Investigational New Drugs (2019) 37:646–657
https://doi.org/10.1007/s10637-018-0667-9

PRECLINICAL STUDIES

Relationship between expression of XRCC1 and tumor proliferation, migration, invasion, and angiogenesis in glioma

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Received: 16 August 2018 / Accepted: 14 September 2018 / Published online: 17 October 2018

Summary

Recently, XRCC1 polymorphisms were reported to be associated with glioma in Chinese population. However, only a few studies reported on the XRCC1 expression, and cancer progression. In this study, we investigated whether XRCC1 plays a role in glioma pathogenesis. Using the tissue microarray technology, we found that XRCC1 expression is significantly decreased in glioma compared with tumor adjacent normal brain tissue ($P < 0.01$, χ^2 test) and reduced XRCC1 staining was associated with WHO stages ($P < 0.05$, χ^2 test). The mRNA and protein levels of XRCC1 were significantly downregulated in human primary glioma tissues ($P < 0.001$, χ^2 test). We also found that XRCC1 was significantly decreased in glioma cell lines compared to normal human astrocytes ($P < 0.01$, χ^2 test). Overexpression of XRCC1 dramatically reduced the proliferation and caused cessation of cell cycle. The reduced cell proliferation is due to G1 phase arrest as cyclin D1 is diminished whereas p16 is upregulated. We further demonstrated that XRCC1 overexpression suppressed the glioma cell migration and invasion abilities by targeting MMP-2. In addition, we also found that overexpression of XRCC1 sharply inhibited angiogenesis, which correlated with down-regulation of VEGF. The data indicate that XRCC1 may be a tumor suppressor involved in the progression of glioma.

Keywords XRCC1 · Proliferation · Migration · Invasion · Angiogenesis · Glioma

Introduction

Glioma is the most common type of primary brain tumors in adults, including astrocytomas, oligodendrogliomas, oligoastrocytomas, and glioblastoma. Among them, the glioblastoma (GBM) is the most frequent and malignant histologic type, with a current median survival of approximately 15 months in patients with newly diagnosed disease following treatment with surgery, chemotherapy and radiotherapy [1]. The invasion nature of GBM has been frequently implicated as a key feature of GBM's resistance to therapy. Many studies found that many factors were involved in the of GBM, including adhesion molecules, extracellular matrix (ECM), protease system and angiogenesis [2, 3], yet the exact molecular mechanism and process of the invasion growth of GBM remain poorly understood. Therefore, there is an urgent need to clarify the molecular mechanisms underlying invasion, migration and angiogenesis behavior of human glioma.

Although many studies have been conducted on the etiology of glioma, it is still not completely understood. Ultraviolet radiation induced DNA damage and genomic instability is one of the leading causes for glioma [4]. To protect DNA against damage, humans have developed a set of complex DNA repair systems. There are four major DNA repair pathways, including base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR) and double strand break repair (DSBR) [5]. X-ray repair cross-complementing group 1 (XRCC1) is one of the DNA repair genes encoding a scaffolding protein that participate in base excision repair (BER) pathway [6]. Different XRCC1 polymorphisms may increase the risk of cancers by impairing interaction with enzymatic

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proteins and consequently altering DNA repair activity, and result in carcinogenesis [7, 8]. Increasing number of single-nucleotide polymorphisms (SNPs) in XRCC1 with varying degree of association have been reported in an array of cancer types, including glioma [9–15]. Recently, XRCC1 polymorphisms were reported to be associated with glioma in Chinese population [14–16]. However, only a few studies reported on the XRCC1 expression, and cancer progression. To our knowledge, the role of XRCC1 in glioma progression has never been studied and more importantly, XRCC1 expression has never been analyzed in glioma patients.

In our study, we report that XRCC1 was significantly downregulated in glioma cells and clinical glioma tissues, compared to normal human astrocytes (NHA) and non-tumor associated tissues. Moreover, we observed that enforced overexpression of XRCC1 inhibited glioma cell proliferation, invasion, migration and angiogenesis abilities. In addition, we investigated the molecular mechanisms underlying XRCC1 actions in glioma cells.

Materials and methods

Patients and samples

A glioma TMA was purchased from Shanxi Alenabio Biotechnology (xi'an, China), No: GL2083a. Pathologic grades of tumors were defined according to the 2000 WHO criteria as follows: 134 cases of benign tumor (Grade I and II), 58 cases of malignant tumor (Grade III and IV), 8 cases of tumor adjacent normal brain tissue and 8 cases of normal brain tissue. The array dot diameter was 1.0 mm, and each dot represented a tissue spot from one individual specimen that was selected and pathologically confirmed. Four human glioma tissues and four nontumorous brain tissues (internal decompression in cerebral trauma) were obtained from the Department of Neurosurgery, the affiliated hospital of Xuzhou Medical University.

Immunohistochemistry of TMA

The TMA slides were dewaxed by heating at 55 °C for 30 min and by three washes, 5 min each, with xylene. Tissues were rehydrated by a series of 5 min washes in 100, 95, and 80% ethanol and distilled water. Antigen retrieval was performed by heating the samples at 95 °C for 30 min in 250 ml of 10 mmol/L sodium citrate (pH 6.0). Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide for 20 min. Nonspecific binding was blocked with goat serum for 30 min. The primary monoclonal rabbit anti-XRCC1 antibody (Abcam, Cambridge, MA) was diluted 1:400 using goat serum and incubated at room temperature for 1 h. After three washes, 2 min each with PBS, the sections were incubated with a biotinylated goat anti-rabbit secondary antibody for 30 min (Zhongshan Biotech, Beijing, China), followed by the incubation with streptavidin-peroxidase (Zhongshan Biotech, Beijing, China) for an additional 30 min. After rinsing with PBS 3 times for 2 min, the sections were stained using DAB (Zhongshan Biotech, Beijing, China) for 15 min, rinsed in distilled water and counterstained with hematoxylin. Dehydration was then performed following a standard procedure, and the sections were sealed with cover slips. Negative controls were performed by omitting XRCC1 antibody during the primary antibody incubation.

Evaluation of immunostaining

The XRCC1 staining was examined double-blinded by two independent pathologists, and a consensus was reached for each core. The expression of XRCC1 was graded as positive when over 5% of tumor cells showed immunopositivity. Biopsies with less than 5% tumor cells showing immunostaining were considered as negative.

RNA extraction and reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNA of brain samples and glioma cell lines were extracted using the Trizol reagent (Tiangen Biotech) according to the manufacturer's instructions. RT-PCR was performed using a Takara RNA PCR Kit (AMV) version 3.0 (Takara, Shiga, Japan) according to the manufacture's protocol. Actin served as an internal control. The primers used were: forward, 5'-TCATCGCACTTCTTCTGTGGA-3′; reverse, 5'-GCCAACCCAGATAGCAACATC-3′ (for XRCC1); forward, 5'-GCGCGGCTACAGCTTCAC-3′; reverse, 5'-GGGGCCGGACTCGTCATA-3′ (for Actin).

Cell culture and transfection

Primary normal human astrocytes (NHA) were purchased from the KeyGEN Biotech Company (Nanjing, China) and cultured under the conditions as instructed by the manufacturer. Human glioma cell lines (U251, U87, T98G, SHG44, A172) were purchased from the Institute of Biochemistry and Cell Biology, Chinese Academy of Science. All glioma cells were cultured in DMEM supplemented with 10% fetal bovine serum (Invitrogen, Shanghai, China) at 37 °C in 5% CO_2.

The pFlag-control and pFlag-XRCC1 expression plasmids were a gift from Dr. Shouyu Wang (Nanjing Medical University, China). Transfection of the pFlag-control and pFlag-XRCC1 plasmids into the U251 and U87 glioma cells were carried out using Lipofectamine 2000

transfection reagent (Invitrogen, Shanghai, China) following the manufacturer's instructions. 6 h after transfection, the medium containing transfection reagents was removed. The cells were rinsed twice with PBS and incubated in fresh medium. 24 h after transfection, cells were lysed for Western blot assay, and cell proliferation assay, cell matrigel invasion assay, migration assay and HUVEC growth and tube-formation assay.

Western blot analysis

Western blotting analysis was performed according to standard methods as previously described [17], using rabbit anti-Cyclin D1, anti-p16, anti-MMP-2, anti-TIMP-2, anti-VEGF antibodies (all from Cell Signaling Technology, Danvers, MA, USA) and rabbit anti-XRCC1 (Abcam, Cambridge, MA),. The membranes were stripped and re-probed with an anti-β-actin antibody (Zhongshan Biotech, Beijing, China) as a loading control.

Cell proliferation assay

Cell proliferation was analyzed using a WST-8 Cell Counting Kit-8 (Beyotime, Nantong, China); 3×10^3 cells suspended in 100 μl DMEM medium containing 10% fetal bovine serum were seeded in 96-well plates and incubated for 24, 48, 72 and 96 h; 10 μl CCK-8 solution was added to each well and the cultures were incubated at 37 °C for 1 h. Absorbance at 450 nm was measured on an ELX-800 spectrometer reader (Bio-Tek Instruments, Winooski, USA).

Transwell invasion and migration assays

Cell invasion and migration assays were performed using a transwell system (Corning, NY) according to the manufacturer's protocol. To assess invasion, filters were precoated with matrigel (BD Biosciences, NJ, USA). 5×10^4 cells were seeded in serum-free medium in the upper chamber. After 24 h incubation at 37 °C, cells in the upper chamber were carefully removed with a cotton swab and the cells that had traversed the membrane were fixed in methanol, stained with Giemsa and photographed in five independent × 100 fields for each well. Three independent experiments were done and used to calculate fold invasion relative to control. The same experimental design was used for migration experiments except that the filters were not precoated with matrigel.

Wound healing assay

24 h after U251 and U87 glioma cells were transfected with pFlag-XRCC1, a rectangular lesion was created using a plastic pipette tip and the monolayer was rised twice with PBS and incubated in serum-free media. At the designated time, five randomly selected fields at the lesion border were acquired under an inverted microscope (Olympus).

HUVEC growth and tube-formation assay

For HUVEC growth assay, 2×10^4 HUVECs suspended in 100 μl conditioned medium from either negative control cells or XRCC1 overexpressed cells. HUVECs were seeded at a density of 2×10^4 in a 96-well culture plate and incubated at 37 °C in a humidified atmosphere containing 5% CO_2 for 24 h. Then, cell proliferation was detected according to the CCK-8 manufacturer's instructions. For tube-formation assay, U251 and U87 cells were cultured in 6-well plate with fresh complete medium for 24 h, and the medium was collected and centrifuged to remove any cells debris before its use as a conditioned medium. 48-well plate was coated with Matrigel and kept in 37 °C for 30 min. Then, 2×10^4 HUVECs were suspended in 100 μl conditional medium and applied to the pre-coated 48-well plate. After incubation at 37 °C for another 24 h, the number of capillary-like tubes from three randomly chosen fields was counted.

Gelatin zymography

5×10^5 cells were seeded in 6-well plate for 24 h and transfected with the XRCC1 and negative control. Thereafter, cells were incubated in serum-free for an additional 24 h. Then performed as described previous [17].

ELISA assay for VEGF

The protein levels of VEGF in the supernatant were measured using the Quantikine human VEGF ELISA kit (NeoBioscience, Shanghai, China) according to the manufacturer's instruction. In brief, the cells were seeded in 6-well plates and cultured to 90% confluence, and then cells were switched to fresh medium. The supernatants were collected and the number of cells in each well was counted after 24 h. VEGF in the supernatant (100 μl) was determined, and normalized to the cell number. A serial dilution of human recombinant VEGF was included in each assay to obtain a standard curve.

Statistical analysis

All experiments were performed three times, and data were analyzed with SPSS 16.0 software (SPSS, Chicago, IL). The two-tailed Student's t test was used to evaluate the significance of the differences between two groups of data in all pertinent experiments; Differences were considered significant when $P < 0.05$.

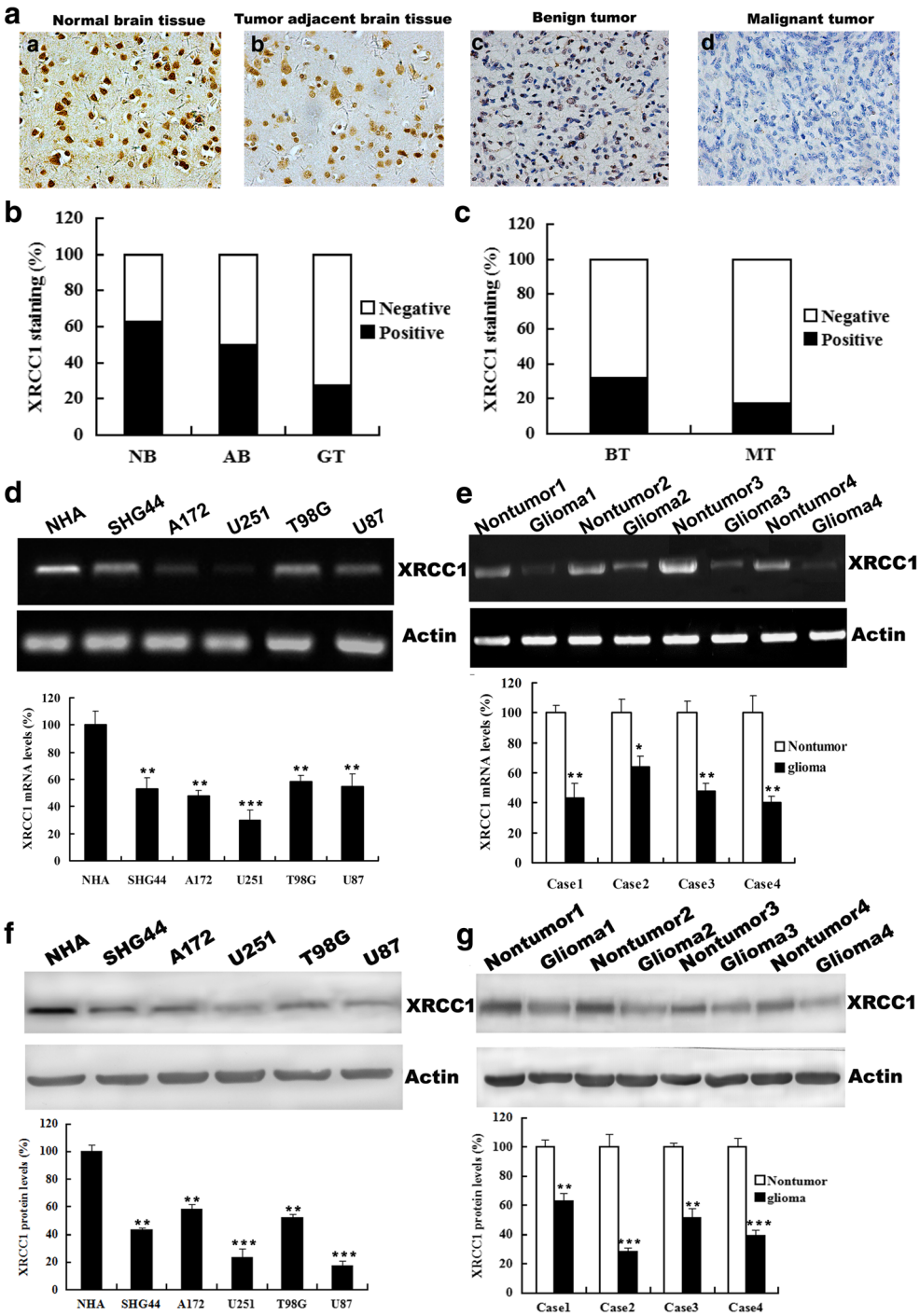

Fig. 1 Expression of XRCC1 in human glioma samples and glioma cell lines. A Representative images depict XRCC1 immunohistochemical staining. **a** Positive XRCC1 staining in normal brain tissue (NB); **b** Positive XRCC1 staining in adjacent normal brain tissue (AB); **c** Negative XRCC1 staining in benign tumor (BT); **d** Negative XRCC1 staining in malignant tumor (MT). B A significant difference in XRCC1 staining was observed between normal brain tissue and glioma tissue (GT) ($P < 0.01$, χ^2 test) and between tumor adjacent normal brain tissue and glioma tumor ($P < 0.01$, χ^2 test). C XRCC1 staining was dramatically decreased in malignant tumor compared with benign tumor ($P < 0.05$). D RT-PCR analysis of XRCC1 expression in normal human astrocytes NHA and glioma cell lines, including SHG44, A172, U251, T98G, U87. E Representative RT-PCR analysis of total. RNA extracted from human glioma tissues and glioma adjacent nontumorous. Actin served as the internal control. F Western blot analysis of XRCC1 expression in normal human astrocytes NHA and glioma cell lines, including SHG44, A172, U251, T98G, U87. G Whole-cell protein extracts were further prepared from four paired tumor adjacent normal glioma tissues and glioma tissues. The XRCC1 protein level was determined by Western blot analysis.. All experiments were carried out in triplicate. Data are shown as mean ± SD. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Original magnification (a-d) × 400

Results

XRCC1 expression is downregulated in glioma tissues and glioma cell lines

In order to investigate whether XRCC1 expression is changed in glioma, we utilized a TMA to evaluate the XRCC1 expression in normal brain tissue, tumor adjacent normal brain tissue, benign tumor (Grade I and II) and malignant (Grade III and IV). The representative pictures presented in Fig. 1a showed that XRCC1 protein mainly localized in nuclei was stained in brown. XRCC1 positive staining was observed in 5 of 8 (62.5%) normal brain tissue, 4 of 8 (50%) tumor adjacent normal brain tissue, 48 of 192 (25%) glioma tissue (Fig. 1b). A significant difference in XRCC1 staining was observed between normal brain tissue and glioma tissue ($P < 0.01$, χ^2 test)

Table 1 XRCC1staining and clinicopathological characteristics of 190 glioma patients

Variables	XRCC1 staining			
	Negative, No. (%)	Positive, No. (%)	Total	*P* *
Age				
< 45 years	68(75.6%)	22(24.4%)	90	0.260
≥ 45 years	72(72.0%)	28(28.0%)	100	
Gender				
Male	89(78.1%)	25(21.9%)	114	0.721
Female	55(72.4%)	21(27.6%)	76	
WHO Grade				
Benign(I-II)	90(68.2%)	42(31.8%)	132	0.046
Malignant(III-IV)	48(82.8%)	10(17.2%)	58	
Histologic type				
Astrocytoma	102(75.6%)	33(24.4%)	135	0.704
Glioblastoma	24(77.4%)	7(22.6%)	31	
Oligoastrocytoma	12(75%)	4(25%)	16	
Ependymoma	6(75%)	2(25%)	8	

* χ^2 test

and between tumor adjacent normal brain tissue and glioma tissue ($P < 0.01$, χ^2 test). To further confirm these observations, RT-PCR and Western blot assay was done using four glioma tissues and paired non-tumor tissues. It was clear that the glioma tissue had a drastic decrease of XRCC1 expression as compared with the non-tumor tissues (Fig. 1e, g), which was consistent with the level of XRCC1 protein expression determined by immunohistochemical staining. In addition, RT-PCR and Western blot analyses showed that expression of XRCC1 was markedly lower in all 5 analyzed glioma cell lines, including SHG44, A172, U251, T98G, U87, as compared with that in normal human astrocytes (NHA) (Fig. 1d, f). Collectively, our results suggest that XRCC1 is downregulated in gliomas.

XRCC1 expression is correlated with clinicopathological parameters

The clinicopathologic features of 192 glioma biopsies were summarized in Table 1. WHO grade and histologic type are known to be important prognostic markers for patients with glioma. We studied whether XRCC1 expression correlates with these markers. We found XRCC1 positive staining in 39 of 134 (29.1%) benign tumor and 9 of 58 (15.5%) malignant tumor. Therefore, XRCC1 staining was dramatically decreased in WHO stages III-IV compared with stages I-II ($P < 0.05$, χ^2 test, Fig. 1c). However, we did not find significant correlations between XRCC1 expression and histologic type (Table 1). There is also no significant correlations between XRCC1 expression with other clinicopathologic variables, including patient age and gender (Table 1).

XRCC1 overexpression inhibits glioma cells proliferation and cell cycle

To investigate the biological role of XRCC1 in glioma progression, we first transiently transfected pFlag-XRCC1 into U251 and U87 glioma cells. 24 h after transfection, cells were harvested for western blot or subjected to cell proliferation. Western blot results confirmed significant increase of XRCC1 in either U251 or U87 cells transfected with pFlag-XRCC1 (Fig. 2a). CCK-8 cell proliferation assays revealed that cell growth was reduced in XRCC1 overexpressed U251 and U87 cells compared with control cells (Fig. 2b and c).

To determine if the reduced cell proliferation of XRCC1 overexpressed cells is due to cell cycle arrest, we performed flow cytometry analysis. The results showed that overexpression of XRCC1 in either U251 or U87 cells resulted in an increase of cell population at G1 phase (Fig. 2d and e). Moreover, immunoblot analysis showed increased p16 expression but decreased levels of cyclin D1 in glioma U251 and U87 cells that overexpression of XRCC1 (Fig. 2f, g).

XRCC1 overexpression inhibits glioma cells migration, invasion and MMPs activity

The association of XRCC1 expression with progression of glioma I-II to III-IV prompted us to verify the inhibitory role of XRCC1 on glioma cell migration and invasion. First, we investigated the role of XRCC1 in migration of glioma cells by wound-healing assay and migration assay. We found that there was significant delay in wound closure after XRCC1 re-expression compared with pFlag-control transfected group

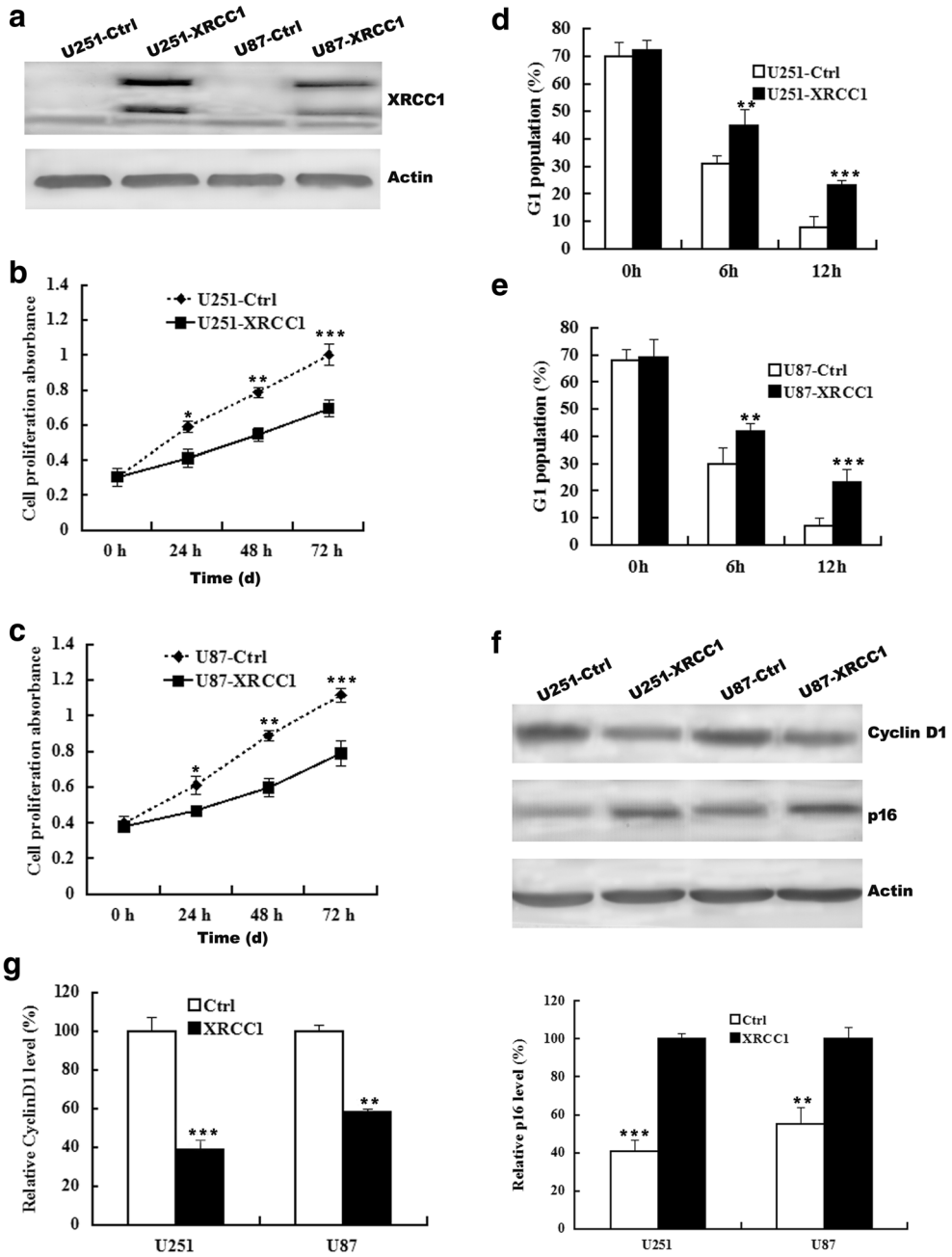

Fig. 2 Overexpression of XRCC1 reduces glioma cell proliferation in vitro. **a** 24 h after transfection, the expression of XRCC1 in U251 and U87 glioma cells was evaluated by western blot. **b**, C CCK-8 assays revealed that upregulation of XRCC1 reduced cell proliferation of U251 and U87 glioma cells, compared to negative (NC)-transfected cells. **d**, **e** overexpression of XRCC1 in U251 and U87 cells resulted in an increase of cell population at G1 phase by flow cytometry analysis. F Western blot analysis of the relative protein of cyclin D1 and p16 in XRCC1 overexpression and NC group of U251 and U87 cells. β-Action was used as a whole cell protein loading control. G Quantitative analysis of relative protein level of cyclin D1, and p16 in glioma U251 and U87 cells. All experiments were carried out in triplicate. Data are shown as mean ± SE. $^{**}P < 0.01$; $^{***}P < 0.001$

(Fig. 3a, b). In addition, in cell migration assay, our results showed that U251-XRCC1 cells and U87-XRCC1 cells decreased the ability to migrate through Boyden chamber by 65 and 54%, respectively, compared with the control cells (Fig. 3c, d). In cell invasion assay, XRCC1 overexpression inhibits cell invasive ability of U251 and U87 cells in matrigel-coated Boyden chamber by 59 and 43%, respective (Fig. 4a, b).

Since MMPs play a crucial role in cell migration and invasion, we then carried out the zymography assay to compare the activity of MMPs in XRCC1-overexpressing and control cells. As shown in Fig. 4c, MMP-2 gelatinolytic activity was dramatically decreased in XRCC1-overexpressing U251 and U87 cells compared with the control cells, respectively. Then, we performed western blot to examine the MMP-2 and TIMP-2 expression in glioma cells. Western blot results showed that inhibition of MMP-2 is correlated to increased expression of TIMP-2 in U251 and U87 cells increasing XRCC1 (Fig. 4d, e).

Expression of XRCC1 in glioma cells inhibited growth and tube formation of human umbilical vein endothelial cells and VEGF activity

To further determine the effect of XRCC1 overexpression on angiogenic potential of human glioma cells, the

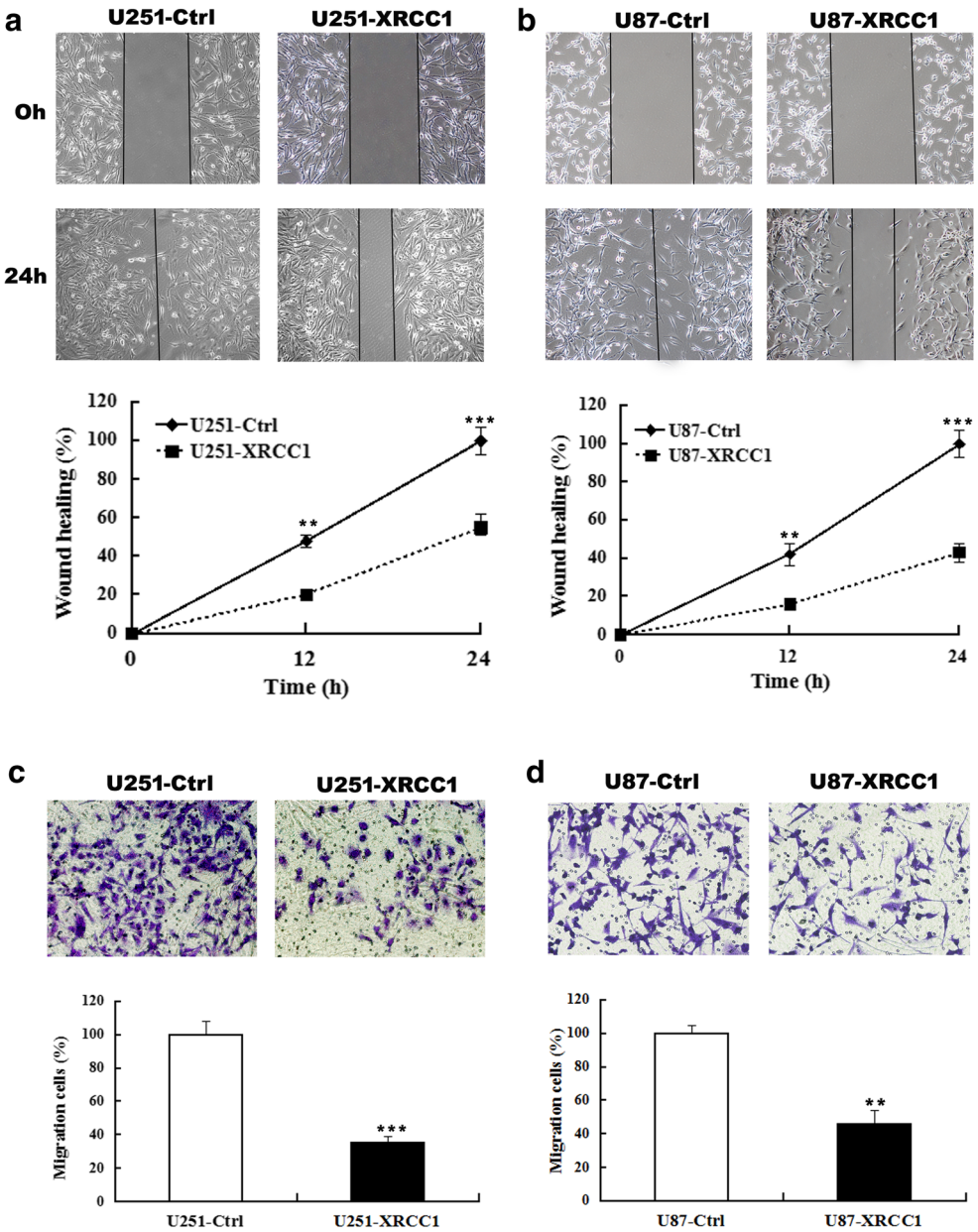

Fig. 3 Overexpression of XRCC1 inhibits glioma cell migration. **a**, **b** Wound-healing assay was done on monolayers of U251and U87 glioma cells after 36 h of transfection. The photographs were taken at 0, 12 and 24 h after wounds were made. **c**, **d** Cell migration assay was performed after XRCC1 restoration in glioma U251 and U87 cells. All experiments were carried out in triplicate. Data are shown as mean ± SD. $^{**}P < 0.01$; $^{***}P < 0.001$

angiogenic potential of the supernatant of U251 and U87 cells transfected with pFlag-control or pFlag-XRCC1 were determined by human umbilical vein endothelial cells (HUVECs) growth assay and tube formation assay. The growth of HUVECs in conditioned medium from XRCC1-overexpression U251 and U87 cells was inhibited by 62 and 73%, respectively, when compared with the corresponding negative control (Fig. 5a and b). The average number of complete tubular structures formed by HUVECs was significantly decreased by 72 and 78% in conditioned medium from XRCC1-overexpressing U251 and U87 compared with negative control cells, respectively (Fig. 5c and d).

To investigate the mechanism of XRCC1 regulating angiogenesis, we performed western blot and ELISA to detect the VEGF levels in glioma cells. Our data showed that overexpression of XRCC1 was dramatically reduced VEGF protein level in U251 and U87 cells (Fig. 5e). A significant inhibition in VEGF secretion was observed in conditioned medium from U251 and U87 cells after overexpression of XRCC1 (Fig.5f).

Discussion

Cancer develops through interactions between polygenic and environmental factors, and changes in DNA repair pathway

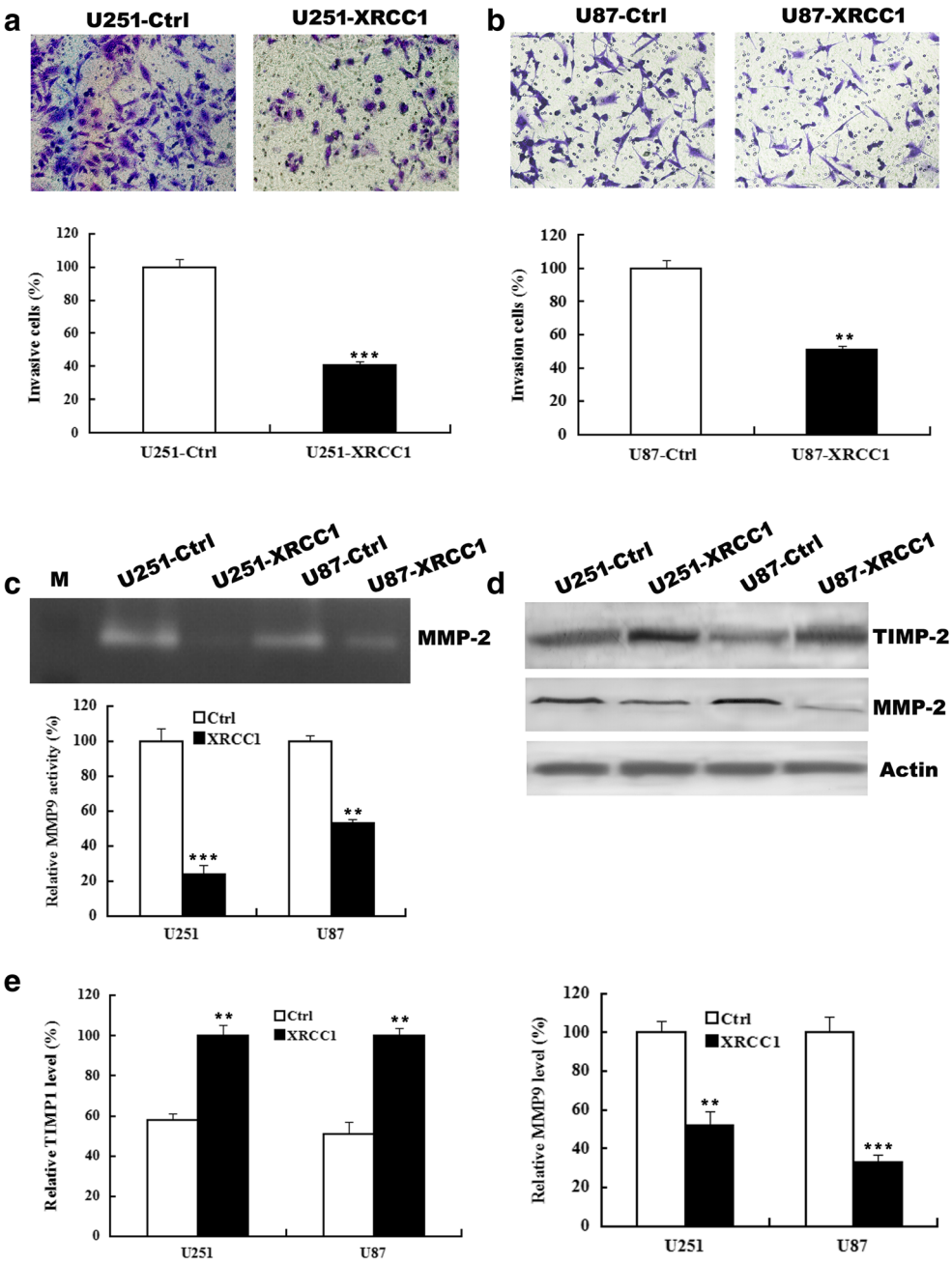

Fig. 4 Effect of XRCC1 overexpression on cell invasion. **a**, **b** Reintroduction of XRCC1 inhibited the invasion ability of U251 and U87 cell by matrigel cell invasion assay. **c** Gelatin zymography analysis of the relative enzyme activities of MMP-2 in XRCC1 overexpression and NC group for both U251 and U87 cell lines. **d** Western blot analysis of the relative protein levels of TIMP-2 and MMP-2 in XRCC1 overexpression and NC group of U251 and U87 cells. **e** Quantitative analysis of relative protein level of TIMP-2, and MMP-2 in glioma U251 and U87 cells. All experiments were carried out in triplicate. Data are shown as mean ± SE. $^{**}P < 0.01$; $^{***}P < 0.001$

can increase susceptibility to tumours [18]. X-ray repair cross-complementing group 1 (XRCC1) is located on chromosome 19q13.2–13.3 and is 33 kb in length, encoding a scaffolding protein in BER pathway that functions in the repair of single-strand breaks, which is the most common lesion of cellular DNA injury [19]. The original studies showed that the cells lacking XRCC1 were sensitive to ionizing radiation, hydrogen peroxide, camptothecin, and alkylating agents [20–23]. The current studies of XRCC1 mainly focused on the relationship between gene polymorphisms and cancer susceptibility. Several studies have reported the association of XRCC1 polymorphisms with the risk in non-small-cell lung cancer (NSCLC) [24], colorectal cancer [25], gastric cancer [26] and prostate cancer [27]. In recently years, XRCC1 gene is considered as an important candidate gene for influencing the susceptibility to glioma, and several association studies have been conducted to evaluate the role of Arg194Trp, Arg280His, and Arg399Gln genetic polymorphisms on the susceptibility to glioma [28, 29]. Though there are many studies reporting on the XRCC1 polymorphisms in cancer, relatively fewer studies have reported on the XRCC1 expression. In this study, our data first demonstrated that XRCC1 was significantly decreased in glioma compared with tumor adjacent normal brain tissue by TMA and Immunohistochemical

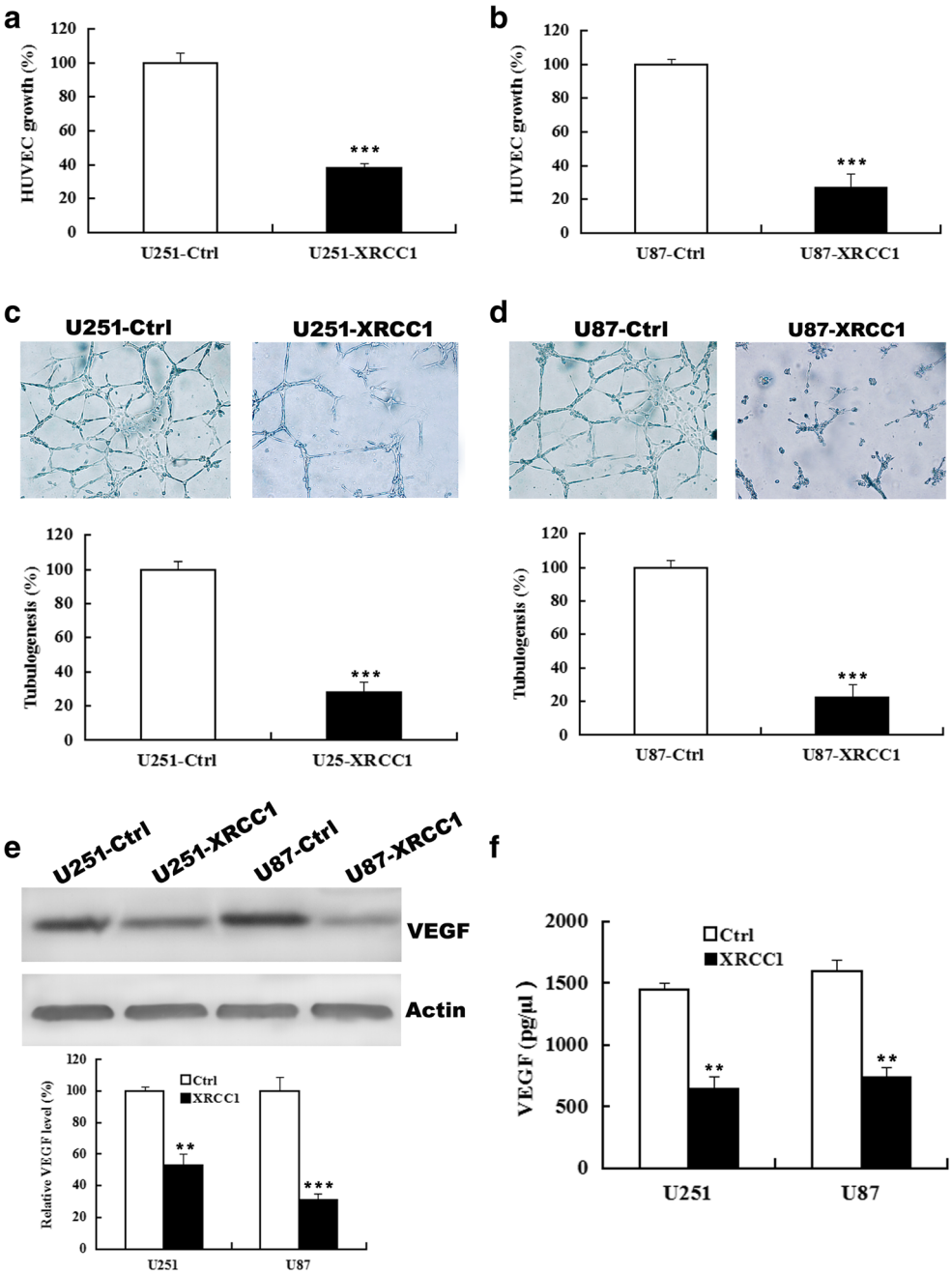

Fig. 5 Reduction of angiogenesis in U251 and U87 cells by restoration of XRCC1 expression. **a**, **b** CCK-8 cell proliferation assay was performed to detect the HUVECs proliferation. **c**, **d** representative pictures were taken in situ for tube formation in the supernatant of U251 and U87 cells. The degree of tube formation was assessed as the percentage of cell surface area versus total surface area. **e** Western blot analysis of the relative protein levels of VEGF in XRCC1 overexpression and NC group of U251 and U87 cells. **f** The secretion of VEGF was determined by ELISA assay. All experiments were carried out in triplicate. Data are shown as mean ± SE. $***P < 0.001$

technique (Fig. 1a, b). In addition, XRCC1 expression is downregulated in high grade (III-IV) glioma in comparison to low grade (I-II) glioma (Fig. 1c). To further confirm these observations, RT-PCR and Western blot analyses showed that expression of XRCC1 was markedly lower in 4 clinical glioma tissues and all 5 glioma cell lines, (Fig. 1d-g). Consistent with previous studies, XRCC1 has been reported to be downregulated in a variety of different tumor types including melanoma [30], pancreatic adenocarcinoma [31], renal cell carcinoma [32], bladder cancer [33], and gastric cancer [34]. However, our results are in contrast to the results from ovarian cancer [35] and head and neck squamous cell cancer [36]. We think that the cancer pathogenesis is different for different kinds of cancer and this could be the reason why XRCC1 acted as an oncogene in ovarian Cancer and head and neck cancer.

Disturbed regulation of cell cycle is the essential mechanism on abnormal proliferation of cell and incidence of cancer [37]. The development of a tumor requires both the activation of oncogenes and the inactivation of tumor suppressor genes, which triggers the uncontrolled proliferation of cancer cells. We found that the ability of cell proliferation was drastically decreased after XRCC1 overexpression in glioma cells, which is due to inhibition of cell cycle progression by arresting cell

cycle at G1 Phase [37]. Cell cycle progression is strictly controlled by cyclins and cyclin-dependent kinase (CDK) inhibitors [38]. Cyclin inhibitors such as p16 plays an important role in cell cycleregulation by decelerating cells progression from G1 phase to S phase, and therefore acts as a tumor suppressor that is implicated in the prevention of cancers, including glioma [39]. We found that overexpression of XRCC1 increased p16 expression and decreased cyclin D1 expression, which may result in glioma cells to arrest at G1 phase (Fig. 2d-g). This might be due to the elevated expression of p16 which inhibits the expression of cyclin D1. Our finding is contrast with Bhandaru et al. [30] and Liu et al. [32] who found that XRCC1 did not influence the melanoma and renal cell carcinoma cell cycle.

Previous research found XRCC1 mainly involved in DNA repair and thus it was presumed to be involved in the initial stages of cancer development. Our TMA results illustrated the importance of XRCC1 in later stages, especially in advancement from WHO stages I-II to stages III-IV. In vitro assay, our studies found that overexpression of XRCC1 significantly inhibited the wound healing abilities of gliomas cells. Simultaneously, in the migration and invasion assays of gliomas cells, we also found that XRCC1 remarkably reduced the capabilities of gliomas cell migration and invasion. As we all know, glioblastoma cells not only are highly proliferative but also readily invade surrounding brain structures. This is a multistep process that involves the detachment from the primary tumor mass, migration through the extracellular matrix (ECM) and colonization of surrounding sites [40]. Matrix metalloproteinases (MMPs) play a critical role in tumor invasion by cleaving the ECM components. TIMP-2 is a main negative regulator of MMP-2 enzyme activity and involved in several tumor metastasis processes, including glioma [41]. Hence, we checked the expressions of MMP-2 and TIMP-2 in gliomas cells with XRCC1 over-expression. Our results indicate that XRCC1 could negatively regulate MMP-2 expression and enzyme activity, whereas it was positively related to the expression of TIMP-2, which also validated the findings that MMP-2 and TIMP-2 have been proved to perform an important role in gliomas aggression. This result is in agreement with the report by Bhandaru et al. [30] demonstrating that silencing of XRCC1 enhances MMP2 activity in melanoma cells. However, it remains to be elucidated how XRCC1 regulates MMP-2 expression and activity and its signal pathway to regulate glioma cell invasion.

Angiogenesis is an important basis for the growth of solid tumors, including gliomas. Glioma cells clearly need the vasculature for the delivery of nutrients and oxygen for tumor growth. High grade gliomas show two types of infiltration into the normal brain [42]. The first is a diffuse infiltrative migration of single cells into the brain parenchyma. The second type is a clear perivascular migration along the microvasculature. Brain tumor infiltration has been shown to be one of the main reasons for tumor recurrence [43]. These infiltrated cells cannot be resected by surgery and are known to be resistant to current chemo- and radiotherapies. Malignant gliomas exhibit striking angiogenesis with elevated expression of vascular endothelial growth factors (VEGFs), which promote blood vessel formation by endothelial precursors [44]. VEGFs are secreted or cell surface–bound proteins expressed by tumor cells that bind to specific high-affinity transmembrane receptors primarily expressed on endothelial cells to promote endothelial cell proliferation, migration, and survival [45]. In the present study, we found that XRCC1 inhibited HUVECs growth and tube formation in vitro (Fig. 5a-d). Then, we detected the expression and secretion of VEGF after XRCC1 overexpression. We found that VEGF expression and secretion was decreased by restoration of XRCC1 (Fig. 5e, f). These results suggested that XRCC1 suppresses blood vessel formation by regulating VEGF secretion.

Above all, we have shown the association of XRCC1 expression with glioma progression and demonstrated the inhibitory role of XRCC1 on glioma cell proliferation, migration, invasion and angiogenesis. Our results imply that targeting of the XRCC1 pathway may constitute a potential treatment modality for glioma.

Funding This project is supported by grants from the National Natural Science Foundation of China (No.81502160), and Jiangsu Provincial Medical Youth Talent(No.QNRC2016785), and The Project of Invigorating Health Care through Science, Technology and Education (No.CXTDA2017034).

Compliance with ethical standards

Conflict of interest The authors have declared that no competing interests exist.

Ethical approval This study was performed under a protocol approved by the Institutional Review Boards of The Affiliated Hospital of Xuzhou Medical University.

Informed consent Informed consent was obtained from all individual participants included in the study.

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