Original Paper

XRCC2-Deficient Cells are Highly Sensitive to 5-Fluorouracil in Colorectal Cancer

Yong-zhou Zhang^a Ji-Hong An^a Yu-Xin Liu^a Xian-Chuang Wu^a
Shan-Shan Han^a Xue-qun Ren^b Chang-Jiang Qin^b

^aDepartment of Pharmacy, Huai He Hospital of He Nan University, Kaifeng, ^bDepartment of Gastrointestinal Surgery, Huai He Hospital of He Nan University, Kaifeng, China

Key Words

Xrcc2 • Colorectal cancer • 5-fluorouracil • Chemotherapy

Abstract

Background/Aims: Inhibition of the repair of 5-fluorouracil (5-FU)-induced DNA lesions may improve the responses of tumors to anticancer agents. XRCC2 is a key factor in DNA repair. However, the role of XRCC2 in the chemoresistance of colorectal cancer (CRC) treated with 5-FU remains unclear. The aim of this study is to investigate whether XRCC2 expression affects the chemosensitivity of colorectal cancer. **Methods:** XRCC2 expression in CRC tissues was assessed, and the outcomes were analyzed to determine the clinical importance of XRCC2 expression. Following treatment with 5-FU, the effect of XRCC2 on proliferation was evaluated via a CCK-8 assay, the effects on cell cycle distribution and apoptosis were analyzed using flow cytometry, and γH2AX foci formation assays were performed to examine the influence of 5-FU on DNA Double-strand breaks (DSBs) repair in CRC cells. **Results:** XRCC2 expression in CRC tissues was significantly higher than that in normal tissues, and this increased XRCC2 expression was associated with advanced T staging, M staging, TNM staging, Duke's staging, and greater liver and lymph node metastases. XRCC2 expression might be an independent prognostic indicator for CRC patients. Patients with negative XRCC2 expression exhibit greater sensitivity to treatment with 5-FU-based chemotherapy than those with positive XRCC2 expression. Moreover, our observations revealed that the knockdown of XRCC2 in CRC cells increased the sensitivities to 5-FU in terms of cell proliferation, apoptosis and cell cycle arrest. DNA DSBs repair was slower in the XRCC2-deficient cells than in the XRCC2-wild type cells. **Conclusion:** Our study demonstrated that XRCC2 might play an important role in CRC and function as a novel prognostic indicator and that the down-regulation of XRCC2 may be useful for sensitizing CRC cells during 5-FU chemotherapy.

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Y. Zhang and J. An contributed equally to this work.

Chang-Jiang Qin
and Xue-Qun Ren

Department of Gastrointestinal Surgery, Huai He Hospital of
He Nan University, Kaifeng, (China)
E-Mail hhyqj@163.com, hhyrxq@126.com

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Introduction

Colorectal cancer (CRC) is the third-most common carcinoma and the fourth leading cause of cancer-related mortality worldwide [1, 2]. In China, the incidence of CRC is approximately 3.7 million cases, CRC is the fifth-most common cancer, causes approximately 1.9 million deaths and is the fifth leading cause of cancer-related mortality [3].

Double-strand breaks (DSBs) are the most deleterious DNA lesions that occur in cells after treatment with chemotherapy and/or radiation [4, 5]. The repair of these DSBs largely determines the outcome of cancer therapy [6, 7]. When incorrectly repaired or left unrepaired, DSBs may lead to cell death [8]. 5-Fluorouracil (5-FU) is widely used in clinical cancer therapy [9, 10]. It is commonly used either alone or in combination with other drugs for the treatment of CRC, breast cancer, head and neck cancers and other types of carcinomas [11-16]. Preoperative chemotherapy (PCT) based on 5-FU is an essential treatment option for advanced colorectal cancer [17-19]. However, the efficacy of this treatment is highly debated because responses to PCT have been found to vary among individuals. Some biomarkers for predicting PCT responses in CRC patients have been investigated, although the clinical applications remain unclear [20-23]. Thus, it is imperative to investigate novel biomarkers for the prediction of chemosensitivity and the tailoring of treatments to individual patients.

Some studies have reported that treating cervical cancer or lung cancer cells with 5-FU results in the activation of the Chk1 protein and the phosphorylation of the H2AX protein (the marker of DSB induction), which indicates the generation of DNA DSBs due to 5-FU treatment [24, 25]. In colorectal cancer, Liu et al. demonstrated that mir-149 increases the sensitivity of colorectal cancer cells to 5-FU by targeting FOXM1 [26]. However, it remains unclear whether XRCC2 expression affects chemosensitivity.

In this study, XRCC2 expression was detected in a TCGA cohort and samples in our medical cohort, and the relationships between XRCC2 expression and clinical features were evaluated. Moreover, the effects of 5-FU on XRCC2-deficient CRC cells were detected in *in vitro* experiments.

Materials and Methods

Patients and tissue specimens

Resection specimens were collected from patients with primary rectal cancer who underwent gastrointestinal surgery without PCT between January 2009 and July 2010 at the Huai He Hospital of He Nan University. Follow-ups were continued until December 2015. Additionally, 97 patients who were clinically diagnosed with locally advanced colorectal cancer (T3-4 and/or N0-2 lesions) were recruited between January 2010 and December 2015. Biopsy tissue samples were obtained before these patients received PCT involving at least 4 cycles of 5-FU-based chemotherapy that included modified FOLFOX-6 (oxaliplatin 85 mg/m², leucovorin 400 mg/m², and a bolus of 5-FU 400 mg/m² followed by a 48-hr infusion of 2400 mg/m²) every 3 weeks. Radical surgery (low anterior resection or abdominoperineal resection) was performed 6-8 weeks after the completion of PCT at the Huaihe Hospital of Henan University. The use of all tissue blocks and serum samples for this study was approved by the Institutional Ethics Review Board of the Huai He Hospital of He Nan University, and written consent was obtained from each patient.

Bioinformatics analysis

The expression data, which was download from the UCSC Cancer Genomics Browser [27], were collected from The Cancer Genome Atlas (TCGA)[28] and were obtained as processed RNA-seq data in the form of RNA-seq via expectation maximization (RSEM). Data comprising a total of 50 non-tumorous and 380 tumorous tissues were used in this study.

Cell culture and retroviral infection

The colorectal cancer cell line SW480 was obtained from the American Type Culture Collection (Manassas, VA). The cells were cultured in DMEM (Life Technologies, Carlsbad, CA) supplemented with 10%

fetal bovine serum (HyClone, Logan, UT) and 1% penicillin/streptomycin. All cells were maintained in a humidified atmosphere at 37°C containing 5% CO₂.

Several lentiviral-based plasmids containing a short hairpin RNA (shRNA) from human XRCC2 were purchased from RiboBio Co., Ltd (Guangzhou, China). The SW480 cell infections with human XRCC2 shRNA were performed as described previously [29, 30]. The knockdown efficiency of XRCC2 was examined by quantitative qRT-PCR and Western blot.

Immunohistochemical (IHC) staining and tumor regression grading (TRG)

IHC staining and tumor regression grading were analyzed as described previously [29, 30].

Western blot

Total protein was extracted with cell lysis buffer (KeyGene, Nanjing, China), and the protein concentration was quantified using an Enhanced BCA Protein Assay Kit (KeyGene, Nanjing, China). The protein was separated by 8–10% SDS-PAGE and electrotransferred to PVDF membranes (Millipore, Billerica, MA, USA). The membrane was blocked for 1 h with 5% BSA in TBS-T and probed with the corresponding primary antibodies overnight at 4°C followed by incubation with rabbit and mouse radish peroxidase-coupled secondary antibodies for 1 hour. Specific bands were detected on autoradiographic film using an enhanced chemiluminescence reagent (Millipore, Billerica, MA, USA).

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was isolated using RNA Plus Reagent (TaKaRa, Japan). Complementary DNA was prepared using oligo T primers according to the protocol supplied with the Primer Script™ RT Reagent (TaKaRa, Japan). The expression of XRCC2 was determined by quantitative real-time PCR using Power SYBR Green PCR master mix (Applied Biosystems).

Proliferation assay

The Cell Counting Kit-8 (CCK-8) assay was used to detect cell proliferation. In brief, the cells were seeded onto 96-well cell culture cluster plates (KeyGene, Nanjing, China) at a density of 5×10³ cells/well in 100 µL of culture. Then, 10 µL of CCK-8 reagent (Dongjido, Japan) was added to each well for 2 h of incubation at 37 °C according to the manufacturer's instructions. The absorbance was read at a wavelength of 450 nm with an automated plate reader (M200 Pro, Tecan). The experiments were repeated at least three times.

Flow cytometry analyses of cell apoptosis and the cell cycle

Cell apoptosis and cell cycle analyses by flow cytometry were performed as described previously [29, 30].

Immunofluorescence

A previously described immunofluorescence (IF) protocol was used [30]. The cells were treated with 5-FU for 24 h after they were plated into 6-well plates with slides. The cells were fixed with 4% paraformaldehyde for 15 min and then washed for 3 min 3 times with PBS. The cell membranes were ruptured with 0.2% Triton at room temperature for 15 min, and the non-specific antigen-binding sites were blocked with 5% BSA for 30 min. The cells were then incubated with anti-γ-H2AX overnight at 4°C. After three more washes with PBS, the cells were incubated with DyLight 488 AffiniPure goat anti-mouse IgG (Abbkine, Redlands, CA, USA) at room temperature in the dark. After 1 h, the cells were washed with PBS three times before being counterstained with 1 µg/ml 4',6-diamidino-2-phenylindole (DAPI) for 3 min in the dark. The cells were observed with a fluorescence microscope.

Statistical analyses

SPSS version 18.0 (SPSS Inc., Chicago, IL) was used for the analyses. The relationships between XRCC2 expression and the features of tumor progression were analyzed using the chi-square and Fisher's exact tests. Kaplan–Meier survival curves were constructed, and the log-rank test was performed for the univariate analyses. A multivariate analysis was performed using Cox's proportional hazards model. A *P*-value of 0.05 was considered statistically significant for all analyses.

Results

Increased levels of XRCC2 in the tumor tissues of the CRC patients

The XRCC2 levels from the CRC tissues from the TCGA database (N=380) and the normal tissues (N=50) were evaluated. The mRNA levels were found to be significantly increased in the CRC tissues (0.02770 ± 0.02352) compared with the normal tissues (0.005885 ± 0.002884 ; $P < 0.001$, Fig. 1A). The up-regulation of XRCC2 mRNA was validated independently in the CRC tissues and paired adjacent normal tissues of the TCGA cohorts (N=30, $P < 0.001$, Fig. 1B). Furthermore, we analyzed the XRCC2 expression levels in the paired CRC and normal tissues in our medical center (Fig. 1C & 1D). XRCC2 was up-regulated in 23 primary CRC tumors compared with their paired normal tissues as determined by qRT-PCR (Fig. 1C) and Western blot (Fig. 1D).

Association between XRCC2 protein expression and clinicopathologic factors in CRC patients

We evaluated XRCC2 protein expression in 101 CRC cases by immunohistochemistry (IHC). Representative XRCC2 IHC stainings are illustrated in Figs. 1A & B. According to the IHC staining, all patients were divided into an XRCC2-negative group (N=33) and an XRCC2-positive group (N=68). As illustrated in Table 1, a chi-square test suggested that positive XRCC2 expression was significantly correlated with advanced T staging ($P = 0.009$), M staging ($P = 0.010$), TNM staging ($P = 0.001$) and Duke's staging ($P < 0.001$). Moreover, positive XRCC2 expression also predicted more liver ($P = 0.040$) and lymph node ($P = 0.021$) metastasis. Interestingly, negative XRCC2 expression predicted a larger tumor size ($P = 0.022$). However, there were no statistically significant relationships of positive XRCC2 expression with patient gender ($P = 0.864$), age ($P = 0.156$), tumor location ($P = 0.450$), differentiation grade ($P = 0.349$), N staging ($P = 0.083$), vascular invasion ($P = 0.535$) or peritoneal metastasis ($P = 0.079$).

Fig. 1. Increased XRCC2 expression in CRC. (a) Analysis of XRCC2 expression in normal tissues (N=50) and CRC tissues (N=380) in the TCGA cohort. (b) Analysis of XRCC2 expression in paired normal and CRC tissues (N=30) in the TCGA cohort. (c) XRCC2 mRNA expression levels in the paired normal and tumor tissues (N=23) in the Huaihe cohort. (d) XRCC2 expression was measured in the CRC tissues and respective adjacent non-tumor tissues in 2 typical patients

XRCC2 up-regulation is associated with poor prognosis in patients

We next evaluated the relationship between XRCC2 expression and the prognoses of the CRC patients in our medical center. For all CRC patients in this study, the follow-up periods ranged from 8 to 72 months with a mean survival time of 47.6 months. Kaplan-Meier plots were used to compare survival between the two groups, and the OS and RFS in the XRCC2-negative group were significantly better than those in the XRCC2-positive group (Fig. 2B, $P < 0.0001$ and Fig. 2C, $P = 0.0053$).

As illustrated in Table 2, univariate analyses revealed that age (HR=0.564, 95% CI 0.296-1.876, $P = 0.004$), tumor size (HR=0.467, 95% CI 0.241-0.903, $P = 0.024$), TNM staging (HR=1.528, 95% CI 0.468-14.647, $P = 0.029$), Duke's staging (HR=1.293, 95% CI 0.505-10.414, $P = 0.023$), lymph node metastasis (HR=0.890, 95% CI 0.560-2.185, $P = 0.002$), peritoneal metastasis (HR=6.703, 95% CI 3.093-18.165, $P = 0.001$) and XRCC2 expression (HR=1.996, 95% CI 1.168-5.396, $P = 0.0012$) were significantly associated with the prognoses of the CRC patients. Furthermore, a multivariate analysis demonstrated that XRCC2-positive expression was an independent prognostic predictor among the CRC patients (HR=2.689, 95% CI 1.3-5.564, $P = 0.008$).

Correlations of XRCC2 expression and histological tumor regression grade with preoperative chemotherapy

The association between XRCC2 expression in the pretreatment biopsy tissue samples and postoperative histological tumor regression was evaluated in 97 CRC patients who received PCT. As illustrated in Table 3, 61.86% (60/97) of the patients exhibited positive XRCC2 expression, and 38.14% (37/97) of the patients exhibited negative XRCC2 expression. Following PCT treatment, 59/97 (60.82%) of the cases exhibited showed poor responses ($\text{TRG} \leq 2$) (Fig. 3A), whereas in 38/97 (39.18%) of the cases, good pathologic responses were achieved ($\text{TRG} \geq 3$; Fig. 3B). Of the latter, 26/37 (72.27%) of the cases were negative for XRCC2 expression, and 12/60 (20.00%) of the cases were positive for XRCC2 expression. These results demonstrated that patients with negative XRCC2 expression exhibit greater sensitivity when treated with 5-FU-based chemotherapy compared to those with positive XRCC2 expression (Table 3).

Knockdown of XRCC2 increased 5-fluorouracil-induced cytotoxicity in colorectal cancer cells

In our previous studies, we established an XRCC2-knockdown SW480 colorectal cancer cell line [29, 30]. XRCC2 expression was detected again via Western blot (Fig. 4A, left

Table 1. Relationships of XRCC2 expression with clinicopathological characteristics in CRC patients

Characteristic	N	XRCC2 expression		χ^2 Value	P Value
		Negative (N=33)	Positive (N=68)		
Gender					
Male	60	20	40	0.029	0.864
Female	41	13	28		
Age					
≤ 60 y	62	17	45	2.015	0.156
> 60 y	39	16	23		
Tumor Location					
Ascending colon	29	7	22	3.691	0.450
Transverse colon	4	2	2		
Descending colon	7	4	3		
Sigmoid colon	2	1	1		
Rectum	59	19	40		
Tumor size					
< 5 cm	62	15	47	5.248	0.022
≥ 5 cm	39	18	21		
Differentiation grade					
Well	1	1	0	2.107	0.349
Moderately	76	24	52		
Poorly	24	8	16		
T staging					
T1	1	0	1	11.557	0.009
T2	16	11	5		
T3	61	16	45		
T4	23	6	17		
N staging					
N0	60	24	36	4.977	0.083
N1	24	7	17		
N2	17	2	15		
M staging					
M0	89	33	56	6.609	0.010
M1	12	0	12		
TNM staging					
I	11	8	3	15.770	0.001
II	41	16	25		
III	37	9	28		
IV	12	0	12		
Duke's staging					
A	16	13	3	24.232	< 0.001
B	26	11	25		
C	37	9	28		
D	12	0	12		
Vascular invasion					
No	96	32	64	0.384	0.535
Yes	5	1	4		
Liver metastasis					
No	93	33	60	4.216	0.040
Yes	8	0	8		
Lymph node metastasis					
No	57	24	33	5.291	0.021
Yes	44	9	35		
Peritoneal metastasis					
No	95	33	62	3.096	0.079
Yes	6	0	6		

Fig. 2. The up-regulation of XRCC2 predicts worse overall survival (OS) and recurrence-free survival (RFS) in CRC patients. (a) A representative image of CRC tissue that was positive for XRCC2 expression according to immunohistochemistry ($\times 200$ magnification). (b) A representative image of CRC tissue that was negative for XRCC2 expression according to immunohistochemical analysis ($\times 200$ magnification). (c) XRCC2-positive expression predicted a poor OS in the CRC patients ($P < 0.001$). (d) XRCC2-positive expression predicted a poor RFS in the CRC patients ($P = 0.0053$).

panel) and qRT-PCR (Fig. 4A, right panel). To determine the cytotoxicity of 5-FU in colorectal cancer cells, we first detected the IC₅₀ of SW480 in 5-FU at different concentrations (Fig. 4B, left panel). To detect the viability of SW480 cells during 5-FU treatment, the cells were treated with 5-FU at different concentrations (0 $\mu\text{g}/\text{ml}$, 10 $\mu\text{g}/\text{ml}$, 25 $\mu\text{g}/\text{ml}$, and 50 $\mu\text{g}/\text{ml}$) for 24 h. The viabilities of the CRC cells significantly decreased (Fig. 4B). According to the IC₅₀ and viability of SW480 cell to 5-FU, the 5-FU concentration of 10 $\mu\text{g}/\text{ml}$ was selected for further study. To determine the role of XRCC2 in 5-FU treatment in CRC, cell proliferation was detected with CCK-8 assays. As illustrated in Fig. 4C, a down-regulation of XRCC2 in CRC cells decreased cell proliferation. Following the treatment of XRCC2-deficient cells with 5-FU, the viability and cell proliferation significantly decreased. Taken together, these findings indicate that XRCC2-deficiency can increase 5-FU-induced cytotoxicity.

Table 2. Univariate and multivariate analyses of the influences of clinicopathological factors on the prognoses of patients with CRC

Variables	Univariate analysis				Multivariate analysis			
	HR	95% CI Lower	95% CI Upper	P value	HR	95% CI Lower	95% CI Upper	P value
Gender	0.772	0.409	1.459	0.424				
Age	0.564	0.296	1.876	0.004	0.479	0.279	0.822	0.008
Tumor location	0.921	0.769	1.102	0.366				
Tumor size	0.467	0.241	0.903	0.024				
Differentiation grade	1.428	0.756	2.698	0.272				
T staging	1.244	0.775	1.995	0.366				
N staging	1.001	0.529	1.894	0.997				
M staging	0.168	0.006	5.069	0.305				
TNM staging	1.528	0.468	13.647	0.029				
Duke's staging	1.293	0.505	10.414	0.023	3.199	2.109	4.825	<0.001
Vascular invasion	0.385	0.041	3.614	0.404				
Liver metastasis	1.709	0.131	22.307	0.683				
Lymph node metastasis	0.890	0.560	2.185	0.002	0.498	0.276	0.898	0.021
Peritoneal metastasis	6.703	3.093	18.165	0.001				
XRCC2 expression	1.996	1.168	5.396	0.012	2.689	1.3	5.564	0.008

Table 3. Correlation between XRCC2 expression and tumor response to PCT treatment according to tumor regression grade (TRG) in colorectal cancer patients who underwent surgery after PCT. Good response: TRG ≥ 3 ; poor response: TRG ≤ 2 . *TRG ≥ 3 , #TRG ≤ 2

XRCC2 expression	Good response*	Poor response#	P value
Positive	12	48	<0.001
Negative	26	11	

Fig. 3. TRG can be used to stratify the pathological response in terms of the biological effect of PCT on tumors. (a) Positive XRCC2 expression in a representative biopsy specimen with a poor TRG (TRG $\leq$ 2). (b) Negative XRCC2 expression in a representative biopsy specimen with a good TRG (TRG $\geq$ 3).

Fig. 4. Knockdown of XRCC2 increased 5-fluorouracil-induced cytotoxicity and impaired the repair of DNA DSBs. (a) Western blotting (left panel) and qRT-PCR (right panel) were used to detect XRCC2 knockdown efficiency in SW480 cells. (b) The IC₅₀ of SW480 in 5-FU at different concentrations (left panel); SW480 cell viabilities in response to different concentrations of 5-FU (right panel). (c) The knockdown of XRCC2 increased 5-fluorouracil-induced cytotoxicity in SW480 cells. (d) Typical images of γ-H2AX in XRCC2-deficient and parental cells at the indicated time-points after a 24-h 5-FU treatment (upper panel). The average number of γ-H2AX foci per nucleus was calculated based on the number of γ-H2AX foci that were detected in more than 100 cells in the treatment group (bottom panel). (e) γ-H2AX protein expression in XRCC2-deficient and parental cells at the indicated time-points after a 24-h 5-FU treatment.

The down-regulation of XRCC2 increased 5-fluorouracil-induced DSBs in colorectal cancer cells

γ-H2AX IF staining is an extremely sensitive method for the detection of DSB repair efficiency, and this method was used to examine the presence of γ-H2AX foci induced by 5-FU^[31]. A typical image (Fig. 4D) shows the γ-H2AX foci in the XRCC2-deficient cells and in the parental cells after treatment with 5-FU. In the parental cells, the γ-H2AX foci in the nuclei disappeared 24 h after 5-FU treatment. In contrast, in the XRCC2-deficient cells, the γ-H2AX foci in the nuclei were still present at 24 h after 5-FU treatment. The γ-H2AX protein expression levels were similar in the IF assays (Fig. 4E). These results suggest that XRCC2 may play a role in enhancing the capacity of cells to repair 5-FU-induced DNA DSBs.

Fig. 5. The down-regulation of XRCC2 increases 5-FU-induced cell apoptosis. (a) Levels of apoptosis in XRCC2-deficient and control cells that were treated with 5-FU for 24 h. (b) The percentages of cells that underwent apoptosis following 5-FU treatment were quantitated, and the data are expressed as the means $\pm$ the SDs from three independent experiments. (c) Detection of cleaved-PARP, cleaved caspase-9, cleaved caspase-3, and Bcl-2 protein levels by Western blot.

Deficient XRCC2 enhanced 5-FU-induced cell apoptosis

In our previous study, XRCC2 overexpression was found to inhibit colorectal cancer cell apoptosis [30]. To determine whether XRCC2-mediated chemoresistance in colorectal cancer cells is also due to inhibition of apoptosis, cell apoptosis was detected by flow cytometry. As illustrated in Figures 5A and 5B, the SW480 cells exhibited a significant reduction in 5-FU-induced apoptosis compared with the XRCC2 knockdown cells ($P < 0.01$). Moreover, we detected possible apoptosis-related proteins including poly adenosine diphosphate ribose polymerase (PARP), Caspase-9, Caspase-3 and BCL-2. We found the cells with XRCC2 knockdown and 5-FU treatment exhibited higher levels of cleaved-PARP, cleaved-Caspase-9 and cleaved-Caspase-3 compared with the cells with XRCC2 knockdown or 5-FU treatment alone (Fig. 5C). In contrast, a significant decrease in the BCL-2 level was detected in the sh-XRCC2 cells following 5-FU treatment (Fig. 5C). Taken together, these findings demonstrated that the knockdown of XRCC2 may increase 5-FU-induced apoptosis in colorectal cancer.

XRCC2 knockdown increased 5-FU-induced G2/M arrest

Cells in the G2 and M phases of the cell cycle are sensitive to interventions such as 5-FU and radiation [30, 32]. To determine the cell cycle progression of colorectal cancer cells after treatment with 5-FU, exponentially growing cancer cells were exposed to 5-FU and then were analyzed by flow cytometry. We found that the knockdown of XRCC2 did not affect cell cycle progression, whereas the treatment of the cells with 5-FU increased G2/M phase arrest compared to the control group. Moreover, the knockdown of XRCC2 in colorectal cancer cells increased cell G2/M phase arrest after treatment with 5-FU (Fig. 6A and 6B). Our findings indicated that the down-regulation of XRCC2 in colorectal cancer cells increased the percentage of shXRCC2 cells in the G2/M phase compared with the control

Fig. 6. XRCC2 knockdown increased 5-FU-induced G2/M arrest. (a) Cell cycle stages of the XRCC2-deficient cells and control cells that were treated with 5-FU for 24 h. (b) The percentages of cells that underwent cell cycle arrest following 5-FU treatment were quantitated, and the data are expressed as the means $\pm$ the SDs from three independent experiments. (c) Detection of phosphorylated Chk2-Thr68 protein levels by Western blot.

cells (Fig. 6B). To confirm these findings, changes in Chk2, which is a key effector protein regarding the G2/M checkpoint, were detected. As illustrated in Fig. 6C, higher levels of Chk2-Thr68 phosphorylation were detected in the sh-XRCC2 cells than in the control cells, and the phosphorylation of Chk2-Thr68 was increased in the shXRCC2 cells treated with 5-FU compared with the other group (Fig. 6C).

Discussion

XRCC2, a member of the mammalian RAD51 gene family, is a homologous recombination (HR) repair gene. XRCC2 has an important role in endogenous DNA damage repair in the developing embryo, the formation of chromosome rearrangements in embryonic stem cells, cell senescence and aging [33-35]. XRCC2 has been explored and determined to play some roles in various types of human tumors, including breast cancer, thyroid cancer, pancreatic cancer and other types of carcinomas [30, 36-41]. Low levels of XRCC2 have been detected in breast cancer [42], whereas greater XRCC2 expression is present in rectal cancer and gastric cancer [30, 43]. It has been hypothesized that XRCC2 expression is related to the initiation or progression of carcinogenesis [44]. In the presents study, we found that XRCC2-deficient cells exhibit a high sensitivity to 5-FU, and a higher XRCC2 level indicated poor prognoses in patients with CRC.

In previous studies, greater XRCC2 expression has been found to be associated with advanced TNM stage in rectal cancer and to predict worse OS [30]. In the present study,

we found that the XRCC2 level was significantly higher in the CRC tumor tissues than in the normal tissues; moreover, greater expression of XRCC2 has been found to be related to larger tumor size, advanced T staging, M staging, TNM staging, Duke's staging, liver metastasis and lymph node metastasis in the Huai He Hospital. Additionally, immunohistochemical analysis revealed that the XRCC2 protein existed in the nucleus and cytoplasm and that patients with high levels of XRCC2 expression have worse OSs and RFSs compared with those of patients with low expression levels. These results suggest that XRCC2 may play an important role in the progression of CRC and that XRCC2 expression might be an independent predictor of poor survival in CRC patients.

5-FU-based chemotherapy strategies are widely used in colorectal cancer, gastric cancer and other types of carcinomas [14, 17, 18]. However, some patients present resistance to 5-FU, which results in the failure of treatment. Therefore, it is of great significance to further understand the molecular mechanism of the control of CRC growth to contribute to the creation of individualized treatment. By treating patients with preoperative 5-FU-based chemotherapy strategies, we found that patients with negative XRCC2 expression in the tumor tissues have exhibit better responses than those who are positive for XRCC2 expression. Therefore, we hypothesize that XRCC2, as a biomarker of 5-FU-based chemoresistance, could predict curative effects in patients with CRC.

Classically, 5-FU belongs to the class of antimetabolite chemotherapeutics and is thought to be an inhibitor of the enzyme thymidylate synthase, which plays a role in nucleotide synthesis. Recently, several reports have described 5-FU-induced DNA lesions and their repair in CRC cells [45]. XRCC2 plays an important role in the repair of DSBs based in the HRs and thereby decreases the sensitivity to 5-FU. When we examined whether XRCC2 knockdown suppressed the repair of DNA DSBs, we observed that the number of γ -H2AX foci, which are used to measure DNA DSBs, was higher in the XRCC2 knockdown cells than in the control cells after 24 h of 5-FU treatment. These results suggest that XRCC2 makes a major contribution to the repair of DSBs induced by 5-FU.

HR operates mainly via the use of intact sister chromatids during the late S and G2 phases but not during the G1 phase [46, 47]. Correspondingly, when XRCC2 was knocked down in the SW480 cells, greater numbers of cells were arrested in the G2/M phase after 24 h of 5-FU treatment. Plausibly, the XRCC2-mediated changes in the cell cycle may have consequently affected the sensitivity of tumor cells to 5-FU therapy.

Apoptosis is one of the major mechanisms of cell death in response to chemotherapy drugs [48]. In the present study, XRCC2 inhibition significantly increased the extent of 5-FU-induced apoptosis, which supports the hypothesis that XRCC2 plays a role in apoptosis evasion. Moreover, we detected potentially apoptosis-related proteins in the XRCC2 knockdown cells that were treated with 5-FU, and significantly higher levels of cleaved PARP, caspase-9, and caspase-3 were detected, in addition to lower levels of Bcl-2, compared with the control cells. Thus, our results also give credence to the presently used clinical regimen of combining 5-FU-based chemotherapy.

In this study, we only detected the sensitivity of the colorectal cancer cell line SW480, which is a stable XRCC2 knockdown cell line that we constructed in a previous study, to 5-FU. While in our previous study, we found that the knockdown of XRCC2 in other colorectal cancer cell lines results in changes in cell function and viability that are similar to those observed in the SW480 cells. Therefore, in this study, we used only a single cell lines to study whether XRCC2 deficiency results in a high sensitivity to 5-FU. These results may have supported our hypothesis.

In summary, the present study demonstrated that XRCC2 expression is associated with poor survival among CRC patients. XRCC2 expression status could provide a useful prognostic predictor and a potential therapeutic target for individualized treatment. The knockdown of XRCC2 impaired 5-FU-induced DNA DSB repair *in vivo* and increased 5-FU-induced cell death, apoptosis and G2/M phase arrest. Our data suggest that XRCC2 may serve as a molecular target that could improve the efficacy of 5-FU therapy.

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Disclosure Statement

The authors declare no conflicts of interest.

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