Translational Oncology 13 (2020) 100804

Contents lists available at ScienceDirect

Translational Oncology

journal homepage: www.elsevier.com/locate/tranon

Low Expression of Smurf1 Enhances the Chemosensitivity of Human Colorectal Cancer to Gemcitabine and Cisplatin in Patient-Derived Xenograft Models

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ARTICLE INFO

Article history:
Received 30 March 2020
Received in revised form 8 May 2020
Accepted 11 May 2020
Available online xxxx

ABSTRACT

Despite the side effects, chemotherapy is one of the most common treatments in colorectal cancer (CRC). An open-ended question about CRC chemotherapy, which has been discussed quite often, is with respect to the validation of prognostic or predictive factors. It is believed that personalized chemotherapy can improve the treatment outcome of patients with colorectal tumors. Though, Smurf1 is highly expressed in multiple tumors and plays a critical role in the occurrence and development of multiple cancers, it's role in the susceptibility of CRC response to chemotherapy is still unknown, Therefore, the study aimed to understand the role of Smurf1 in the susceptibility of CRC response to chemotherapy. The study showed that the knockdown of Smurf1 increases gemcitabine and cisplatin-induced HCT116 cells apoptosis in vitro. Furthermore, in vivo experiments showed that tumors that had low Smurf1 expression exhibited enhanced gemcitabine, cisplatin, and gemcitabine plus cisplatin anti-tumor effects in HCT116 cell-derived xenograft (CDX) models and patient-derived xenograft (PDX) models. In conclusion, the results indicated that Smurf1 inhibits the chemosensitivity of CRC to gemcitabine, cisplatin, and gemcitabine plus cisplatin. Therefore, downregulati1ng the Smurf1 expression is a potential strategy to increase the efficacy of gemcitabine and cisplatin in CRC patients.

Introduction

Colorectal cancer (CRC) is a common gastrointestinal malignant tumor, which is the third most common cancer in terms of incidence and second most common in terms of mortality [1]. In the recent years, new and improved generation of chemotherapeutic agents and molecular targeted agents for treating CRC have emerged [2,3]. However, some CRC patients are insensitive to chemotherapeutic drugs.

Smad ubiquitination regulatory factor 1 (Smurf1) is a HECT-type E3 ubiquitin-protein ligase, which belongs to the neuronal precursor cell-expressed developmentally down-regulated 4 (Nedd4) family. Smurf1 is highly expressed in multiple cancers, including CRC [4], ovarian cancer [5], and breast cancer [6]. It plays a critical role in the occurrence and development of multiple tumors by targeting diverse substrates such as RhoA [7], DAB2IP [8], and ARHGP26 [5] for ubiquitin-dependent degradation and regulates tumor cell growth and metastasis. However, it is significantly unclear if Smurf1 regulates the sensitivity of cancer cells to chemotherapeutic drugs.

To improve drug development, National Cancer Institute (NCI), in the 1970s, used human cancer models for drug screening. However, over 90% of the drugs with preclinical activity failed during the clinic trail owing to insufficient efficacy [9]. During the last 40 years, several methods or models have been developed for preclinical testing of anti-tumor agents

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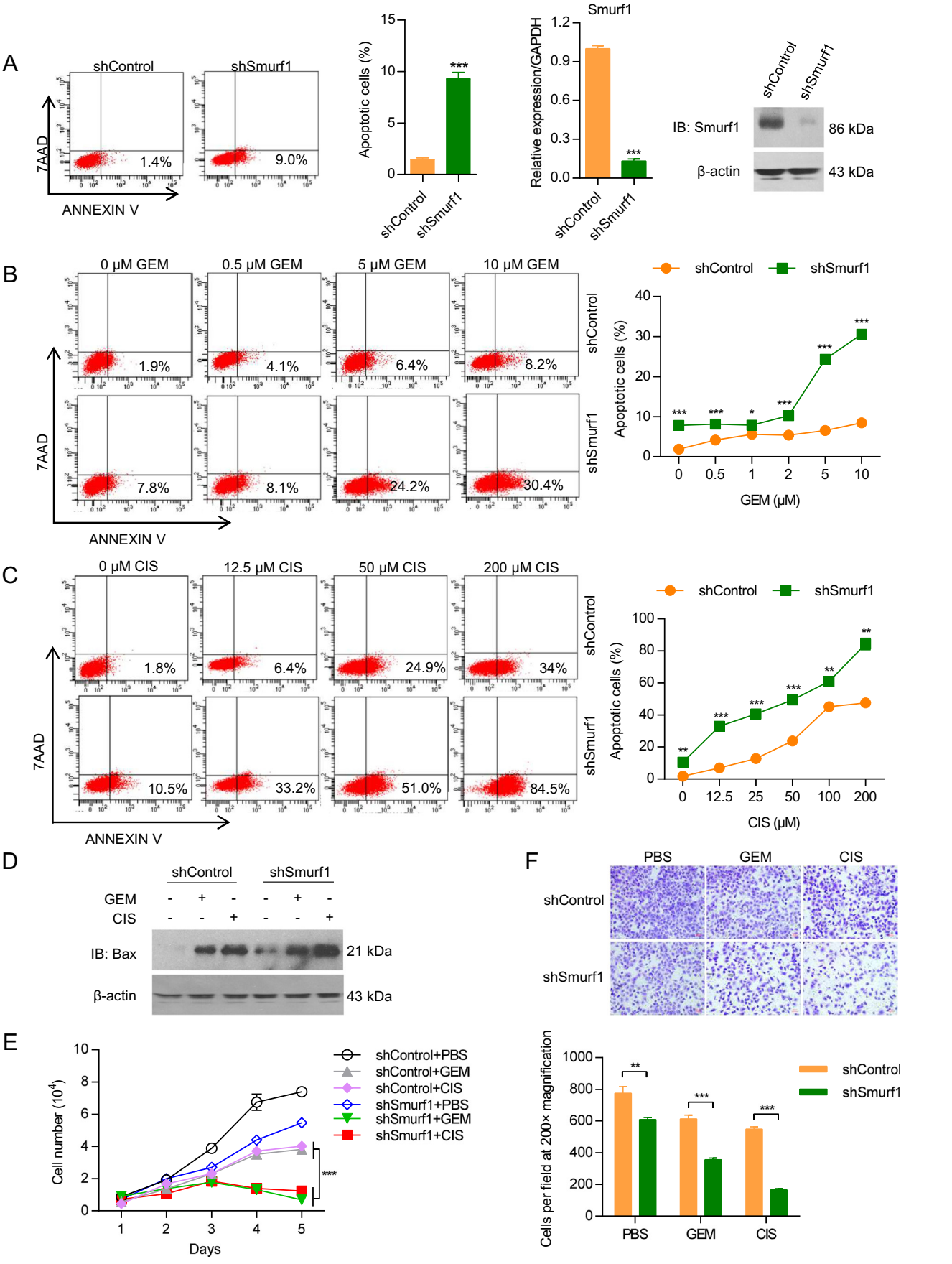
http://dx.doi.org/10.1016/j.tranon.2020.100804



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both in vitro and in vivo [10]. Recently, the development and application of patient-derived tumor xenograft (PDX) models for preclinical research have gained ample popularity. PDX models, established from tumor tissue samples directly implanted into immunodeficient mice, maintain the histopathologic architecture and genetic characteristics of their donor tumor, as well as the interplay with stromal components and other cells fluxing into the tumor environment [10–12]. Therefore, PDX models offer a potential solution to maximize the success of drug development and ultimately allow for personalized treatment of patients.

In the present study, we investigated if the knockdown of Smurf1 could enhance the chemosensitivity of colorectal cancer cells to gemcitabine, cisplatin, and gemcitabine plus cisplatin in cell-derived xenograft (CDX) and PDX models. We determined that the knockdown of Smurf1 in HCT116 cell line exhibited enhanced chemosensitivity to gemcitabine, cisplatin, and gemcitabine plus cisplatin, alongside that of CDX and PDX models. To the best of our knowledge, this is the first description of the relationship between Smurf1 and chemosensitivity in human cancers.

Materials and Methods

Cell Culture and Cell Lines

HCT116, MCF7, HeLa and A549 cells were cultured in RPMI 1640 medium (Gibco) supplemented with 10% fetal bovine serum (Gibco), 100 units mL^{-1} penicillin and streptomycin, at 37°C in a humidified 5% CO_2 incubator. All cell lines used in this study were purchased from American Type Culture Collection (ATCC, Manassas, VA).

Antibodies and Chemical Reagents

Anti-Smurf1 antibody (ab57573) was purchased from Abcam; anti-PCNA and anti-Cyclin D1 antibodies were purchased from Cell Signaling Technology. Gemcitabine was purchased from MCE; cisplatin was purchased from APExBIO.

Establishment of Stable Smurf1 Knockdown Cell Lines

To knock down endogenous Smurf1 expression in HCT116, MCF7, HeLa and A549 cells, lentiviruses containing Smurf1 or control shRNA were purchased from GeneChem Co., Ltd., Shanghai, China. And the stable cell lines with Smurf1 knockdown were constructed as previously described [13]. The knockdown efficiency was detected by western blot and real-time PCR.

RNA Extraction and Analysis

Smurf1 knockdown cell lines, including HCT116, MCF7, HeLa and A549 cells, were homogenized in TRizol and total RNA purified, DNase treated, quantified and subjected to reverse transcription PCR, quantitative Real-time PCR (qPCR) and calculating gene expression levels relative to GAPDH ($2^{-\triangle\triangle Ct}$). Quantitative Real-time PCR was performed using an ABI StepOne Plus system (Applied Biosystems, Foster City, CA). Primer sequences used in this study were as follows: Smurf1 F, 5′-ctaccagcgtttggatctat-3′ and Smurf1 R, 5′-tgtctcgggtctgtaaact-3′; GAPDH F, 5′-aggtcggtgtgaacggatttg-3′ and GAPDH R, 5′-tgtagaccatgtagttgaggtca-3′. F and R represent forward and reverse primer, respectively.

Apoptosis Assays for Flow Cytometry

2×10^5 HCT116 cells stably knocked down for Smurf1 or not were washed with PBS and stained with PE Annexin V and 7-AAD according to the manufacture's protocol of PE Annexin V Apoptosis Detection Kit I (BD Pharmingen, San Diego, CA, USA). Apoptotic cells were then determined by flow cytometry.

Transwell Assays

5×10^4 control or Smurf1-depleted HCT116 cells were added in the upper well of the 24-well transwell plate with 8-mm polyethylene terephalate membrane filters (Corning) in 200 μL serum-free RPMI 1640 medium supplemented with 1 μM GEM or 6.25 μM CIS. The bottom reservoir was filled with 500 μL RPMI 1640 medium plus with 10% fetal bovine serum (FBS) and 100 U mL^{-1} penicillin. Cells were cultured at 37°C in a humidified 5% CO_2 incubator. After 24 h, cells on the lower membrane surface were fixed with methanol for 10 min and stained with 0.1% Crystal Violetin 20% (v/v) methanol for 15 min, photographed and the cell number on each filter was counted in five randomly selected 200× fields.

Histochemistry

Tissues were dissected in 10% formalin for 48 h and processed for 3.5 μm paraffin wax sections. Hematoxylin and Eosin staining was performed using standard histology techniques. Primary antibodies used were against PCNA, Cyclin D1 and Smurf1. Signal detection was performed using DAB substrate kit (ZSGB-BIO, Beijing, China) according to the manufacturer's instructions.

Nude Mice Xenograft Models (CDX Models)

BALB/c nude mice (6-7 weeks old, 18.0 ± 2.0 g) were obtained from Beijing HFK Bioscience CO., LTD. HeLa cells (8×10^5/site) were inoculated subcutaneously into both sides of the front armpits of the mice. Tumor size and body weight were measured weekly and tumor volume (TV) was calculated as follows: TV (mm^3) = $(a \times b^2)/2$, where a and b were the largest and smallest diameters, respectively. Once the tumor had attained a size of 100 mm^3, mice were injected intraperitoneally (i.p.) with gemcitabine at 50 mg/kg, cisplatin at 2.5 mg/kg and gemcitabine (25 mg/kg) plus cisplatin (1.25 mg/kg) weekly.

All animals were sacrificed after treated or not for 3 weeks. Tumors were removed, weighed and fixed for histological examination. All experimental procedures were approved by the Animal Care and Use Committee (IACUC) of Institute of Laboratory Animal Sciences (Institute of Laboratory Animal Science, CAMS & PUMC). Then histochemical analysis was performed as described in Histochemistry method.

Construction of PDX Models

Experiments were performed after receiving patient informed consent and approval from the Research Ethics Committee of Peking University People's Hospital. All samples identities are renamed with codes (such as CRC1, CRC2, CRC3, CRC4, CRC5, CRC6) instead of the patient's name, and hospital number. The animal care protocol for this study was approved by the Institutional Animal Care and Use Committee (IACUC) of the Laboratory of Animal Research at the Institute of Laboratory animal Sciences, CAMS&PUMC. Fresh

← **Figure 1. Knockdown of Smurf1 promotes gemcitabine or cisplatin-induced HCT116 cells apoptosis. A.** Flow cytometry was performed to evaluate the cell apoptosis of control or Smurf1-depleted HCT116 cells. And the percentage of apoptotic cells was statistically analyzed. The knockdown efficiencies of Smurf1 were detected by real-time PCR and western blot. Data represent three independent experiments. **B and C.** The cell apoptosis of control or Smurf1-depleted HCT116 cells with or without treated with the indicated concentration of gemcitabine (0.5, 1, 2, 5, 10 μM) or cisplatin (12.5, 25, 50, 100, 200 μM) for 48 h. And the percentage of apoptotic cells was statistically analyzed. **D.** Western blot analysis of the expression of apoptosis protein Bax in control or Smurf1-depleted HCT116 cells treated with 10 μM GEM or 200 μM CIS for 48 h. **E.** The cell numbers of control or Smurf1-depleted HCT116 cells cultured in medium supplemented with 1 μM GEM or 6.25 μM CIS were calculated in the indicated time. **F.** The cell migration assays were performed in transwell plates. Data represent three independent experiments. GEM, gemcitabine; CIS, cisplatin. Data represent the mean ± S.D. $^{**}P < .01$, $^{***}P < .001$.

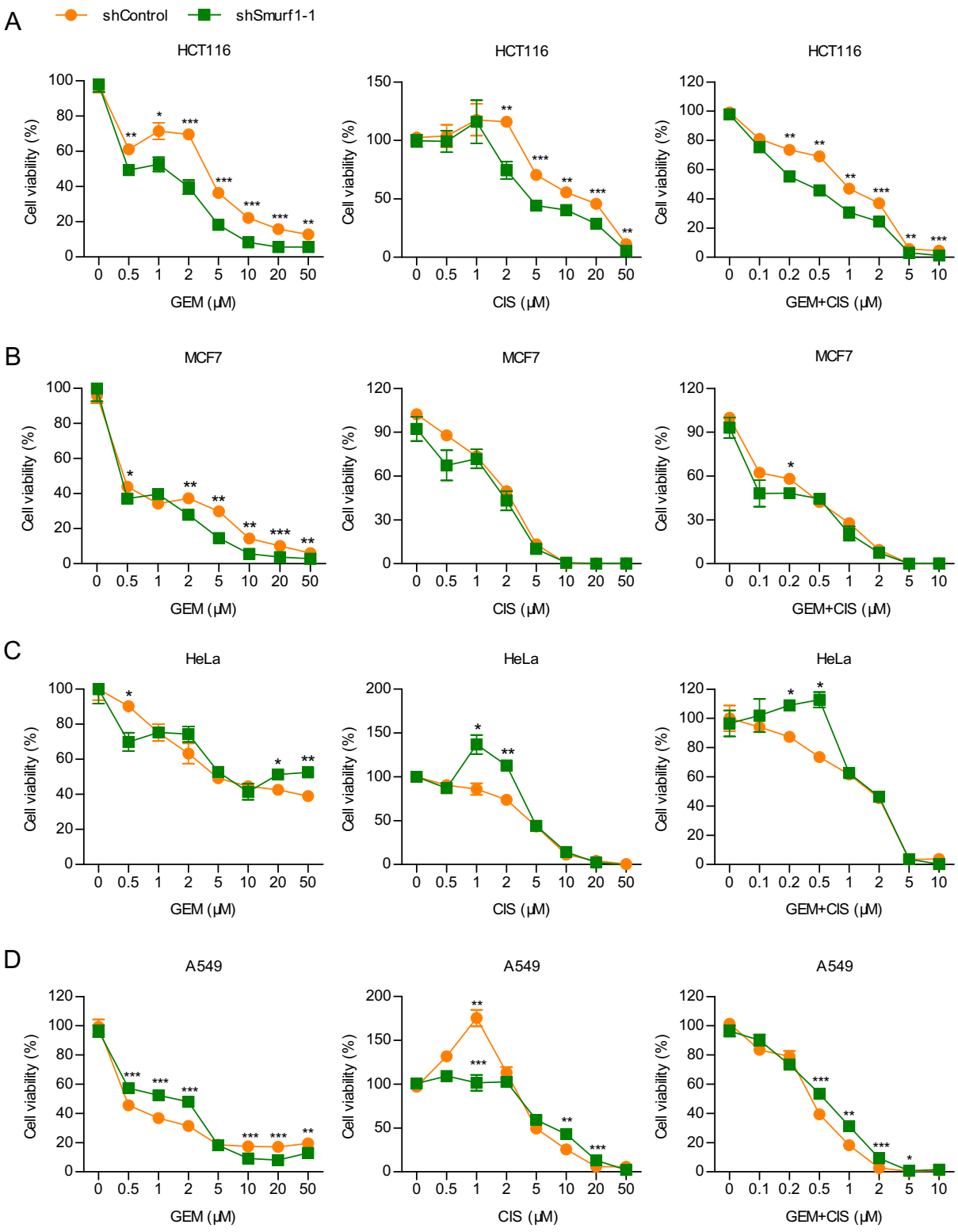


Figure 2. Smurf1 inhibits chemosensitivity of HCT116 cells to gemcitabine and cisplatin in vitro. CCK-8 assay was used to detect cell viability of HCT116 (**A**), MCF7 (**B**), HeLa (**C**) and A549 (**D**) cells. Cells were treated with the indicated concentrations of gemcitabine (0, 0.5, 1, 2, 5, 10, 20, 50 μM), cisplatin (0, 0.5, 1, 2, 5, 10, 20, 50 μM) or both (0, 0.1, 0.2, 0.5, 1, 2.5, 10 μM) for 48 h. Data represent three independent experiments. Data represent the mean ± S.D. $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$.

colorectal cancer tissues were sliced into about 2-3 mm^3 fragments in cold PBS and six-week-old male NSG mice were subjected to tumor engraftment. Once the xenograft tumor had attained a size of 100 mm^3, 50 mg/kg gemcitabine, 2.5 mg/kg cisplatin and 25 mg/kg gemcitabine plus 1.25 mg/kg cisplatin were given via intraperitoneal injection (i.p.) weekly. Body weight and tumor growth were monitored weekly for five weeks and PDX mice were sacrificed. These tumor tissues were fixed in 10% formalin for 48 h and processed for 3.5 μm sections. Then histochemical analysis was performed as previously described.

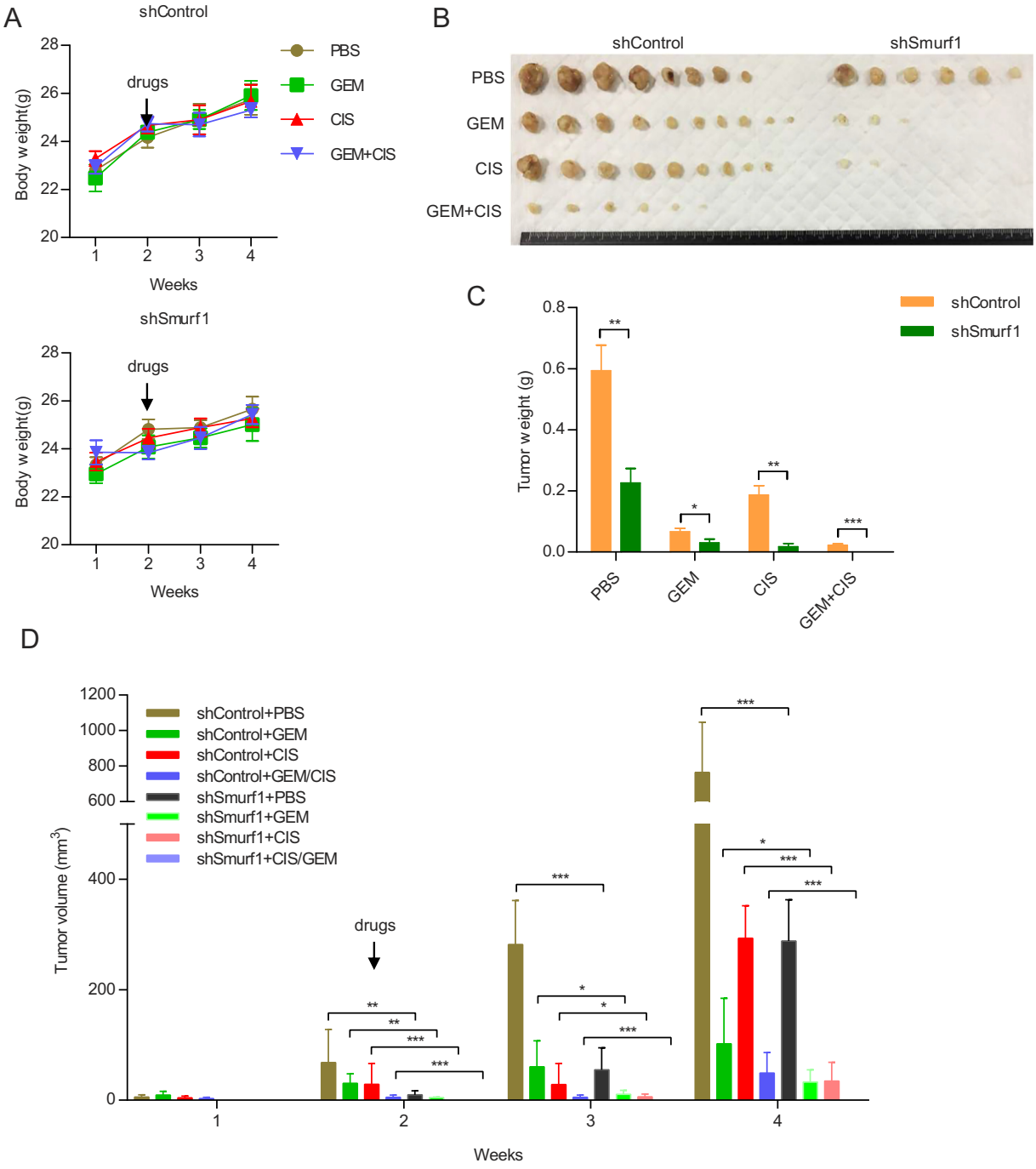


Figure 3. Depletion of Smurf1 enhances the chemosensitivity of human colorectal cancer to gemcitabine and cisplatin in HCT116 cell-derived xenograft models. **A.** Body weight was analyzed at the indicated time points. **B.** Volume of xenografts derived from HCT116 cells (8×10^5 per site) subcutaneously inoculated into both sides of the front armpits of the mice (n = 6 per group) and treated with or without gemcitabine, cisplatin or both for 3 weeks. The transplanted tumors were removed and photographed. Tumor weight (**C**) and tumor volume (**D**) were determined. Tumor volume was measured using a caliper at the indicated time points. Data are shown as the mean ± S.D. *$P < .05$, **$P < .01$, ***$P < .001$.

Statistical Analysis

Data were analyzed using GraphPad Prism5 (GraphPad Software). Two-tailed Student's t-test was applied to analyze continuous variables. Statistically significant differences were presented as either *P < 0.05, **P < 0.01, or ***P < 0.001. Three independent replicates were performed for each experiment. All results were calculated as the mean ± standard deviation (SD).

Results

Gemcitabine and Cisplatin Increase Smurf1-Depleted HCT116 Cells Apoptosis

Smurf1 is highly expressed in colorectal tumor tissues and correlates with cancer progression [4]. It was determined that the knockdown of Smurf1 leaded to a significant increase in apoptosis in HCT116 cells (Figure 1*A*). To investigate the role of Smurf1 in

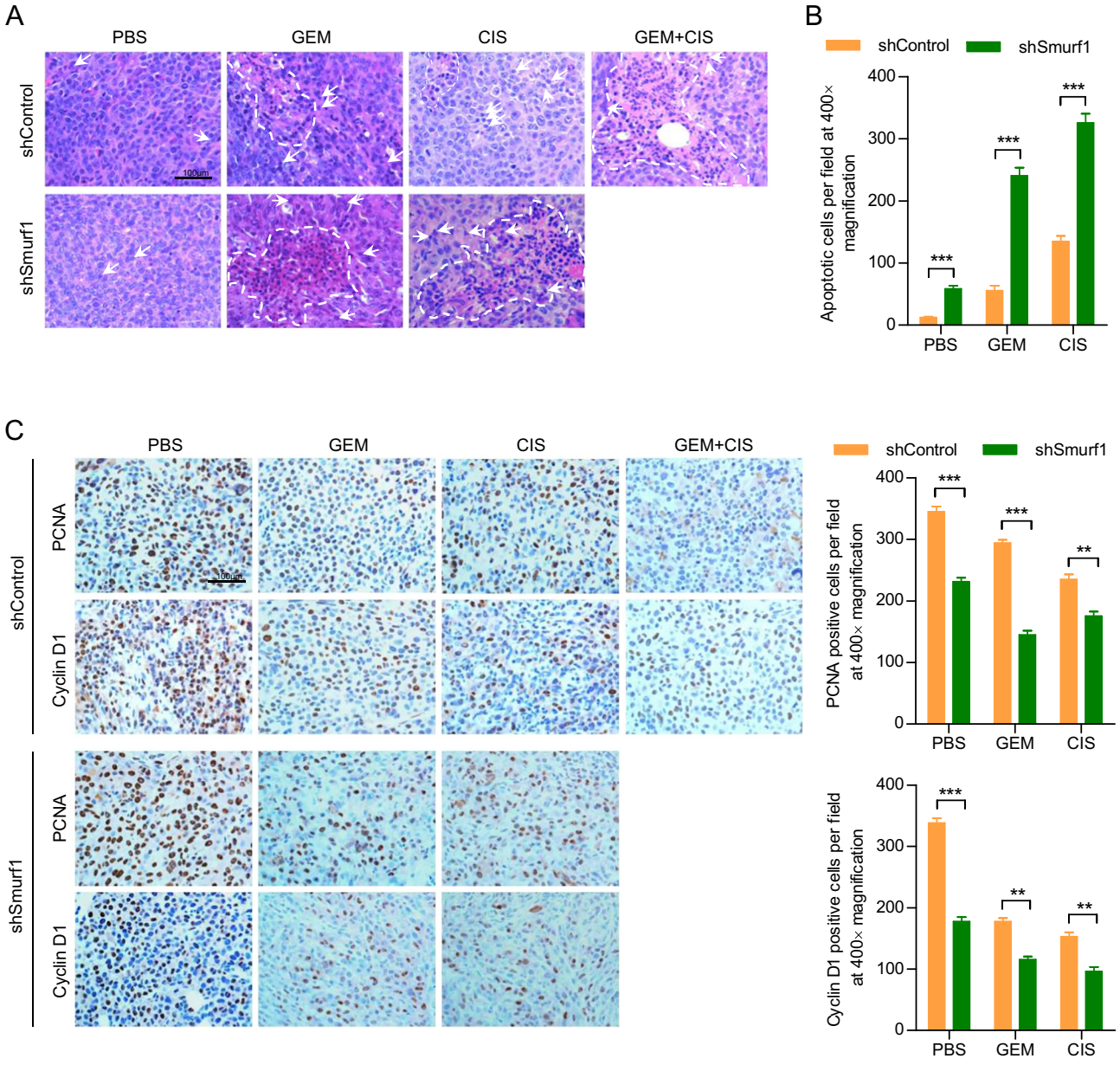


Figure 4. Histological analysis of colon tumors from HCT116 cell-derived mouse xenografts. A. The histology of xenograft tumors was determined via H&E staining (magnification, 400×). Apoptotic cells were shown as the white arrows indicated. And the white dashed boxes showed the area of necrosis. **B.** Apoptotic cells were determined through TUNEL staining. **C.** Immunohistological staining was analyzed using antibodies against PCNA and Cyclin D1 to determine cell proliferation of xenograft tumors from shControl or shSmurf1 HCT116 cells. The positive cell number was counted in five randomly selected 400× fields. Scale, 100 μm. Data represent the mean ± S.D. $^{**}P < .01$, $^{***}P < .001$.

terms of the sensitivity of human colorectal cancer cells to chemotherapeutic drugs, we firstly identified if gemcitabine could significantly induce apoptosis of Smurf1-depleted HCT116 cells. As shown in Figure 1B, the percentage of apoptotic cells increased with an increase in the gemcitabine concentration. Similarly, cisplatin was found to increase the rate of apoptotic cells (Figure 1*C*). Gemcitabine or cisplatin enhanced the expression of apoptosis-related protein, Bax, in Smurf1-depleted HCT116 cells (Figure 1*D*). Furthermore, gemcitabine or cisplatin reduced the proliferation and migration of Smurf1-depleted HCT116 cells (Figure 1, *E* and *F*). These findings revealed an inhibitory function of Smurf1, which was suppressing the chemosensitivity of human colorectal cancer cells to gemcitabine and cisplatin.

The Knockdown of Smurf1 Enhances the Sensitivity of Colorectal Cancer Cells to Gemcitabine and Cisplatin In Vitro

Based on the above-mentioned results, we demonstrated that Smurf1 plays a critical role in HCT116 cells' resistance to gemcitabine and cisplatin. As listed in the Supplementary Table S1, we found that the half maximal inhibitory concentration (IC_{50}) data for HCT116 significantly decreased for gemcitabine, cisplatin, and gemcitabine plus cisplatin treatment in the Smurf1-depleted HCT116 cells compared to the control cells following drug treatment. The effect observed was most significant in the combination group. Furthermore, we investigated to determine similar effects in other types of cancer cells. We established breast cancer MCF7, cervical cancer HeLa, and lung adenocarcinoma A549 cell lines with lentivirus-

Table 1
Patient information

Case no.	Gender	Age	Position	Diagnosis	IHC
CRC1	M	76	Colon	Adenocarcinoma	HER2: negative MLH1: positive MSH2: positive MSH6: positive PMS2: positive c-MET: positive (+)
CRC2	M	61	Right hemicolon	Adenocarcinoma	HER2: negative MLH1: positive MSH2: positive MSH6: positive PMS2: positive c-MET: positive (++)
CRC3	M	34	Rectum	Adenocarcinoma	HER2: positive MLH1: positive MSH2: positive MSH6: positive PMS2: positive c-MET: positive (+)
CRC4	M	59	Colon	Adenocarcinoma	HER2: positive MLH1: positive MSH2: positive MSH6: positive PMS2: positive c-MET: positive (++)
CRC5	M	46	Rectum	Adenocarcinoma	HER2: positive MLH1: positive MSH2: positive MSH6: positive PMS2: positive c-MET: positive (++)
CRC6	F	59	Right hemicolon	Adenocarcinoma	HER2: positive MLH1: positive MSH2: positive MSH6: positive PMS2: positive c-MET: positive (+++)

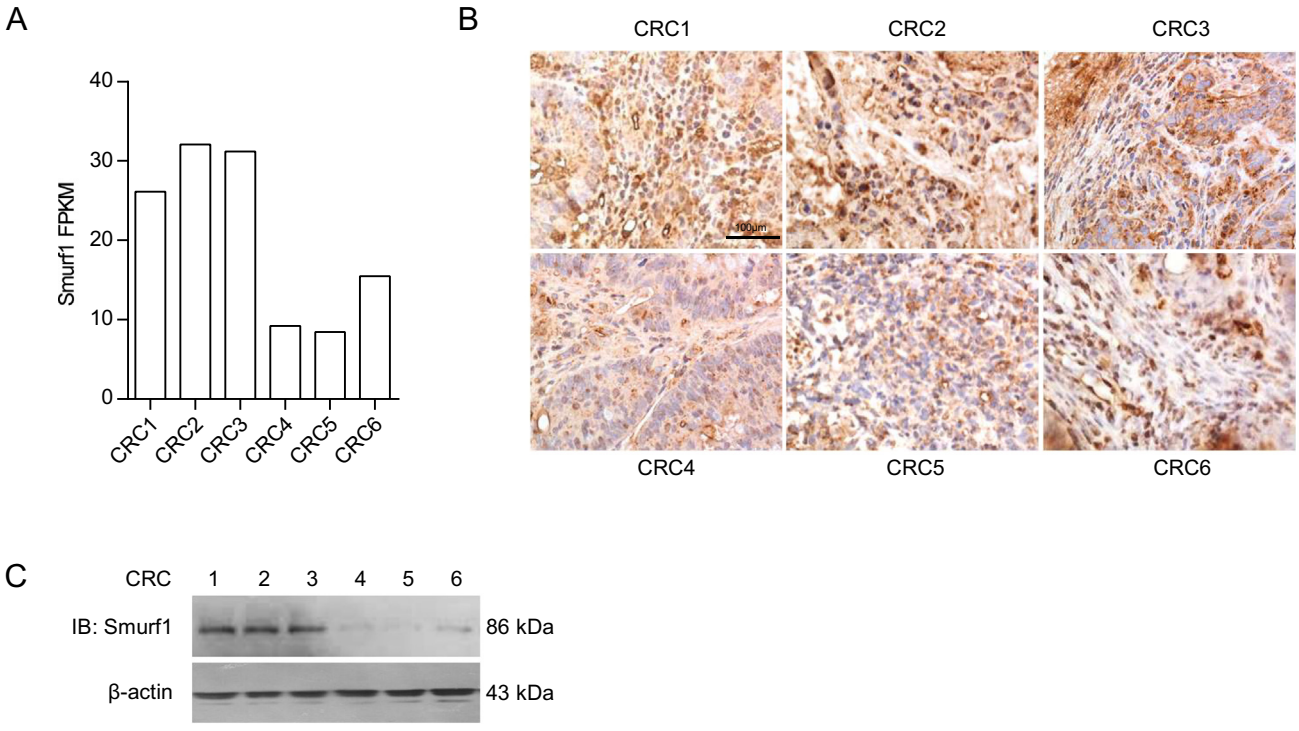
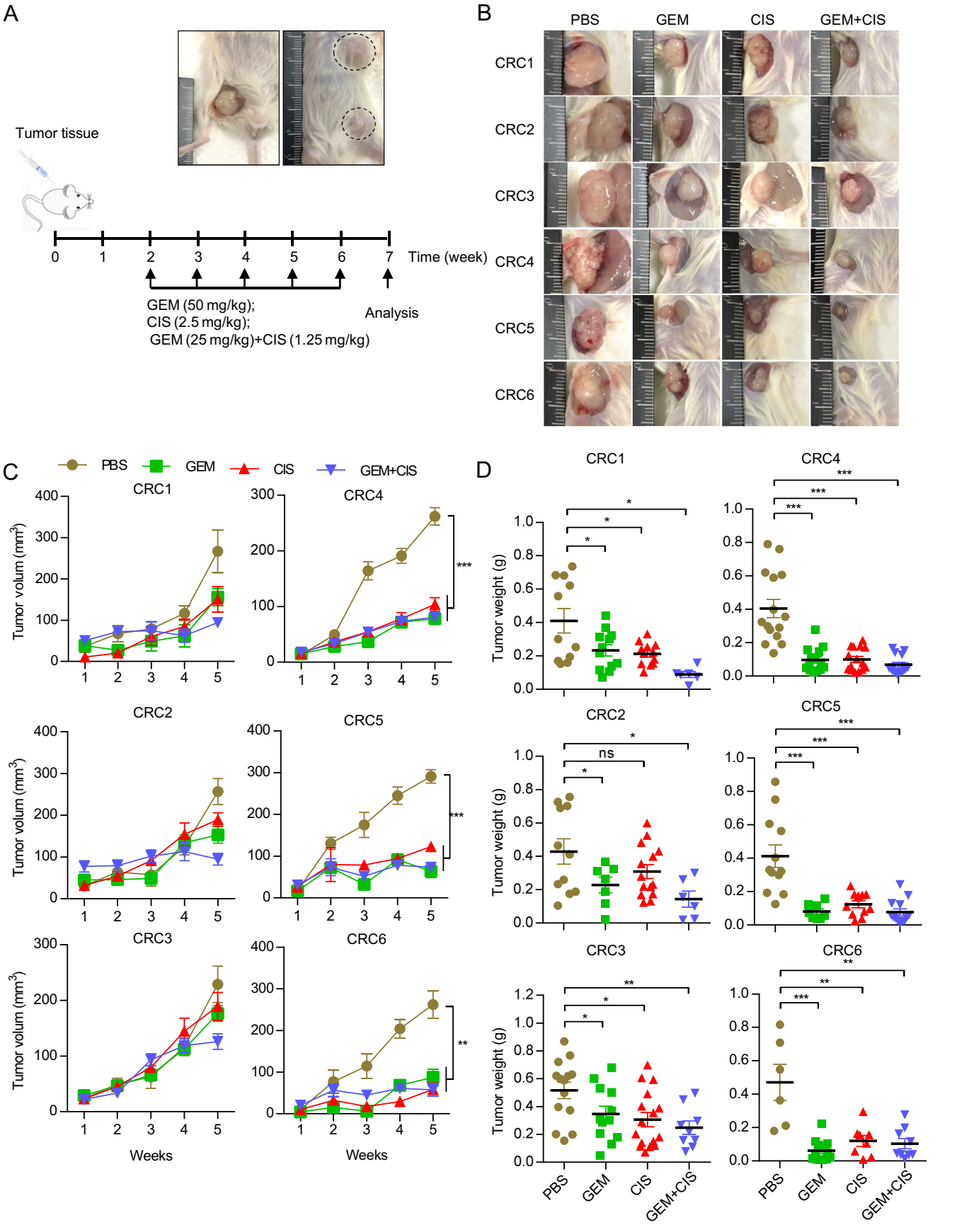


Figure 5. Analysis of Smurf1 expression in CRC tissues. A. Smurf1 RNA levels were analyzed in the indicated 6 CRC tissues. RNA-seq analysis was performed. **B.** Immunohistological analysis of the CRC tissues and representative images of Smurf1 staining were shown at 400× magnification. Scale, 100 μm. **C.** Western blot analysis of Smurf1 expression in the CRC tissues.



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mediated Smurf1 stably silencing as Smurf1-depleted HCT116 cells. However, contrary to our expectations, the knockdown of Smurf1 showed no significant enhanced effects on the chemosensitivity of MCF7, HeLa, and A549 cells to gemcitabine, cisplatin, or gemcitabine plus cisplatin (Supplementary Table S1). These results confirmed that the knockdown of Smurf1 specifically enhanced the sensitivity of the CRC cells to gemcitabine and cisplatin. Furthermore, the cell viability assays were performed by CCK-8. Smurf1-depleted HCT116 (Figure 2*A*), MCF7 (Figure 2*B*), HeLa (Figure 2*C*), and A549 (Figure 2*D*) cells were planted into 96-well plates and then treated with the indicated concentration of gemcitabine, cisplatin, and gemcitabine plus cisplatin. The cell viability was analyzed at OD_{450nm} of CCK-8. While maintaining consistency with the previously obtained results, compared to the control cells, the cell viability of HCT116, unlike that of MCF7, HeLa, and A549 cells treated with gemcitabine, cisplatin, or gemcitabine plus cisplatin, significantly declined. When considered together, the data indicated that Smurf1 specifically promoted CRC resistant to chemotherapy.

The Knockdown of Smurf1 Enhances the Sensitivity of Colorectal Cancer Cells to Gemcitabine and Cisplatin in HCT116 Cell-Derived Xenograft Models

We compared the efficacy of gemcitabine and cisplatin in inhibiting Smurf1-depleted and control HCT116 cell-derived xenograft tumor growth. Smurf1-depleted and control HCT116 cells were subcutaneously inoculated on both the sides of the front armpits of BALB/c nude mice. The treatment was performed for three weeks. Thereafter, the body weight, photographed tumor size, tumor weight, and tumor volume of the mice treated with gemcitabine, cisplatin, and gemcitabine plus cisplatin were analyzed. The indicated treatment showed no significant change in terms of the body weight in the Smurf1-depleted and control groups (Figure 3*A*). We found that Smurf1 depletion significantly enhanced the chemosensitivity of HCT116 cell-derived xenograft tumors to gemcitabine and cisplatin, especially in combination groups, assessed via photographed tumor size (Figure 3*B*), tumor weight (Figure 3*C*), and tumor volume (Figure 3*D*). The results suggested that Smurf1 inhibited the sensitivity of colorectal cancer cells to chemotherapy.

Hematoxylin and eosin (H&E) staining of xenografts from each group showed varying degrees of necrosis and apoptosis in the tumor tissues. As shown in Figure 4*A*, tumors from Smurf1-depleted and control cells treated with PBS had identical pathological characteristics. However, Smurf1-depleted tumors treated with gemcitabine or cisplatin, when compared to control tumors, showed an increase in apoptosis with extensive necrosis. TUNEL staining further confirmed that depletion of Smurf1 significantly increased apoptosis in the gemcitabine and cisplatin-treated tumors as compared to the control tumors (Figure 4*B*). Proliferating cell nuclear antigen (PCNA) and Cyclin D1 staining showed a significant decrease in cell proliferation in Smurf1-depleted tumors in the gemcitabine and cisplatin-treated groups compared to the control tumors (Figure 4*C*). The in vivo data confirmed that Smurf1 suppressed the chemosensitivity of HCT116 cell-derived xenograft tumors to gemcitabine and cisplatin. When considered together, these results conclude that the knockdown of Smurf1 enhanced the sensitivity of the CRC cells to gemcitabine or cisplatin.

Gemcitabine and Cisplatin Significantly Inhibit Low Smurf1 Expression Tumor Growth in Patient-Derived Xenograft Models

To further prove that Smurf1 suppresses the chemosensitivity of human colorectal cancer cells to gemcitabine and cisplatin, we selected tumor samples from 6 colorectal cancer patients, aged between 34 and 76 years. These samples enabled us to demonstrate the effect of Smurf1 expression level on the chemosensitivity of human colorectal cancer cells to chemotherapy. The 6 tumor samples were labeled as CRC1, CRC2, CRC3, CRC4, CRC5, and CRC6, respectively. Pathologically, all the 6 tumors were adenocarcinomas. However, histologically, 4 out of the 6 tumors were positive for HER2 and all the 6 tumors were positive for the known markers, MLH1, MSH2, MSH6, PMS2, and c-MET (Table 1). Firstly, we analyzed the mRNA levels of Smurf1 from the RNA-Seq data of the 6 clinical tumor samples, assessed with FKPM. As shown in Figure 5*A*, CRC1, CRC2, and CRC3 expressed higher mRNA levels of Smurf1 compared to CRC4, CRC5, and CRC6. Furthermore, we evaluated the protein expression of Smurf1 by IHC and western blot in all the tumor samples. It was observed that CRC1, CRC2, and CRC3 consistently expressed higher protein levels of Smurf1 compared to CRC4, CRC5, and CRC6 (Figure 5, *B* and *C*).

The 6 colorectal tumor PDX models were successfully established by subcutaneous implantation (Figure 6*A*). The PDX mice were treated with gemcitabine, cisplatin, and a combination of gemcitabine and cisplatin. Figure 6*B* shows the images of the tumors which clearly display the significant difference in tumor size. The tumor volumes of CRC4, 5 and 6 with low Smurf1 expression were smaller than CRC1, 2 and 3 with high Smurf1 expression after treatments with gemcitabine, cisplatin, gemcitabine plus cisplatin. With the indicated treatment, the tumor sizes of CRC1, CRC2, and CRC3 were significantly larger than that of CRC4, CRC5, and CRC6. Furthermore, the tumor volume and weight were also analyzed once the tumor was isolated from the mice. Consistently, tumors that had low Smurf1 expression in CRC4, CRC5, and CRC6 showed greater chemosensitivity to gemcitabine, cisplatin, and gemcitabine plus cisplatin compared to tumors that had high Smurf1 expression in CRC1, CRC2, and CRC3, assessed via tumor volume and weight (Figure 6, *C* and *D*).

A histological analysis examined if the characteristics of the patient-derived xenograft tumors treated with gemcitabine and cisplatin were consistent with the results from HCT116 cell-derived xenograft (Figure 4). As shown in Figure 7*A*, these xenografts retained the characteristics of their original adenocarcinomas. Xenografts from these 6 patients treated with PBS showed similar pathological characteristics. However, tumors with low Smurf1 expression in CRC4, CRC5, and CRC6, treated with gemcitabine, cisplatin, or their combination showed an increase in apoptosis and extensive necrosis compared to tumors with high Smurf1 expression in CRC1, CRC2, and CRC3. Furthermore, PCNA and Cyclin D1 staining showed a significant decrease in cell proliferation in tumors of CRC4, CRC5, and CRC6 that were treated with gemcitabine, cisplatin, and their combination compared to tumors of CRC1, CRC2, and CRC3 (Figure 7*B*). When considered together, these results demonstrated that low Smurf1 expression enhanced the chemosensitivity of human colorectal cancer to gemcitabine and cisplatin in patient-derived xenograft models.

Discussion

CRC is one of the most common malignancies with 1.36 million new cases worldwide each year, and is also one of the leading causes of cancer mortality [14]. Although chemotherapy is a common treatment for colorectal cancer therapy, the side effects of systemic chemotherapy, including neurotoxicity and gastrointestinal toxicity, may significantly impact the quality of life of the patients. Personalized chemotherapy may improve the treatment outcome for patients with colorectal tumors [15]. To ensure this, identification of effective molecular markers capable of predicting the response to chemotherapy is essential.

Smurf1 plays a critical role in the occurrence and development of multiple tumors [5,7,8]. Smurf1 is highly expressed in CRC and correlates with cancer progression [4]. However, the role of Smurf1 in the susceptibility of CRC response to chemotherapy has not been evaluated yet. In this study, we firstly identified that the knockdown of Smurf1 causes a significant increase in the apoptosis of HCT116 cells (Figure 1*A*), and gemcitabine and cisplatin

← **Figure 6. PDXs with low Smurf1 expression exhibit more sensitive to gemcitabine and cisplatin. A.** Schematic view of xenograft tumors from human colorectal cancer and experimental design to evaluate the anti-tumor effects of gemcitabine, cisplatin or their combination in Smurf1 highly or lowly expressed PDX models. Fresh tumor specimens were minced with scissors and subcutaneously implant into both sides of the front armpits of the NSG mice. **B.** Representative images of xenograft tumors from the indicated 6 colorectal PDXs after treated with or without gemcitabine, cisplatin or both for 5 weeks. Tumor growth curve (volume, mm^3) (**C**) and tumor weight (**D**) were determined. Tumor volume was measured using a caliper at the indicated time points. Data are shown as the mean ± S.D. $^*P < .05$, $^{**}P < .01$, $^{***}P < .001$.

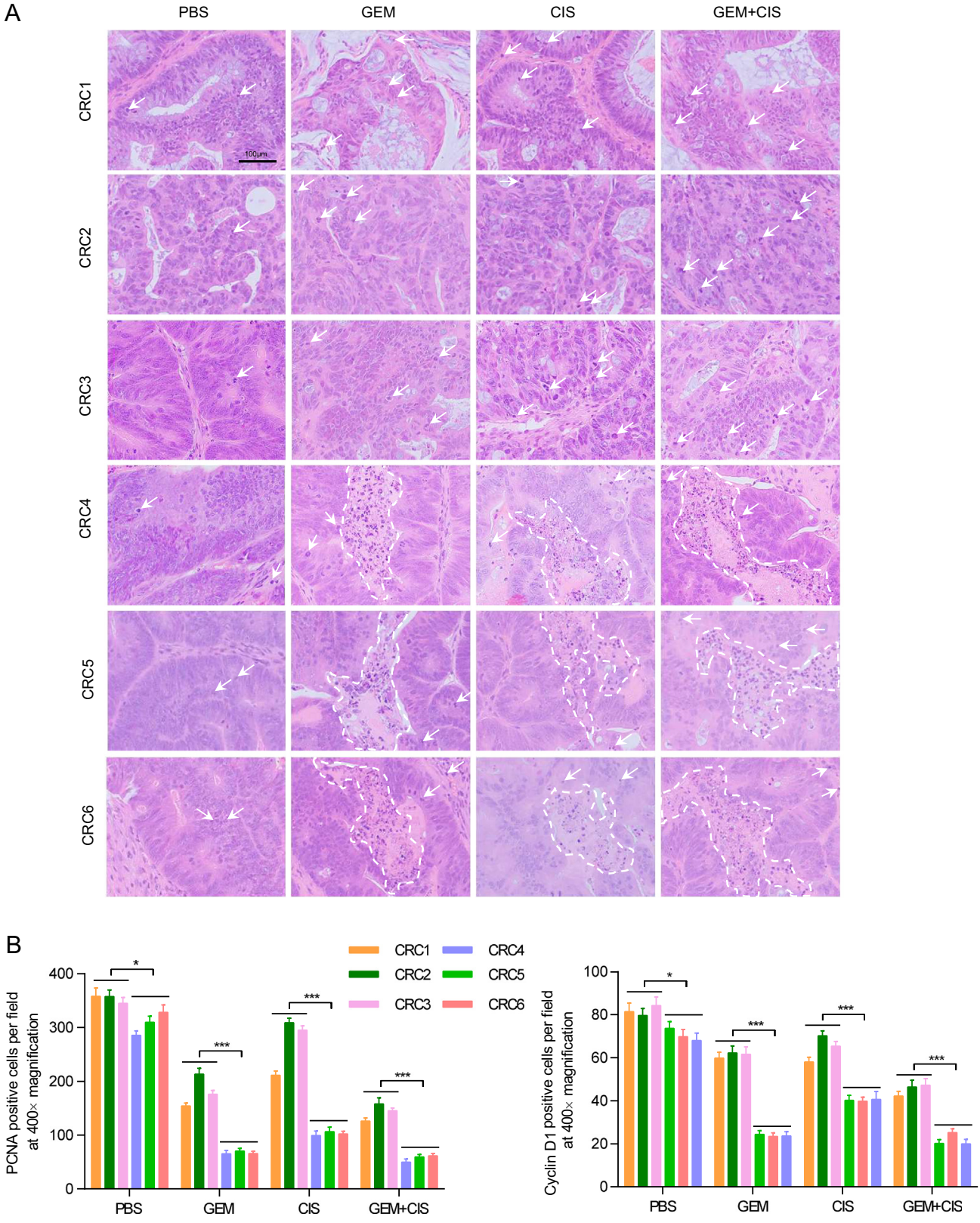

Figure 7. Histological analysis of xenograft tumors from colorectal PDXs after chemotherapeutic treatment. A. H&E staining of the tumor tissues as the indicated treatment (magnification, 400×). Apoptotic cells were shown as the white arrows indicated. And the white dashed boxes showed the area of necrosis. Scale, 100 μm. **B.** Immunohistochemistry of xenografts from mice treated as indicated and evaluated for proliferation by stained with anti-PCNA and anti-Cyclin D1 antibodies. The PCNA and Cyclin D1 positive cells were counted in five randomly selected 400× fields and statistically analyzed. Data represent the mean ± S.D. $^{*}P < .05$, $^{***}P < .001$.

enhance this effect (Figure 1, *B* and *C*). Furthermore, we investigated the association between Smurf1 expression levels and the susceptibility of CRC response to gemcitabine and cisplatin in CDX and PDX models. Preclinical mouse models with high translatability are essential for cancer research and anti-tumor drug development. PDX models are considered ideal as they are capable of retaining the molecular and histopathological features from the originating tumors [9,16].

In our previous study, 67 cases of patient-derived colorectal tumor were subcutaneously transplanted, and 59 xenografts were successfully established. In the current study, 6 cases with high or low Smurf1 expressions were chosen to test the effect of Smurf1 expression levels on the susceptibility of CRC response to chemotherapy. The obtained results suggest that Smurf1 plays the role of a chemosensitivity suppressor of human colorectal cancer cells to gemcitabine and cisplatin. The screening of other cancer therapeutic agents needs to be the focus of future studies. In addition, the mechanism underlying the inhibiting effect of Smurf1 on chemosensitivity also needs to be investigated. As demonstrated, Smurf1 plays a critical role in multiple biological processes, including embryonic development, cell cycle, cell polarity, and motility by targeting numerous substrates, such as MEKK2, Wee1, Securin, Prickle 1, and RhoA, for ubiquitination and degradation. However, the role of Smurf1 substrates in chemosensitivity regulation is not known and, therefore, needs to be investigated further.

In conclusion, the relationship between Smurf1 expression levels and the chemosensitivity of human colorectal cancer to gemcitabine and cisplatin in HCT116 cell-derived xenografts and colorectal patient-derived xenograft models is demonstrated. A new inhibitory function of Smurf1 is expressed in the form of a chemosensitivity suppressor of human colorectal cancer cells to gemcitabine and cisplatin.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tranon.2020.100804.

Acknowledgements

We thank Prof. Shan Wang and Prof. Zhanlong Shen for collecting patient tumor samples from Peking University People's Hospital.

Author Contributions

JG performed the experiments; JG and CM analyzed the data; RW and GX designed the experiments; RW wrote the manuscript.

Funding

This study was supported by National Natural Science Foundation of China (81601375), CAMS Innovation Fund for Medical Sciences (2016-I2M-3-019, 2019-I2M-1-003), the National Key Research and Development Program of China (2017YFF0204706), the Fundamental Research Funds for the Central Universities (FRF-MP-18-005) and Overseas Expertise Introduction Project for Discipline Innovation or 111 Project (T2018012).

Conflicts of Interest

The authors declare no competing financial interests.

References

[1] F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre, A. Jemal, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA-Cancer J. Clin. 68 (2018) 394–424.

[2] X. Chen, S. Chen, Y. Li, Y. Gao, S. Huang, H. Li, Y. Zhu, SMURF1-mediated ubiquitination of ARHGAP26 promotes ovarian cancer cell invasion and migration, Exp. Mol. Med. 51 (2019) 46.

[3] H.H. Chung, B.I. Jang, [A perspective: role of targeted therapy in colon cancer], Korean J. Gastroenterol. 61 (2013) 128-135.

[4] M. Hidalgo, F. Amant, A.V. Biankin, E. Budinska, A.T. Byrne, C. Caldas, R.B. Clarke, S. de Jong, J. Jonkers, G.M. Maelandsmo, et al., Patient-derived xenograft models: an emerging platform for translational cancer research, Cancer Discov. 4 (2014) 998–1013.

[5] M. Hidalgo, E. Bruckheimer, N.V. Rajeshkumar, I. Garrido-Laguna, E. De Oliveira, B. Rubio-Viqueira, S. Strawn, M.J. Wick, J. Martell, D. Sidransky, A pilot clinical study of treatment guided by personalized tumorgrafts in patients with advanced cancer, Mol. Cancer Ther. 10 (2011) 1311–1316.

[6] S. Hussain, A. Singh, S.U. Nazir, S. Tulsyan, A. Khan, R. Kumar, N. Bashir, P. Tanwar, R. Mehrotra, Cancer drug resistance: a fleet to conquer, J. Cell. Biochem 120 (2019) 14213–14225.

[7] K.A. Johnson, P.H. Brown, Drug development for cancer chemoprevention: focus on molecular targets, Semin. Oncol. 37 (2010) 345–358.

[8] J.H. Kim, Chemotherapy for colorectal cancer in the elderly, World J. Gastroenterol. 21 (2015) 5158–5166.

[9] A. Kwon, H.L. Lee, K.M. Woo, H.M. Ryoo, J.H. Baek, SMURF1 plays a role in EGF-induced breast cancer cell migration and invasion, Mol. Cells 36 (2013) 548–555.

[10] M.G. Lee, S.I. Jeong, K.P. Ko, S.K. Park, B.K. Ryu, I.Y. Kim, J.K. Kim, S.G. Chi, RASSF1A directly antagonizes RhoA activity through the assembly of a Smurf1-mediated destruction complex to suppress tumorigenesis, Cancer Res. 76 (2016) 1847–1859.

[11] H. Li, S. Wheeler, Y. Park, Z. Ju, S.M. Thomas, M. Fichera, A.M. Egloff, V.W. Lui, U. Duvvuri, J.E. Bauman, et al., Proteomic characterization of head and neck cancer patient-derived xenografts, Mol. Cancer Res. 14 (2016) 278–286.

[12] X. Li, X. Dai, L. Wan, H. Inuzuka, L. Sun, B.J. North, Smurf1 regulation of DAB2IP controls cell proliferation and migration, Oncotarget 7 (2016) 26057–26069.

[13] J. Stebbing, K. Paz, G.K. Schwartz, L.H. Wexler, R. Maki, R.E. Pollock, R. Morris, R. Cohen, A. Shankar, G. Blackman, et al., Patient-derived xenografts for individualized care in advanced sarcoma, Cancer 120 (2014) 2006–2015.

[14] S. Stintzing, [Personalized Treatment for Colorectal Carcinomas], Dtsch. Med. Wochenschr. 142 (2017) 1652-1659.

[15] E.C. Townsend, M.A. Murakami, A. Christodoulou, A.L. Christie, J. Koster, T.A. DeSouza, E.A. Morgan, S.P. Kallgren, H. Liu, S.C. Wu, et al., The public repository of xenografts enables discovery and randomized phase II-like trials in mice, Cancer cell 29 (2016) 574–586.

[16] P. Xie, M. Zhang, S. He, K. Lu, Y. Chen, G. Xing, Y. Lu, P. Liu, Y. Li, S. Wang, et al., The covalent modifier Nedd8 is critical for the activation of Smurf1 ubiquitin ligase in tumorigenesis, Nat. Commun. 5 (2014) 3733.