2020) Phase 2 study of cabazitaxel as second-line treatment in patients with HER2-negative metastatic breast cancer previously treated with taxanes—a Hellenic Cooperative Oncology Group (HeCOG) Trial. *Br J Cancer* **123**, 355–361.

30 Madsen CV, Adimi P, Jakobsen A and Steffensen KD (2020) Cabazitaxel – a treatment option in recurrent platinum-resistant ovarian cancer. *Anticancer Res* **40**, 5255–5261.

31 Hafner A, Bulkly ML, Jambhekar A and Lahav G (2019) The multiple mechanisms that regulate p53 activity and cell fate. *Nat Rev Mol Cell Biol* **20**, 199–210.
32 Takayama T, Miyanishi K, Hayashi T, Sato Y and Niitsu Y (2006) Colorectal cancer: genetics of development and metastasis. J Gastroenterol 41, 185–192.

33 Lou M, Li R, Lang TY, Zhang LY, Zhou Q and Li L (2021) Aberrant methylation of GADD45A is associated with decreased radiosensitivity in cervical cancer through the PI3K/AKT signaling pathway. Oncol Lett 21, 8.

34 Chu Y, Li D, Zhang H, Ding J, Xu P, Qiu X and Zhang H (2018) PIG3 suppresses gastric cancer proliferation by regulating p53-mediated apoptosis. J Biol Regul Homeost Agents 32, 1185–1189.

35 Do H, Kim D, Kang J, Son B, Seo D, Youn H, Youn B and Kim W (2019) TFAP2C increases cell proliferation by downregulating GADD45B and PMAIP1 in non-small-cell lung cancer cells. Biol Res 52, 35.