Original Article
XRCC2 reduced the sensitivity of NSCLC to radio-chemotherapy by arresting the cell cycle

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Abstract: Objective: This study aimed to reveal the role and mechanism of X-ray repair cross complementing 2 (XRCC2) and bevacizumab combined with radiotherapy in the treatment of non-small cell lung cancer (NSCLC). Methods: Gene Expression Profiling Interactive Analysis (GEPIA) database and Starbase database were used to predict the expression level of XRCC2 in NSCLC tissues and the survival time of patients diagnosed with NSCLC, respectively. Besides, qRT-PCR (quantitative real time polymerase chain reaction) and immunoblotting were conducted to confirm the expression of XRCC2 NSCLC tissues and cells. Moreover, cell viability and colony formation were measured by CCK-8 (cell counting kit-8) assay. Cell migration and invasion capabilities were determined by transwell assay. Flow cytometry analysis was employed to detect cell cycle. Results: XRCC2 was highly expressed in NSCLC tissues and cells. Additionally, bevacizumab combined with radiotherapy significantly inhibited NSCLC cell proliferation, migration and invasion. Knockdown of XRCC2 further aggravated the role of bevacizumab and radiotherapy in NSCLC, while XRCC2 overexpression reversed these effects efficiently. Furthermore, XRCC2 silence exacerbated the arrest of cell cycle induced by bevacizumab combined with radiotherapy in NSCLC cells, whereas overexpression of XRCC2 alleviated the arrest remarkably. Conclusion: Collectively, our research revealed that XRCC2 inhibited the sensitivity of NSCLC to bevacizumab combined with radiotherapy by decreasing cell cycle arrest.

Keywords: Non-small cell lung cancer, lung adenocarcinoma, lung squamous cell lung carcinoma, X-ray repair cross complementing 2, bevacizumab combined with radiotherapy, cell cycle

Introduction

Lung cancer, a kind of heterogeneous cancer, consists of no less than 50 subtypes, among which small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) are the major subtypes [1]. NSCLC is the overriding form of lung cancer, which occupies approximately 80% of lung carcinomas. NSCLC is divided into LUAD (lung adenocarcinoma) and LUSC (squamous cell lung carcinoma) given that the subclassification is vital for developing chemotherapy and molecule-targeted accurate therapy [2]. According to previous records, more than 733 million patients are diagnosed with lung cancer and approximately 610 billion sufferers are killed by the disease in China [3]. Smoking, environmental factors and genetic elements all contribute to the incidence of NSCLC. Additionally, the carcinogenesis of NSCLC is also highly correlated with oncogenes and antioncogenes

[4]. Chest radiotherapy is one of the major treatment methods for patients who have been diagnosed with NSCLC. However, during radiotherapy, tumors frequently develop radio-resistance, thus limiting its efficacy [5, 6]. Meanwhile, since cancer is usually characterized by metastasis and recurrence, it is urgent to explore efficient therapies to improve the outcome of treatment in patients with NSCLC [6].

Bevacizumab acts on VEGF (vascular endothelial growth factor) as a sort of humanized monoclonal antibody, which can reverse forepassed tumor vessels and prevent the generation of new vessels [7, 8]. Therefore, bevacizumab has the capability to improve the therapeutic effect of drug combinations, given that it can increase the permeability of vessels and decrease the pressure of interstitial fluid [9, 10]. Previous reports primarily illustrated that bevacizumab combined with radiotherapy could result in sat-

isfactory effects for patients with severe cancer [11]. Nevertheless, the impacts of bevacizumab and radiotherapy on NSCLC and its detailed mechanism are still completely unknown. Besides, increasing evidence illustrated that revealing the target genes of drugs in cancer is essential to improve their therapeutic effects [12].

XRCC2 belongs to the RAD51 (RAD51 recombinase) family which encodes proteins that are related to DNA damage and repair [13, 14]. It has been reported that XRCC2 is involved in radiotherapy for various cancers, including colon cancer and breast cancer [15, 16]. Park et al. discovered XRCC2 had multiple variants in females with breast cancer in 2021 [17]. In addition, XRCC2 has been involved in several panels testing clinical genes [18, 19]. Present studies have discovered SNPs (single nucleotide polymorphisms) of several genes were highly related to NSCLC which included XRCC2 [20]. However, whether XRCC2 exerts a role in bevacizumab combined with radiotherapy in NSCLC treatment remains to be uncovered.

The goal of our research is to reveal the effect of XRCC2 on the treatment of NSCLC with bevacizumab combined with radiotherapy.

Materials and methods

Clinical samples and ethics statement

NSCLC tissues and normal tissues were collected form 15 patients that experienced primary NSCLC surgery in The Third Affiliated Hospital of Shandong First Medical University. These patients did not receive any other adjuvant therapy. After collection, all tissues were stored in liquid nitrogen for the next experiments. The research was approved of by The Third Affiliated Hospital of Shandong First Medical University Ethics Committee (2019-13) and it abided by the principle of the Declaration of Helsinki. All patients mentioned in this text volunteered to participate in the study and signed the consent form. Every animal experiment is in correspondence with the requirement of the Care and Use of Laboratory Animal via International Committees. The experimental schemes were approved by the Ethical Committee of The Third Affiliated Hospital of Shandong First Medical University Ethics Committee.

Cell culture

A549, H157, HCC827 and H838 cell lines were brought from Qingqi Biotechnology Development Co., Ltd. (Shanghai, China) and cultured in RMPI (Roswell Park Memorial Institute)-1640 medium added with 10% FBS (fetal bovine serum), 100 U^{-ml} streptomycin and 100 U^{-ml} penicillin in the 37°C incubators with 5% CO_2.

Transient and stable transfection

Si (small interfering)-XRCC2-1# (sense-5'-UUGCAACGACACAAACUAUAA-3', antisense-5'-UUAUAGUUUGUGUCGUUGCAA-3'), si-XRCC2-2# (sense-5'-CAGAAAUGCUUUAUCACCUAA-3', antisense-5'-UUAGGUGAUAAAGCAUUUCUG-3') and si-NC (sense-5'-UUCUCCGAACGUGUCAC-GU-3', antisense-5'-ACGYGACACGUUCGGAGAA-3') were obtained from Sangon Biotech (China). PcDNA3.1 and pcDNA3.1/XRCC2 were transfected into cells transiently by utilizing Lipofectamine 2000 (11668019, Invitrogen, USA) according to the manufacturer's protocol. SiRNAs were transduced into NSCLC cells employing 5 ug/mL polybrene (GM-040901, Genomeditech, China) to establish stable cell lines. After 36 h, positive cells were screened by puromycin (2 μg/mL, GM-040401, Genomeditech, China) for 48-72 h. Finally, the transfection effects were determined using qRT-PCR and immunoblotting analysis.

qRT-PCR (quantitative real time polymerase chain reaction)

Total RNA from tissues of patients with NSCLC in addition to cells were extracted using the Trizol kit (10296010, Thermo Fisher Scientific, USA). The RNA was employed to synthesize cDNA using the RevertAid RT kit (K1691, Thermo Fisher Scientific, USA). qRT-PCR was conducted according to the protocol offered by PowerUp SYBR Green Master Mix (A25742, Thermo Scientific, USA) according to the following reaction system: 94°C, 2 min, 94°C, 30 s for 30 cycles, 72°C 60 s. The sequences of primers are listed in **Table 1**. The data was analyzed based on the $2^{-\Delta\Delta Ct}$ method and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) served as the control gene.

Immunoblotting analysis

A549 cells were seeded in 6-well plates and treated with radiotherapy for the indicated

Table 1. The sequence of primer

Gene	qRT-PCT Primer
XRCC2	Forward: 5' AGCGATCTGCCCACCTTGGC-3'
	Reverse: 5'-GCCAAGTGGGCAGATCGCT-3'
GAPDH	Forward: 5'-CCAAAATCAGATGGGGCAATGCTGG-3'
	Reverse: 5'-TGATGGCATGGACTGTGGTCATTCA-3'

time and harvested by SDS (sodium dodecyl sulfate) lysis buffer. The concentration of total protein was measured by BCA Protein Assay Kit (Phygene, China). Next, gel electrophoresis was utilized to separate the target protein, and proteins were transferred to the PVDF (polyvinylidene fluoride) membrane. Subsequently, PVDF membrane was washed with TBST (Tris Buffered Saline Tween) 3 times and blocked by TBST with 3% milk for 0.5-1 hour at room temperature. The primary antibody against GAPDH (2118, Cell Signaling Technology), XRCC2 (sc-365854, Santa Cruz Biotechnology), CyclinD1 (GB13079, ServiceBio), CDK1 (cyclin dependent kinase 1, sc-54, Santa Cruz Biotechnology) was added into TBST and incubated with the PVDF membrane at 4°C overnight. Then Anti-rabbit IgG, HRP-linked Antibody (7074, Cell Signaling Technology) or Anti-mouse IgG, HRP-linked Antibody (#7076, Cell Signaling Technology) and visualizer were used to identify the target band. The protein level was determined using Image J software as described in a previous study [21].

Cell counting kit (CCK)-8 assay

A549 cells were planted into 96-well plates and each well was added with 0.1 mL medium containing serum, followed by changing to fresh medium every 3 days for a total of 7 days. Every treatment was divided into 5 duplicated wells. In addition, a combination was added with medium as the control group. After culturing 12 h, cells were treated by radiotherapy and a 9:1 CCK-8 solution was incubated for 1 h. Then a microplate reader was employed to examine the OD (optical density) value at 450 nm wavelength at indicated time point. The survival curve was plotted on the basis of the OD value to present the viability of cells.

Colony formation assay

A549 cells were planted in 12-well plates and treated by radiotherapy after 12 hours. Then the cells were cultured for 10 days and stained with trypsin and counted using Image J software.

Transwell assay

A549 cells were resuspended in medium without serum and were seeded into the top compartment of the transwell chamber which was 8 µm (CorningInc). The medium was added with 10% fetal bovine serum into the top compartment and incubated with the cells for nearly 48 hours. Then it was fixed in addition to staining the cells existing on the top part of the membrane. In the end, a microscope (Olympus) was used to measure the count of migratory cells. As for invasion, a similar protocol was conducted while embedded Mtrigel (Corning) in the transwell chamber.

Cell cycle analysis

A549 cells were digested and suspended after a series of treatments. Next, the cells were fixed with 75% ethanol at 4°C for 4 hours and the supernatant was discarded, subsequently the cells were incubated with RNA enzyme, including 40% PI (Prodium Iodide, Sigma). Then the cells were washed with PBS 3 times. Flow cytometry (Beckman) was used to detect the cell cycle and data was analyzed using FlowJo software.

Tumor xenograft experiment

Four to five week old nude mice were fostered in a specific pathogen-free (SPF) environment, they were allowed access to water and food freely. A total of 15 mice were divided into 3 groups with 5 mice in each group: blank group (injected with A549-pcDNA3.1 cells); treatment by bevacizumab combined with radiotherapy group (injected with A549-pcDNA3.1 cells); and treatment by bevacizumab combined with radiotherapy group (injected with A549-pcDNA3.1/XRCC2 cells). One hundred µl A549-pcDNA3.1 or A549-pcDNA3.1/XRCC2 cell suspension, including approximately 1×10^7 cells were injected into the left limb of nude mice. After tumor formation, mice were treated with 1.5 mg/kg bevacizumab and 6 MV X-rays from a linear accelerator (Elekta, Sweden) every 3 days for 18 days. The volume of every tumor was measured and recorded every three days from day 9 for a total of 21 days. The data
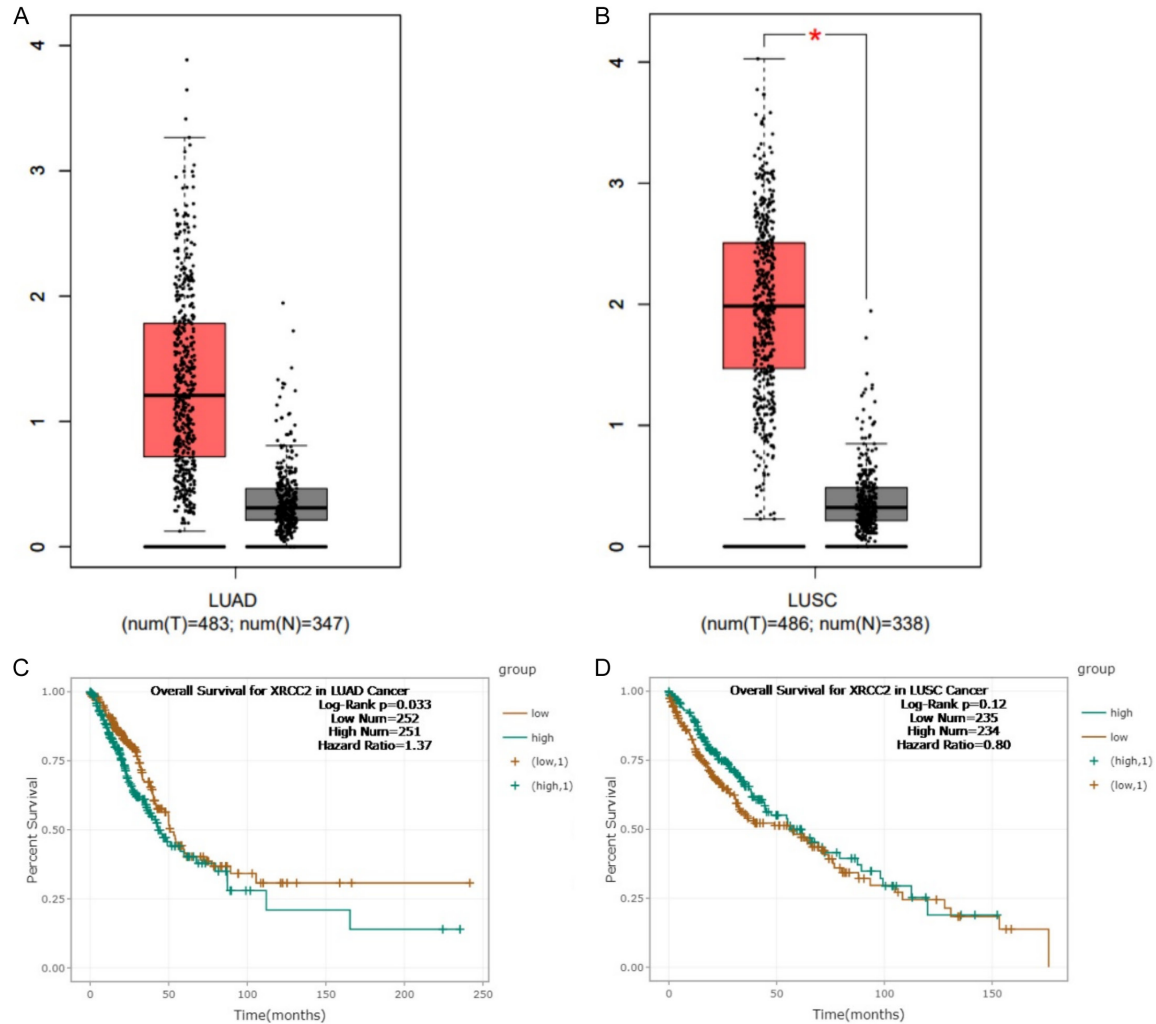
Figure 1. XRCC2 (X-ray repair cross complementing 2) was upregulated dramatically in patients with NSCLC and was related to poor prognosis of patients. A and B: The expression of XRCC2 in LUAD (lung adenocarcinoma) and LUSC (squamous cell lung carcinoma) tissues compared to normal tissues according to GEPIA (Gene Expression Profiling Interactive Analysis); C and D: The relationship between XRCC2 and survival time of patients with LUAD as well as LUSC. *P<0.05, versus normal (N) tissues.

was used to draw the curve of tumors after 21 days. In addition, the weight of tumors was recorded after humane euthanasia of the mice using 1% sodium pentobarbital (0.01 mL/g). The formula for calculating tumor volume is: V (volume) = l (length) * w (width)^2/2.

Statistical analysis

All data were analyzed by Graphpad software and calculated data were presented as $\overline{x}$±sd. Student's t-test (two groups) or ANOVA (one-way analysis of variance) followed by Tukey post hoc tests (multiple groups) were used to analyze the differences between different

groups. Additionally, **P<0.01 represented significant differences between diverse groups.

Results

XRCC2 was upregulated dramatically in patients with NSCLC and was related to poor prognosis of patients

To reveal the effect of XRCC2 on NSCLC, we consulted the GEPIA website and found XRCC2 was upregulated significantly in LUAD and LUSC tissues compared to normal tissues (**Figure 1A**, **1B**). Additionally, high expression of XRCC2 was associated with poor prognosis
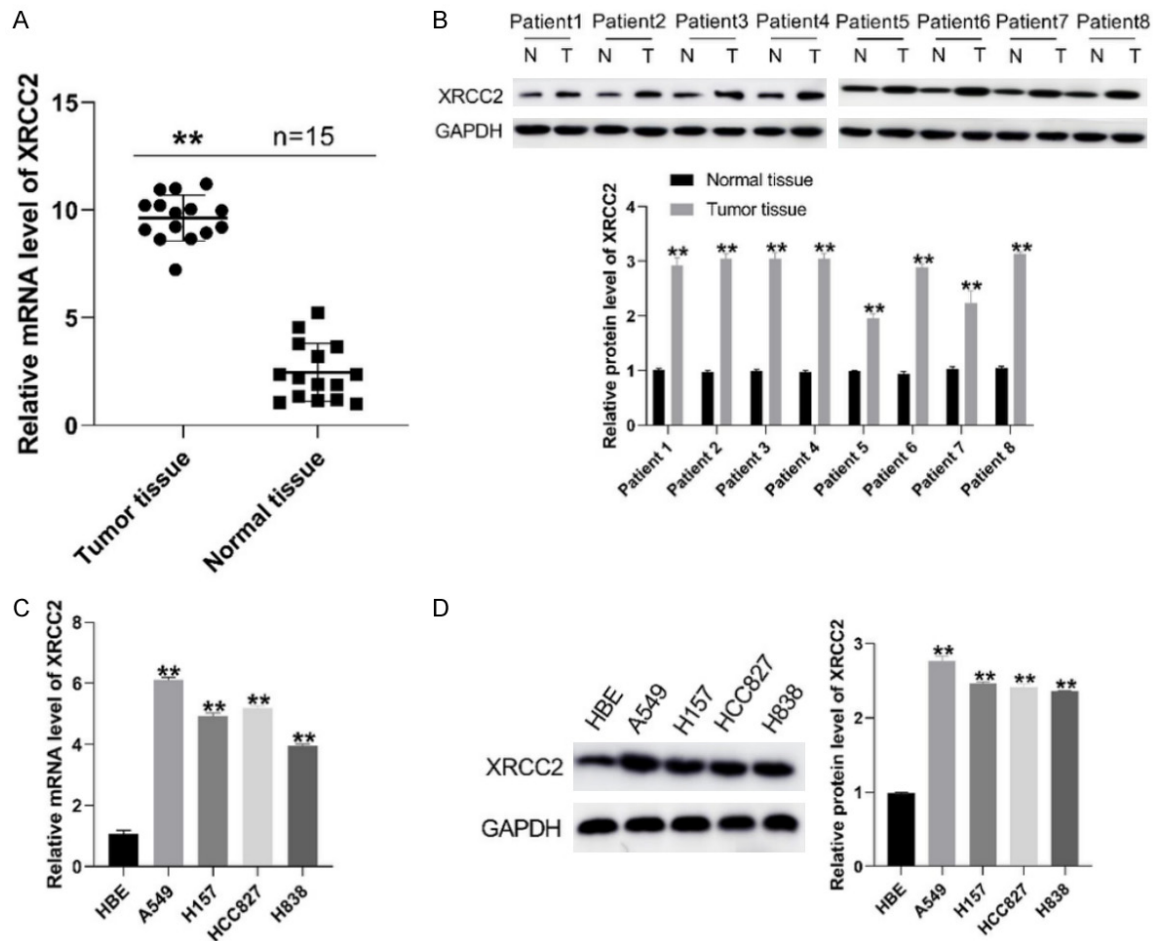
Figure 2. Expression level of XRCC2 (X-ray repair cross complementing 2) was higher in NSCLC (Non Small Cell Lung Cancer) tissues and NSCLC cells. A: The mRNA level of XRCC2 in NSCLC tumor and normal tissue (n=15); B: The protein level of XRCC2 in NSCLC tumor and normal tissue; C and D: The mRNA and protein levels of XRCC2 in NSCLC cells compared with normal cell. $^{**}P<0.01$, compared with normal tissues/cells.

in patients with NSCLC according to the Starbase database (**Figure 1C**, **1D**). Thus, we hypothesized that XRRC2 might promote the development of NSCLC notably.

Expression level of XRCC2 was higher in NSCLC tissues and NSCLC cells

To verify the above-mentioned results obtained from databases, we measured the mRNA and protein levels of XRCC2 in from 15 NSCLC patients offered by our hospital. The data showed that the mRNA and protein levels of XRCC2 in NSCLC tissues were obviously higher than that in normal tissues ($^{**}P<0.01$, **Figure 2A**, **2B**). Consistently, qRT-PCR and western blotting experiments revealed that the mRNA and protein levels of XRCC2 in NSCLC cells (A549, H157, HCC827 and H838) were signifi-

cantly higher than that in normal cells (HBE; $^{**}P<0.01$, **Figure 2C**, **2D**) with the highest level in A549 cells which was then employed for subsequent experiments.

Treatment of bevacizumab combined with radiotherapy suppressed proliferation, migration and invasion of NSCLC cells

Next, A549 cells were seeded in 6-well plates and treated with 5 µg/mL bevacizumab for 24 hours followed with 6 Gy dose X-ray irradiation exposure for another 3 days. Then, CCK-8 and clone formation assays showed that bevacizumab combined with radiotherapy decreased the proliferative ability of NSCLC cells significantly ($^{**}P<0.01$, **Figure 3A**, **3B**). In addition, transwell assay was used to confirm the effect of bevacizumab and radiotherapy on migration
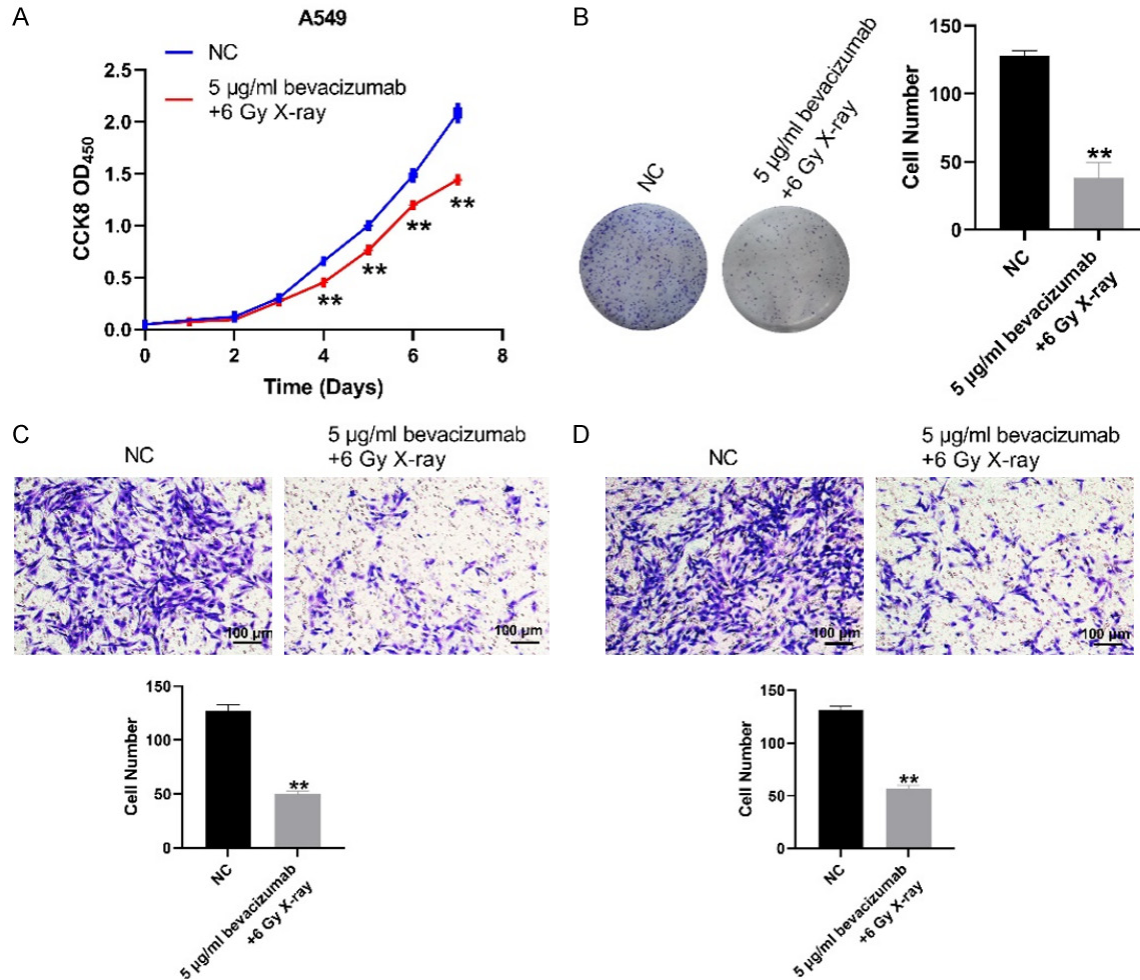
Figure 3. Treatment of bevacizumab combined with radiotherapy suppressed proliferation, migration and invasion of NSCLC (Non Small Cell Lung Cancer) cell. A and B: CCK-8 (cell counting kit-8) and colony formation assay illustrated that treatment by bevacizumab combined with radiotherapy downregulated the proliferation of A549 cells; C and D: Transwell assay showed the migration and invasion of A549 cells were suppressed after treatment of bevacizumab and radiotherapy (200×). **P<0.01 versus NC group.

and invasion in NSCLC cells. According to above-mentioned treatment, the data illustrated migration and invasion of A549 cells were depressed obviously after the treatment (**P<0.01, **Figure 3C**, **3D**). Thus, bevacizumab and radiotherapy can inhibit the proliferation, migration and invasion of NSCLC cells.

Silencing XRCC2 increased the sensitivity of NSCLC cells to bevacizumab combined with radiotherapy

To verify how XRCC2 impacts NSCLC after the treatment of bevacizumab combined with radiotherapy, we transfected 2 siRNAs targeting XRCC2 into A549 cells separately to decrease the load of XRCC2 as shown in **Figure 4A** (**P<0.01). Additionally, the mRNA level of XRCC2 in A549-XRCC2$^{-/-}$-1# was lower than that in A549-XRCC2$^{-/-}$-2#, so we chose A549-XRCC2$^{-/-}$-1# to finish the following study. The A549-XRCC2$^{-/-}$-1# and A549-XRCC2$^{+/+}$ cells were seeded in plates and treated with 5 µg/ml bevacizumab combined with 6 Gy X-ray for an indicated time. Subsequently, CCK-8 assay, colony assay and transwell assay were conducted to detect the effect of XRCC2 knockdown on proliferation, migration and invasion of A549 cells. The results showed that silencing XRCC2 further suppressed the ability of proliferation, migration and invasion of A549 cells treated by bevacizumab combined with radiotherapy (**P<0.01, $^{\&\&}$P<0.01, **Figure 4B**, **4E**).
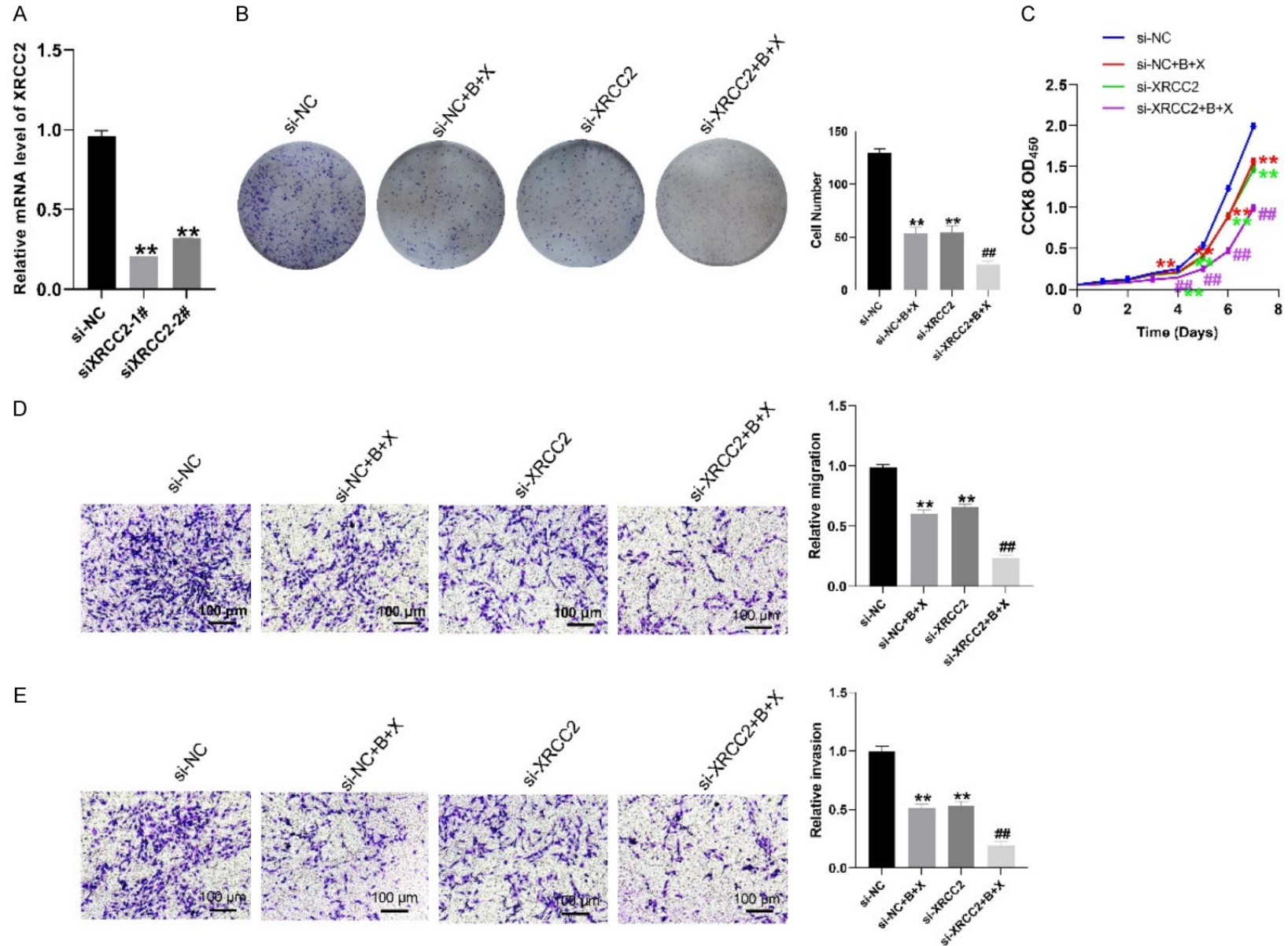
Figure 4. Silencing XRCC2 (X-ray repair cross complementing 2) increased the sensitivity of NSCLC (Non Small Cell Lung Cancer) cells to bevacizumab combined with radiotherapy. A: The mRNA level of XRCC2 in A549; B and C: The proliferation of cells detected by CCK-8 and colony formation assay; D and E: The migration and invasion of cells detected by Transwell assay (200×). **$P<0.01$ versus si-NC group, ##$P<0.01$ versus si-NC+5 µg/ml bevacizumab+6 Gy X-ray group.

Overexpression of XRCC2 reversed the inhibitory effect of bevacizumab combined with radiotherapy on NSCLC cells

Next, we transfected vectors with or without XRCC2 to confirm its effects on the progression of NSCLC after treatment. CCK-8 assay and colony assay demonstrated that overexpression of XRCC2 renovated the proliferative viability decreased by bevacizumab combined with radiotherapy (**P<0.01, **Figure 5B**, **5C**). Then transwell experiments were conducted to verify that transfecting XRCC2 recovered the migration and invasion of A549 after the treatment of bevacizumab combined with radiotherapy consistently (**Figure 5D**, **5E**).

XRCC2 regulated the sensitivity of NSCLC cell to bevacizumab combined with radiotherapy via controlling cell cycle

To further explore the detailed mechanism of XRCC2 on NSCLC cells after treatment, we employed flow cytometry to detect cell cycles in different groups. A549-XRCC2$^{-/-}$ and A549-XRCC2$^{+/+}$ cells were exposed to bevacizumab and radiotherapy at indicated times and harvested for the measurement of cell cycles. The results illustrated treatment with bevacizumab combined with radiotherapy decreased the percentage of cells in G0/G1 phase but increased those in S and G2/M phases. Meanwhile, silencing XRCC2 in cells further arrested cells to S as well as G2/M stage and reduced the number of cells in G0/G1 stage (**P<0.01, **Figure 6A**). As expected, overexpression of XRCC2 in A549 cells could reverse the cell cycle arrest induced by treatment of bevacizumab combined with radiotherapy (**Figure 6B**).

Overexpression of XRCC2 restored the inhibitory effect on tumors in mice induced by the treatment of bevacizumab combined with radiotherapy

An *in vivo* xenograft model was successfully constructed in mice with a success rate of 100%. After tumor formation, mice were treated by 5 mg/kg bevacizumab combined with 6 Gy X-ray. As shown in **Figure 7A**, **7C**, the volume and weight of tumors were decreased by exposure to bevacizumab combined with radiotherapy compared to the control group. Furthermore, the volume and the weight of tumors were renovated by overexpressing XRCC2 in

cells (**P<0.01, **Figure 7A**, **7C**). Collectively, bevacizumab combined with radiotherapy could decrease the volume and the weight of tumors in mice, while overexpression of XRCC2 recovered the growth of tumor to some extent.

Discussion

Lung cancer is still a main reason for cancer deaths worldwide [22]. Non-small cell lung cancer (NSCLC) is regarded as the leading subtype of lung cancer, which is mainly divided into LUAD and LUSC [23]. XRCC2 can encode proteins involved in the repairing of DNA double-strand breaks, which has been found to be in close relationship with NSCLC [24-26]. It has been reported that XRCC2 is highly associated with a variety of cancers, such as glioma, colorectal cancer and ovarian cancer [27-29]. However, the detailed effect and mechanism of XRCC2 on NSCLC were unclear until now. Our team found the expression of XRCC2 in NSCLC tissues was notably higher than that in normal tissue and its overexpression is related to poor prognosis of patients who have been diagnosed with NSCLC according to GEPIA and ENCO-IR database. Besides, qRT-PCR and immunoblotting analysis confirmed that the expression of XRCC2 was upregulated in NSCLC tissues compared with the normal tissues. Accordingly, the load of XRCC2 is elevated in NSCLC cells (A549, H157, HCC827 and H838) than in normal cells, HBE. Based on these results, we primarily hypothesized that XRCC2 exists as an oncogene in NSCLC.

It has been suggested that the reason for cancer cells resisting radiation may be metastasis or recurrence of the tumor after radiotherapy [30]. Considering that cancer cells ability to resist chemotherapeutic drugs and radiation is increasing, and with a propensity for metastasis, more and more treatment failures are happening during the treatment of tumors [31]. Notably, the leading cause of the failure in treating NSCLC is radioresistance [32]. Bevacizumab was approved by the FDA (Food and Drug Administration) as an anti-angiogenetic drug, which initially was used for treating colon cancer combined with classic chemotherapy, subsequently it is employed in various advanced tumors such as kidney, breast and lung cancer [33]. In terms of NSCLC, bevacizumab is useful as the first-line treatment drug, even
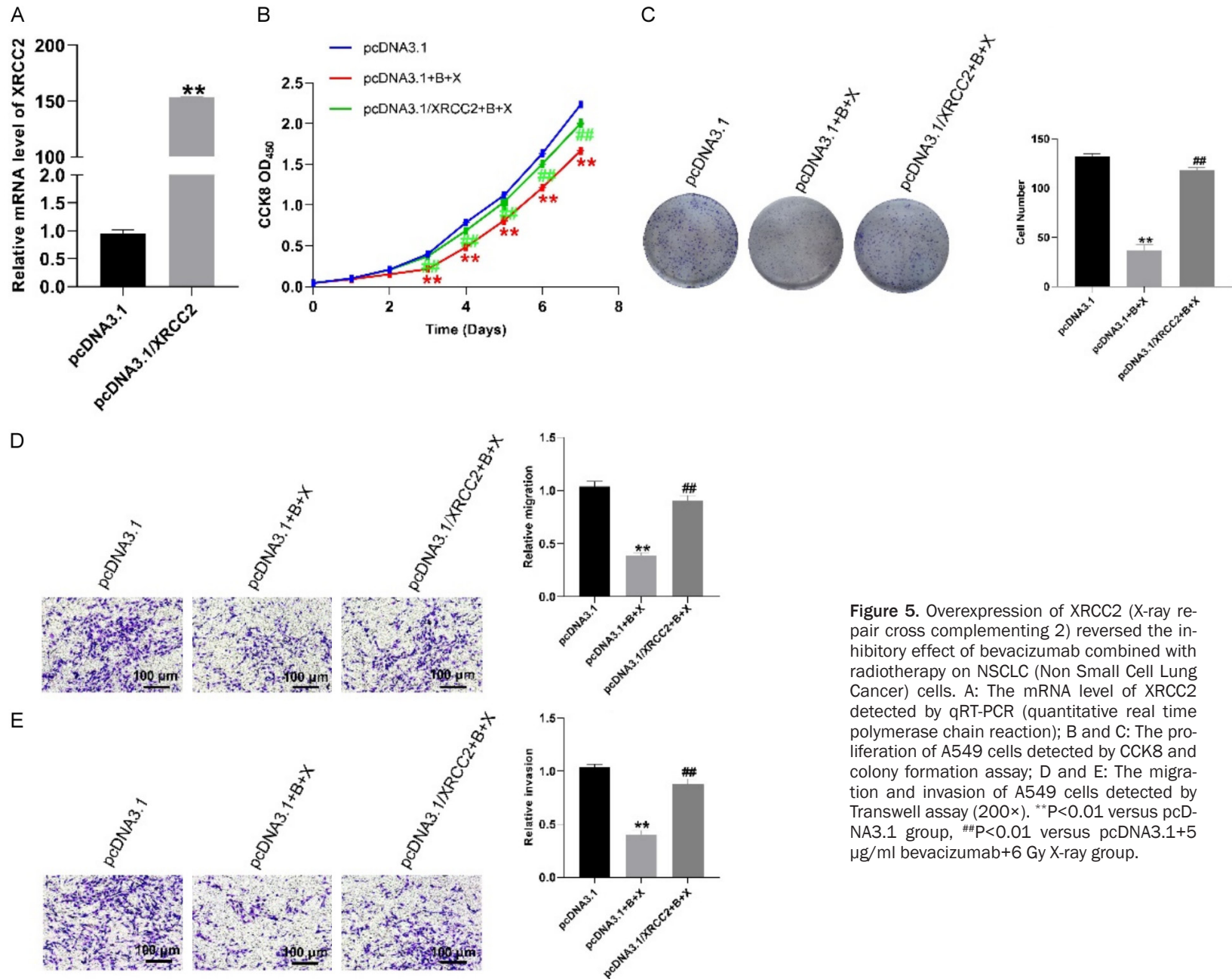
Figure 5. Overexpression of XRCC2 (X-ray repair cross complementing 2) reversed the inhibitory effect of bevacizumab combined with radiotherapy on NSCLC (Non Small Cell Lung Cancer) cells. A: The mRNA level of XRCC2 detected by qRT-PCR (quantitative real time polymerase chain reaction); B and C: The proliferation of A549 cells detected by CCK8 and colony formation assay; D and E: The migration and invasion of A549 cells detected by Transwell assay (200×). **$P < 0.01$ versus pcDNA3.1 group, ##$P < 0.01$ versus pcDNA3.1+5 μg/ml bevacizumab+6 Gy X-ray group.
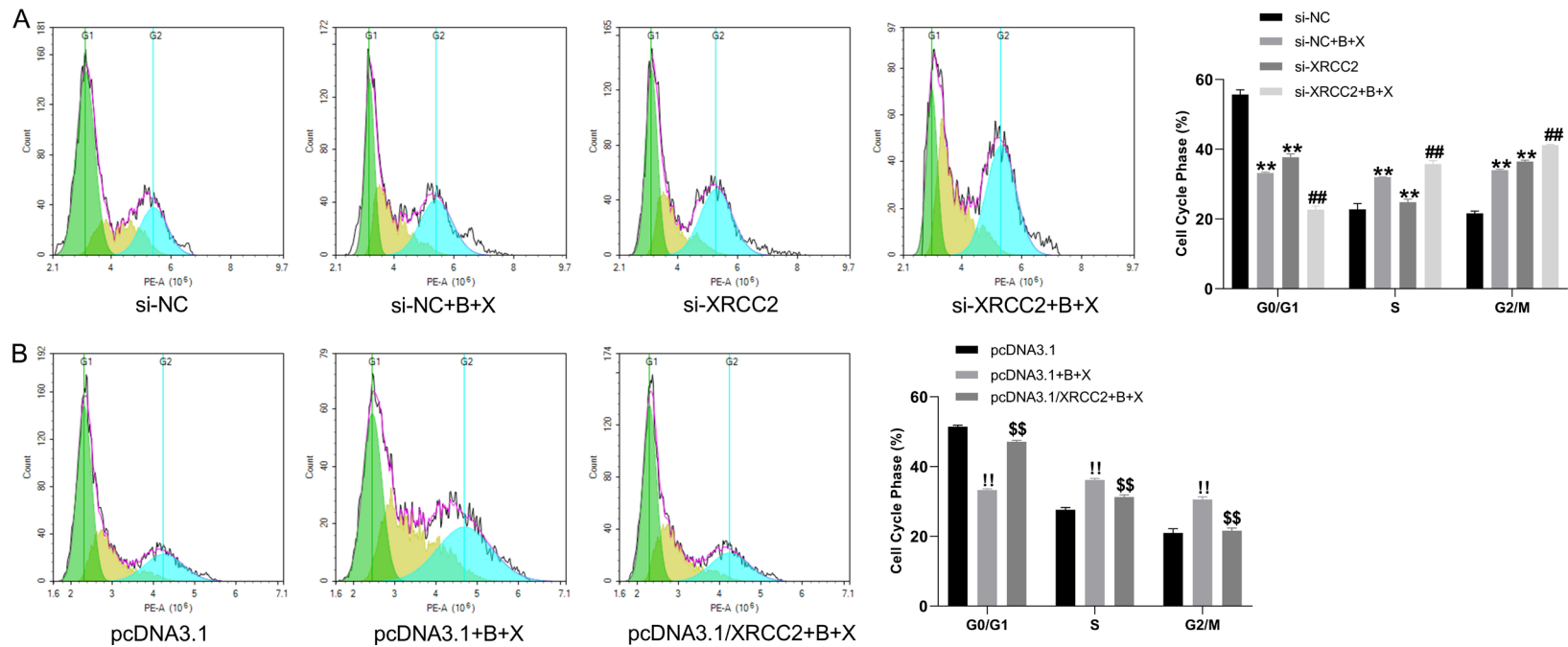
Figure 6. XRCC2 regulated the sensitivity of NSCLC (Non Small Cell Lung Cancer) cells to bevacizumab combined with radiotherapy via controlling cell cycle. A and B: The cell cycle distribution of A549 cells after various treatment. **$P<0.01$ versus si-NC group, ##$P<0.01$ versus si-NC+5 µg/ml bevacizumab+6 Gy X ray group, !!$P<0.01$ versus pcDNA3.1 group, $$$P<0.01$ versus pcDNA3.1+5 µg/ml bevacizumabv+6 Gy X-ray group.
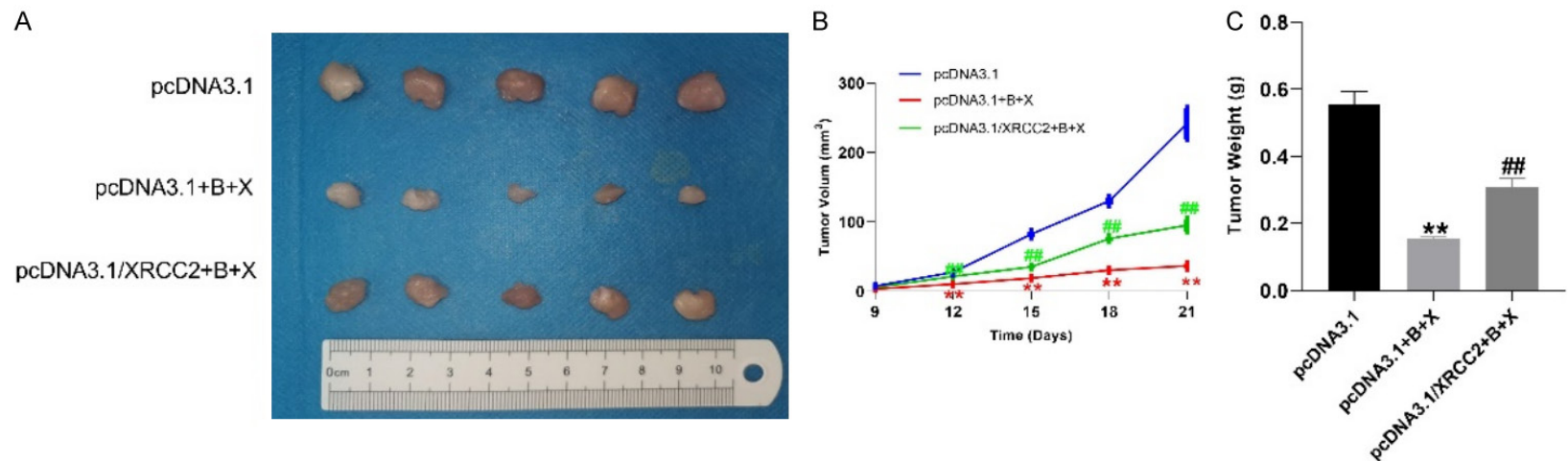
Figure 7. Overexpression of XRCC2 (X-ray repair cross complementing 2) restored the inhibitory effect of bevacizumab combined with radiotherapy on tumor growth in mice. A: The photo of tumor; B: The volume of tumor; C: The weight of tumors. **P<0.01 versus pcDNA3.1 group, ##P<0.01 versus pcDNA3.1+5 mg/kg, bevacizumab+6 Gy X-ray group.

though experts have recommended that doctors should be cautious in choosing bevacizumab as the primary drug [34]. Nevertheless, the role of bevacizumab combined with radiotherapy in NSCLC and its related molecular mechanism has not been elucidated. This investigation showed that treatment with bevacizumab combined with radiotherapy downregulated the proliferative ability of NSCLC cells. Additionally, migration and invasive abilities of A549 cells were inhibited significantly after exposure to bevacizumab and X-ray. These results proved that treatment with bevacizumab combined with radiotherapy could inhibit the progression of NSCLC cells dramatically.

A previous study illustrated that XRCC2 knockdown exhibits an unexpected role in sensitizing colon cancer cells to X-ray irradiation [15]. Butkiewicz et al. discovered that XRCC2 polymorphisms are closely related to the prognosis of NSCLC patients who received radiotherapy [25]. However, whether XRCC2 exerts vital effects on bevacizumab combined with radiotherapy in NSCLC has been uncovered. Our present study verified that knockdown of XRCC2 further aggravated the inhibitory effect induced by bevacizumab and X-ray for the first time. In contrast, overexpressing XRCC2 in NSCLC cells restored its ability of proliferation, migration and invasion inhibited by the above-mentioned treatment.

These findings revealed that XRCC2 increases the sensitivity of NSCLC to bevacizumab and radiotherapy.

Subsequently, to detect the impact of XRCC2 on NSCLC cells during the therapeutic process by bevacizumab combined with radiotherapy, we constructed A549 cell lines silenced expression of XRCC2. A549-XRCC2$^{-/-}$ and A549-XRC22$^{+/+}$ cells were treated with bevacizumab combined with radiotherapy for the indicated time and conducted CCK8, colony formation in addition to transwell assay.

Emerging research has proved that XRCC2 is involved in the progression of diverse cancers by regulating the cell cycle [35]. Consistently,

this investigation verified that treatment by bevacizumab and X-ray arrested cells to S and G2 stages, and decreased the proportion of cells in the G1 stage. Knockdown of XRCC2 further aggravated the blocking effects on the cell cycle, while overexpression of XRCC2 renovated the regulatory impact produced by bevacizumab combined with radiotherapy.

There are still some limitations in this study. For example, whether XRCC2 alone is able to regulate the progression of bevacizumab in NSCLC needs further study. More experiments will be conducted to elucidate how XRCC2 affects the role of bevacizumab and radiotherapy in the development of NSCLC.

In summary, our study indicates that XRCC2 knockdown can improve the efficacy of bevacizumab combined with radiotherapy in the treatment of NSCLC by arresting the cell cycle.

Disclosure of conflict of interest

None.

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References

[1] Osmani L, Askin F, Gabrielson E and Li QK. Current WHO guidelines and the critical role of immunohistochemical markers in the subclassification of Non-Small Cell Lung Carcinoma (NSCLC): moving from targeted therapy to immunotherapy. Semin Cancer Biol 2018; 52: 103-109.

[2] Walia R, Jain D, Madan K, Sharma MC, Mathur SR, Mohan A, Iyer VK and Kumar L. p40 & thyroid transcription factor-1 immunohistochemistry: a useful panel to characterize non-small cell lung carcinoma-not otherwise specified (NSCLC-NOS) category. Indian J Med Res 2017; 146: 42-48.

[3] Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ and He J. Cancer statis-

tics in China, 2015. CA Cancer J Clin 2016; 66: 115-132.

[4] Qiu M, Chen YB, Jin S, Fang XF, He XX, Xiong ZF and Yang SL. Research on Cirp40 & thyroid transcription factor-1 immunohistochemistry: a useful panel to characterize Non-Small Cell Lung Carcinoma-Not Otherwise Specified (NSCLC-NOS) category. Cadian clock genes in non-small-cell lung carcinoma. Chronobiol Int 2019; 36: 739-750.

[5] Henning WC, Azzoli CG, Santivasi WL and Xia F. Basic mechanisms of therapeutic resistance to radiation and chemotherapy in lung cancer. Cancer J 2013; 19: 200-207.

[6] Yang X, Zhang W, Cheng SQ and Yang RL. High expression of lncRNA GACAT3 inhibits invasion and metastasis of non-small cell lungcancer to enhance the effect of radiotherapy. Eur Rev Med Pharmacol Sci 2018; 22: 1315-1322.

[7] Yuan F, Chen Y, Dellian M, Safabakhsh N, Ferrara N and Jain RK. Time-dependent vascular regression and permeability changes in established human tumor xenografts induced by an anti-vascular endothelial growth factory vascular permeability factor antibody. Proc Natl Acad Sci U S A 1996; 93: 14765-14770.

[8] O'Connor JP, Carano RA, Clamp AR, Ross J, Ho CC, Jackson A, Parker GJ, Rose CJ, Peale FV, Friesenhahn M, Mitchell CL, Watson Y, Roberts C, Hope L, Cheung S, Reslan HB, Go MA, Pacheco GJ, Wu X, Cao TC, Ross S, Buonaccorsi GA, Davies K, Hasan J, Thornton P, del Puerto O, Ferrara N, van Bruggen N and Jayson GC. Quantifying antivascular effects of monoclonal antibodies to vascular endothelial growth factor: insights from imaging. Clin Cancer Res 2009; 15: 6674-6682.

[9] Yanagisawa M, Yorozu K, Kurasawa M, Nakano K, Furugaki K, Yamashita Y, Mori K and Fujimoto-Ouchi K. Bevacizumab improves the delivery and efficacy of paclitaxel. Anticancer Drugs 2010; 21: 687-694.

[10] Turley RS, Fontanella AN, Padussis JC, Toshimitsu H, Tokuhisa Y, Cho EH, Hanna G, Beasley GM, Augustine CK, Dewhirst MW and Tyler DS. Bevacizumab-induced alterations in vascular permeability and drug delivery: a novel approach to augment regional chemotherapy for in-transit melanoma. Clin Cancer Res 2012; 18: 3328-3339.

[11] Yu X, Wang QX, Xiao WW, Chang H, Zeng ZF, Lu ZH, Wu XJ, Chen G, Pan ZZ, Wan DS, Ding PP and Gao YH. Neoadjuvant oxaliplatin and capecitabine combined with bevacizumab plus radiotherapy for locally advanced rectal cancer: results of a single-institute phase II study. Cancer Commun (Lond) 2018; 38: 24.

[12] Kanthou C and Tozer G. Targeting the vasculature of tumours: combining VEGF pathway in-

hibitors with radiotherapy. Br J Radiol 2019; 92: 20180405.

[13] Park JY, Virts EL, Jankowska A, Wiek C, Othman M, Chakraborty SC, Vance GH, Alkuraya FS, Hanenberg H and Andreassen PR. Complementation of hypersensitivity to DNA interstrand crosslinking agents demonstrates that XRCC2 is a fanconi anaemia gene. J Med Genet 2016; 53: 672-680.

[14] Berwick M, Satagopan JM, Ben-Porat L, Carlson A, Mah K, Henry R, Diotti R, Milton K, Pujara K, Landers T, Dev Batish S, Morales J, Schindler D, Hanenberg H, Hromas R, Levran O and Auerbach AD. Genetic heterogeneity among fanconi anemia heterozygotes and risk of cancer. Cancer Res 2007; 67: 9591-9596.

[15] Wang Q, Wang Y, Du L, Xu C, Sun Y, Yang B, Sun Z, Fu Y, Cai L, Fan S, Fan F and Liu Q. shRNA-mediated XRCC2 gene knockdown efficiently sensitizes colon tumor cells to x-ray irradiation in vitro and in vivo. Int J Mol Sci 2014; 15: 2157-2171.

[16] Popanda O, Tan XL, Ambrosone CB, Kropp S, Helmbold I, Fournier DV, Haase W, Sautter-Bihl ML, Wenz F, Schmezer P and Chang-Claude J. Genetic polymorphisms in the DNA double-strand break repair genes XRCC3, XRCC2, and NBS1 are not associated with acute side effects of radiotherapy in breast cancer patients. Cancer Epidemiol Biomarkers Prev 2006; 15: 1048-1050.

[17] Park DJ, Lesueur F, Nguyen-Dumont T, Pertesi M, Odefrey F, Hammet F, Neuhausen SL, John EM, Andrulis IL, Terry MB, Daly M, Buys S, Le Calvez-Kelm F, Lonie A, Pope BJ, Tsimiklis H, Voegele C, Hilbers FM, Hoogerbrugge N, Barroso A, Osorio A; Breast Cancer Family Registry; Kathleen Cuningham Foundation Consortium for Research into Familial Breast Cancer, Giles GG, Devilee P, Benitez J, Hopper JL, Tavtigian SV, Goldgar DE and Southey MC. Rare mutations in XRCC2 increase the risk of breast cancer. Am J Hum Genet 2012; 90: 734-739.

[18] Bonache S, Esteban I, Moles-Fernandez A, Tenes A, Duran-Lozano L, Montalban G, Bach V, Carrasco E, Gadea N, Lopez-Fernandez A, Torres-Esquius S, Mancuso F, Caratu G, Vivancos A, Tuset N, Balmana J, Gutierrez-Enriquez S and Diez O. Multigene panel testing beyond BRCA1/2 in breast/ovarian cancer Spanish families and clinical actionability of findings. J Cancer Res Clin Oncol 2018; 144: 2495-2513.

[19] Maksimenko J, Irmejs A, Trofimovics G, Berzina D, Skuja E, Purkalne G, Miklasevics E and Gardovskis J. High frequency of pathogenic non-founder germline mutations in BRCA1 and BRCA2 in families with breast and ovarian cancer in a founder population. Hered Cancer Clin Pract 2018; 16: 12.

[20] Sullivan I, Salazar J, Majem M, Pallares C, Del Rio E, Paez D, Baiget M and Barnadas A. Pharmacogenetics of the DNA repair pathways in advanced non-small cell lung cancer patients treated with platinum-based chemotherapy. Cancer Lett 2014; 353: 160-166.

[21] Zhu Y, You J, Wei W, Gu J, Xu C and Gu X. Down-regulated lncRNA RCPCD promotes differentiation of embryonic stem cells into cardiac pacemaker-like cells by suppressing HCN4 promoter methylation. Cell Death Dis 2021; 12: 667.

[22] Chen W, Yang J, Zhang Y, Cai H, Chen X and Sun D. Regorafenib reverses HGF-induced sorafenib resistance by inhibiting epithelial-mesenchymal transition in hepatocellular carcinoma. FEBS Open Bio 2019; 9: 335-347.

[23] Ramalingam SS, Owonikoko TK and Khuri FR. Lung cancer: new biological insights and recent therapeutic advances. CA Cancer J Clin 2011; 61: 91-112.

[24] Oji Y, Tatsumi N, Kobayashi J, Fukuda M, Ueda T, Nakano E, Saito C, Shibata S, Sumikawa M, Fukushima H, Saito A, Hojo N, Suzuki M, Hoshikawa T, Shimura T, Morii E, Oka Y, Hosen N, Komatsu K and Sugiyama H. Wilms' tumor gene WT1 promotes homologous recombination-mediated DNA damage repair. Mol Carcinog 2015; 54: 1758-1771.

[25] Butkiewicz D, Drosik A, Suwinski R, Krzesniak M, Rusin M, Kosarewicz A, Rachtan J, Matuszczyk I and Gawkowska-Suwinska M. Influence of DNA repair gene polymorphisms on prognosis in inoperable non-small cell lung cancer patients treated with radiotherapy and platinum-based chemotherapy. Int J Cancer 2012; 131: E1100-E1108.

[26] Yin M, Liao Z, Huang YJ, Liu Z, Yuan X, Gomez D, Wang LE and Wei Q. Polymorphisms of homologous recombination genes and clinical outcomes of non-small cell lung cancer patients treated with definitive radiotherapy. PLoS One 2011; 6: e20055.

[27] Liu Z, Zhang W, Cheng X, Wang H, Bian L, Wang J, Han Z, Wang Y, Lian X, Liu B, Ren Z, Zhang B, Jiang Z, Lin Z and Gao Y. Overexpressed XRCC2 as an independent risk factor for poor prognosis in glioma patients. Mol Med 2021; 27: 52.

[28] Li XB, Luo H, Huang J, Zhang JD, Yang ZX and Sun XW. XRCC2 gene polymorphisms and its protein are associated with colorectal cancer susceptibility in Chinese Han Population. Med Oncol 2014; 31: 245.

[29] Liu Y, Xu Y, Jiang M, Chen W and Zhu X. Significant value of XRCC2 and XRCC9 expression in the prognosis of human ovarian carcinoma. J Cancer 2021; 12: 6254-6264.

[30] Sui X, Geng JH, Li YH, Zhu GY and Wang WH. Calcium channel Alpha2delta1 subunit (CACNA2D1) enhances radioresistance in cancer stem-like cells in non-small cell lung cancer cell lines. Cancer Manag Res 2018; 10: 5009-5018.

[31] Wei W, Dong Z, Gao H, Zhang YY, Shao LH, Jin LL, Lv YH, Zhao G, Shen YN and Jin SZ. MicroRNA-9 enhanced radiosensitivity and its mechanism of DNA methylation in non-small cell lung cancer. Gene 2019; 710: 178-185.

[32] Chen X, Xu Y, Liao X, Liao R, Zhang L, Niu K, Li T, Li D, Chen Z, Duan Y and Sun J. Plasma miR-NAs in predicting radiosensitivity in non-small cell lung cancer. Tumour Biol 2016; 37: 11927-11936.

[33] Kazazi-Hyseni F, Beijnen JH and Schellens JH. Bevacizumab. Oncologist 2010; 15: 819-825.

[34] Gridelli C, Ardizzoni A, Douillard JY, Hanna N, Manegold C, Perrone F, Pirker R, Rosell R, Shepherd FA, De Petris L, Di Maio M and de Marinis F. Recent issues in first-line treatment of advanced non-small-cell lung cancer: results of an International Expert Panel Meeting of the Italian Association of Thoracic Oncology. Lung Cancer 2010; 68: 319-331.

[35] Xu K, Song X, Chen Z, Qin C, He Y and Zhan W. XRCC2 promotes colorectal cancer cell growth, regulates cell cycle progression, and apoptosis. Medicine (Baltimore) 2014; 93: e294.