Silencing of FOXG1 confers radio-sensitivity through regulating autophagy in glioma cells

Wenjun Liao
West China Hospital, Sichuan University

Churong Li (crlbiology@yeah.net)
Sichuan Cancer Hospital Institute, Sichuan Cancer Center, School of Medicine, University of Electronic Science and Techology of China, Chengdu, Sichuan 611130 https://orcid.org/0000-0002-4361-1704

Jun Yin
Sichuan Cancer Hospital Institute, Sichuan Cancer Center, School of Medicine, University of Electronic Science and Technology of China.

Hui Huang
Department of Cancer Centerm Sichuan Provincial People's Hospital, Chengdu.

Baisen Li
Sichuan Cancer Hospital Institute, Sichuan Cancer Centerm School of Medicine, University of Electronic Science and Technology of China,Chengdu.

Shichuan Zhang
Sichuan Cancer Hospital Institute, Sichuan Cancer Center, School of Medicine, University of Electronic Science and Technology of China, Chengdu.

Peng Zhang
Sichuan Cancer Hospital Institute, Sichuan Cancer Center, School of Medicine, University of Electronic Science and Technologu of China, Chengdu.

Chuan Yang
Sichuan Cancer Hospital Institute, Sichuan Cancer Center, School of Medicine, University of Electronic Science and Technology of China, Chengdu

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Abstract

Background/Aim forkhead box G1 (FOXG1) has recently been observed in many cancers, while its effect on radio-sensitivity in glioma is still unclear. In this study, we hypothesized that FOXG1 be a major players in radio-resistance of glioma as well as the underlying mechanism.

Methods Immunohistochemistry (IHC) was conducted to assess FOXG1 expression in the glioma tissues and glioma-adjacent tissues. Western Blot was applied to detect the expression of autophagy-related proteins. CCK-8 and flow cytometry assays were applied to assess proliferation and apoptosis, respectively.

Results The present study demonstrated that FOXG1 was highly expressed in glioma tissues. FOXG1 silencing enhances the effect of X-ray irradiation on proliferation inhibition and apoptosis of glioma cells, while FOXG1 overexpression has the opposite effect. Interestingly, the chloroquine (CQ) of autophagy inhibitor enhanced X-ray irradiation induces proliferation inhibition and apoptosis in glioma cells.

Conclusions The present study suggests that FOXG1 is a pivotal molecule for circumventing radiation-induced cell death in malignant glioma cells through the regulation of autophagy and provide a target for the treatment of human brain glioma.

Introduction

Glioma, also known as brain glioma, accounts for 50–55% of primary nervous system tumors and is an aggressive form of brain cancer [1]. Despite therapeutic advances in diagnosis and clinical treatment, the prognosis remains poor and the median survival time after diagnosis of malignant glioma is less than 15 months [2].

Surgery, radiotherapy or chemotherapy is introduced when oncology is certified. Due to its anatomical position, glioma can be difficult to completely remove; therefore, postoperative radiotherapy and chemotherapy are necessary. Radiotherapy is usually conducted for high-grade glioma patients once surgeries had been introduced to these patients and it is also appropriate for some patients who are not eligible for surgeries [3]. Study has shown that the patients who received postoperative radiotherapy had markedly improved 5-year overall and progression-free survival rates [4]. However, gliomas exhibit resistance to radiotherapy; thus, strategies to effectively improve the radio-sensitivity of tumors and increase the postoperative survival rates of patients are of prominent focus in radiation oncology.

Forkhead box G1 (FOXG1), also known as brain factor 1 is an important member of the forkhead box transcription factor family, which is involved in multiple developmental processes, including cell signal transduction, proliferation, differentiation, cell cycle regulation and apoptosis [5]. FOXG1 is upregulated in numerous types of malignant tumor, including glioma, and the expression levels have been positively associated with the progression of glioma [6]. A recent study reported that FOXG1 gene-silencing not only inhibited the invasion and metastasis of colorectal carcinoma cells [7], but also suppressed the

vascularization of hepatoma cells [8]. However, to the best of our knowledge, the effects of the FOXG1 gene on the radio-sensitivity of glioma have not yet been reported.

Autophagy is an important process for cells to degrade damaged organelles and non-functional proteins through lysosomes, which with highly evolutionarily conservative and can cope with various stress responses, including hypoxia, nutrient deficiency and drug induction, played an vital role in cell self-renewal and the maintenance of homeostasis of internal environment [9]. The strength of autophagy determines the survival and death of tumor cells. Moderate autophagy can enhance the ability of tumor cells to cope with external stimuli and promote the survival of tumor cells, while excessive autophagy can lead to tumor cell death [10]. Thus, our study aimed to evaluate whether autophagy involved in FOXG1 gene silencing induced radio-sensitivity in glioma cells.

Materials And Methods

Reagents

Chloroquine (CQ) was purchased from Beijing Solarbio Science & Technology Co., Ltd, which was used at a concentration of 10 μM. Additional reagents employed in the present study were commercially available and of analytical purity.

Tissue samples

Glioma tissues from 19 patients (the age ranged from 23 to 72 years old, with 11 males and 8 females) and glioma-adjacent tissues from 5 cases (the age ranged from 24 to 67 years old, with 3 males and 2 females) were obtained from patients who underwent surgery at Sichuan Cancer Hospital (Chengdu, China) from May 2016 to May 2017. For all patients, the original diagnosis and tumor grading were conducted in a blinded manner by 2 experienced pathologists according to the World Health Organization (WHO) classification system. The inclusion criteria were as follows: i) Patients were diagnosed with glioma at first admission; ii) patients were cooperative; and iii) tumors were classified as grade III-IV. Patients that had received medication or chemotherapy prior to enrollment were excluded. The present study was approved by the Ethics Review Board of The University of Electronic Science and Technology of China (Chengdu, China), and all patients provided written informed consent to participate.

Cell culture and transfection

The glioblastoma cell line U87 (CL-0238) and TG-905 (CL-0309) were purchased from Procell Life Science & Technology Co., Ltd. U87 cells were cultured with Minimum Essential medium (MEM) containing 10% FBS (Gibco; Thermo Fisher Scientific, Inc.) and incubated at 37˚C, 5% CO_2. TG-905 cells were cultured in RPMI-1640 medium supplemented with 10% FBS and 1% penicillin and streptomycin.

Small interfering (si) RNA against FOXG1 (si- FOXG1), negative control siRNA (si-NC), pcDNA-FOXG1 and pcDNA-NC were purchased from Guangzhou RiboBio Co., Ltd. Cells were seeded into a 6-well plate at a

density of $1x10^5$ cells/ well or seeded into a 96-well plate at a density of $7x10^3$ cells/ well allowed to reach 50-60% confluence. Cell transfection was performed using Lipofectamine®2000 (Invitrogen, Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. After transfection for 6 h, fresh medium was replaced. After transfection for 24 h, cells were collected for RT-qPCR or western blotting analyses. The siRNA sequences are presented in Table I.

Immunohistochemistry

Glioma tissue samples were fixed with 4% paraformaldehyde for 48-72 h at room temperature, dehydrated, and embedded in paraffin. The paraffin-embedded tissue samples were cut into 4 μm slices, deparaffinized with xylene and dehydrated using a descending alcohol gradient. Subsequently, sections were soaked for 15 min in 3% hydrogen peroxide at room temperature, and blocked with 5% bovine serum albumin (BSA; Sigma-Aldrich, Merck KGaA, St. Louis, MO, USA) at 37°C for 30 min. The sections were incubated with primary anti-FOXG1 antibody (1:100; ab196868; Abcam, Cambridge, UK) at 4˚C overnight. After incubation, sections were warmed for 30 min at 37˚C and then incubated with biotinylated goat anti-rabbit IgG (BA1003; Wuhan Boster Biological Technology, Ltd., Wuhan, China) for 30 min at 37˚C. The sections were then stained with strept avidin biotin-peroxidase complex (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) at 37˚C for 30 min, and then stained with 3, 3'-diaminobenzidine for 5 min at room temperature. The reaction was terminated with tap water when the cytoplasm was colored yellow-brown. The sections were then counterstained with hematoxylin at room temperature for 2-5 min. PBS was used as a substitute for the primary antibody as a negative control. A Nikon computer image system (Nikon Corporation) was used for image collection (magnification, x400). A total of 10 fields in each sample were randomly selected, and Image ProPlus software (Media Cybernetics, Inc.) was used to determine the average intensity of positive signals.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assay

Total RNA was extracted from U87 and TG-905 cells using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.). The PrimeScript™ RT reagent kit (Takara Biotechnology Co., Ltd, Dalian, China) was used to perform reverse transcription. qPCR was performed using SYBR® Premix Ex Taq™ (Takara Bio, Inc.). The thermocycling conditions were as follows: 3 min at 95˚C; 40 cycles between 95˚C for 5 sec and 55˚C for 30 sec; 72˚C for 30 sec. The relative expressions of FOXG1 were calculated using the $2^{-\Delta\Delta CT}$ method and normalized to the housekeeping gene β-actin [11].The data were analyzed using Bio-Rad CFX Manager software (Bio-Rad Laboratories, Inc.). The primer sequences (Sangon Biotech Co., Ltd.) were as follows: FOXG1 forward, 5'-GGCTCACGCTCAACGGCATCTACGA-3', and reverse, 5'-GCGGCACCTTCACGAAGCACTTGTT-3'; β-actin forward, 5'-GAAGATCAAGATCATTGCTCCT-3', and reverse, 5'-TACTCCTGCTTGCTGATCCA-3'.

Western blot analysis

24 h after transfection, harvested cells were collected and total protein was extracted using RIPA lysis buffer (Wuhan Boster Biological Technology, Ltd.).The protein was quantified with a BCA Protein Assay

kit (Wuhan Boster Biological Technology, Ltd.). Equal amounts of each protein sample (20 µg) were separated using SDS-PAGE on a 10% gel, and transferred onto polyvinylidene fluoride membranes using the Bio-Rad II System (Bio-Rad Laboratories, Inc.). The membranes were blocked with 5% skimmed milk powder for 1 h at room temperature, and subsequently incubated with primary antibodies against β-actin and FOXG1 (1:1,000; ab196868; Abcam) at 4˚C overnight. The membranes were then washed using TBS with Tween-20 (TBS-T) prior to incubation with a goat anti-rabbit IgG, HRP-linked secondary antibody (1:5,000; BA1054; Wuhan Boster Biological Technology, Ltd.) at room temperature for 1 h. The membranes were washed with TBS-T once more and visualized using an enhanced chemiluminescence kit (Wuhan Boster Biological Technology, Ltd.). Images were captured using the ChemiDoc™ MP imaging system (Bio-Rad, Laboratories, Inc.) and quantified using Image-ProPlus 6.0 software (Media Cybernetics, Inc., Rockville, MD, USA), with β-actin as an internal loading control.

Cell Counting Kit-8 (CCK-8) assay

After 6 h post-transfection, U87 or TG-905 cells ($7x10^3$/well) were treated with different doses of X-ray radiation (0, 2, 4, 6 and 8 Gy) and cultured for a further 24 h. Subsequently, the culture medium was replaced with fresh medium mixed with the CCK-8 reagent (Dojindo Molecular Technologies, Inc.) at a ratio of 10:1, of which 100 µl was added to each well. The cells were incubated for an additional hour at 37˚C, and the optical density was determined using a microplate reader at 450 nm (Thermo Fisher Scientific, Inc.).

Annexin-V/propidium iodide (PI) double-staining assay

After transfection for 6 h, U87 or TG-905 cells were subjected to different doses of X-ray radiation (0, 2, 4, 6 and 8 Gy), cultured for 24 h and collected. Briefly, the collected cells were washed with PBS and digested with trypsin, and then gently resuspended in 100 µl binding buffer ($1x10^5$ cells) with Annexin V-fluorescein isothiocyanate (5 µl) and PI staining solution (5 µl). The cells were gently mixed, incubated at room temperature for 15 min and apoptotic cells were detected using a FACSCalibur flow cytometer (BD Biosciences) within 1 h. The data were analyzed using FlowJo 10.07 software (Tree star, Ashland).

Statistical analysis

SPSS analysis software version 19.0 (IBM Corp., Armonk, NY, USA) was used for data processing and statistical analysis. All data are presented as the mean ± standard deviation from three separate experiments. Differences among multiple groups were compared by one-way analysis of variance (ANOVA) with Dunnett's post hoc test or two-way ANOVA with Bonferroni's post hoc test, and differences between two groups were compared by the Student's t-test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

FOXG1 protein is increased in glioma tissues

To investigate the potential dysregulation of FOXG1 in glioma, the endogenous expression level of FOXG1 were detected by immunohistochemistry in glioma tissues. As shown in Fig 1, FOXG1 expression was significantly higher in glioma tissues compared with in glioma-adjacent tissues, indicating that FOXG1 may play a vital role in glioma tumorigenesis and development.

FOXG1 attenuates the effect of X-ray irradiation on proliferation inhibition of glioma cells

To further investigate the biological functions of FOXG1 in glioma, U87 and TG-905 cells were transfected with si-FOXG1 sequence or pcDNA-FOXG1 plasmid. RT-qPCR and western blot results revealed that FOXG1 expression was significantly decreased following transfection with si-FOXG1 sequence, while significantly increased following transfection with pcDNA-FOXG1 plasmid, suggesting high transfection efficiency in U87 and TG-905 cells (Fig 2). CCK-8 assay was performed to determine the proliferation of U87 and TG-905 cells. It was revealed that FOXG1 silencing was significantly enhanced the effect of X-ray irradiation on proliferation inhibition whereas FOXG1 overexpression was significantly attenuates the inhibitory effect of X-ray irradiation on proliferation of U87 and TG-905 cells (Fig 3A and B). These results indicated that FOXG1 may act as an oncogene to regulate the progression of glioma cells.

FOXG1 attenuates the effect of X-ray irradiation on apoptosis of glioma cells

Annexin-V/PI double-staining assay was performed to determine the apoptosis of U87 MG and TG-905 cells. It was revealed that FOXG1 silencing was significantly enhanced the effect of X-ray irradiation on apoptosis of U87 cells, whereas FOXG1 overexpression was significantly attenuates the effect of X-ray irradiation on apoptosis of TG-905 cells (Fig 4). These results indicated that FOXG1 can effectively attenuate the effect of X-ray irradiation on apoptosis of glioma cells.

FOXG1 promotes the effect of X-ray irradiation on the expression of autophagy related proteins in glioma cells

In order to verify the role of autophagy in FOXG1 gene silencing induced radio-sensitivity of U87 cells, Western blot assay was applied to detect the expression of autophagy-related proteins. As shown in Fig 5, the expression of LC3 II and Beclin-1 were significantly increased, and the expression of LC3 I was significantly decreased in 6 Gy and 6 Gy +CQ groups compared with control group. Compared with 6 Gy group, the expression of LC3 II and Beclin-1 were significantly increased, and the expression of LC3 I was significantly decreased in 6 Gy +CQ group, indicating that X-ray irradiation treatment could induce autophagy in U87 and TG-905 cells. Compared with 6 Gy group, the expression of LC3 II and Beclin-1 was inhibited in si-FOXG1+6 Gy group, while increased in pcDNA-FOXG1+6 Gy group, indicating that the FOXG1 may increase autophagy induced by X-ray irradiation in U87 cells.

CQ enhanced radio-sensitivity in FOXG1-silenced U87 cells

As shown in Fig 6 and 7, compared with control group, cell proliferation was significantly reduced and the cell apoptotic rate was significantly increased in the other groups except CQ or pcDNA-FOXG1 groups. Compared with 6 Gy group, the cell proliferation was significantly reduced and cell apoptotic rate was

significantly increased in si-FOXG1+6 Gy and 6 Gy+ CQ groups, while the cell proliferation was significantly increased and cell apoptotic rate was significantly decreased in pcDNA-FOXG1+6 Gy group, indicating that FOXG1 silencing and autophagy inhibition could enhance the radio-sensitivity, while FOXG1 overexpression can attenuate the radio-sensitivity in glioma cells. Compared with si-FOXG1+6 Gy or pcDNA-FOXG1+6 Gy group, the cell proliferation was significantly reduced and the cell apoptotic rate was significantly increased in si-FOXG1+6 Gy+ CQ or pcDNA-FOXG1+6 Gy group, indicating that FOXG1 silencing enhanced the radio-sensitivity by inhibiting cell autophagy.

Discussion

The FOXG1 gene, located in the q12 region of chromosome 14 and encoding 489 amino-acid protein [12], is a transcription factor that plays an important role in brain development, cortex neuron differentiation and neurogenesis [13, 14]. Accumulating evidence has highlighted the role of the FOXG1 gene in a variety of malignancies. An imbalance in FOXG1 gene expression is able to influence the occurrence of medulloblastoma [15], and is also associated with the survival and invasive phenotype of a xenograft model of medulloblastoma [16]. Ji *et al* [11] reported that microRNA–378 promoted the proliferation of non-small cell lung cancer cells by inhibiting FOXG1. Inversely, FOXG1 acts as an oncoprotein inhibiting TGF-β -mediated anti-proliferative responses in ovarian cancer cells [17]. In the present study, it revealed that FOXG1 was highly expressed in glioma tissues, which was consistent with the study of Chen et al [18].

Radiotherapy is an important postoperative treatment for glioma that can prolong the survival of patients; however, there are marked differences in the radio-sensitivity of gliomas. Therefore, improving the sensitivity of glioma to radiotherapy is a key issue that requires further investigation. Chi *et al* [19] reported that the inhibition of clusterin expression increased the radio-sensitivity of patients with prostate cancer. Zhou *et al* [20] reported that RNAi silencing of the ring finger protein 8 gene enhanced the radio-sensitivity in non-small cell lung cancer. Schäfer *et al* [21] reported that low expression of the FOXG1 gene may be a crucial indicator for the prognosis of patients with glioma. The overexpression of FOXG1 resulted in increased cell viability in glioblastoma [22]. However, the effect of FOXG1 on the radio-sensitivity of glioma remains unclear. The present study revealed that FOXG1 silencing significantly enhanced the effect of X-ray irradiation on proliferation inhibition and apoptosis of glioma cells, while the overexpression of FOXG1 has the opposite effect. These results demonstrated that FOXG1 may act as an oncogene, and FOXG1 silencing could suppress glioma development, subsequently increasing radio-sensitivity.

Autophagy is another kind of programmed cell death different from apoptosis, but it is closely related to apoptosis. At present, it is generally believed that there are roughly three kinds of relationship between autophagy and apoptosis, that is, autophagy occurs before apoptosis and is necessary to initiate apoptosis; the occurrence of autophagy can inhibit apoptosis; autophagy and apoptosis can be converted to each other and jointly promote cell death [23]. Therefore, autophagy is seen as a double-edged sword in the process of tumor development. LC3 protein is currently recognized autophagy

molecular markers, from LC3 I to LC3 II shift and LC3 II accumulation can reflect the extent to which the occurrence of autophagy [24]Beclin–1 protein has also been proved to be a key protein in autophagy formation, playing an important role in regulating autophagy, and its expression level directly determines the autophagy activity of cells [25]. At present study, it revealed that X-ray irradiation promoted the LC3 II protein accumulation and beclin–1 protein expression; in addition, CQ treatment further increased the accumulation of LC3 II and the expression of beclin–1, indicating that the X-ray irradiation can induce autophagy in glioma cells. Further it revealed that the effect of X-ray irradiation on the proliferation inhibition and apoptosis of glioma cells was significantly enhanced following CQ pretreatment, suggesting that the X-ray irradiation induced autophagy had a protective effect on the cells.

In conclusion, the present study demonstrated that the sensitivity of glioma U87 cells to radiation was significantly increased following FOXG1 gene silencing. Moreover, the FOXG1 silencing inhibited the activation of autophagy induced by X-ray irradiation in glioma cells. And the inhibition of autophagy could enhance radio-sensitivity in glioma cells. These results suggested that resistance to radiation occurs in glioma may be due to the activation of autophagy, FOXG1 gene silencing and autophagy inhibition have synergistic effects on enhancing radio-sensitivity of glioma cells, which provides a new perspective for the treatment of glioma and drug development.

Declarations

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Availability of data and materials

The datasets used or analyzed during the present study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The present study was approved by the Ethics Review Board of The University of Electronic Science and Technology of China, and written informed consent was obtained from all patients.

Patient consent for publication

Written informed consent was obtained from all patients.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Wenjun Liao and Churong Li proposed the hypothesis, analyzed the results, and wrote the manuscript. Wenjun Liao and Churong Li designed and executed the majority of the experiments. Jun Yin, Hui Huang, Baisen Li, Shichuan Zhang, Peng Zhang and Chuan Yang assisted in the execution of a part of the experiments. All authors read and approved the final version of the manuscript.

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Figures

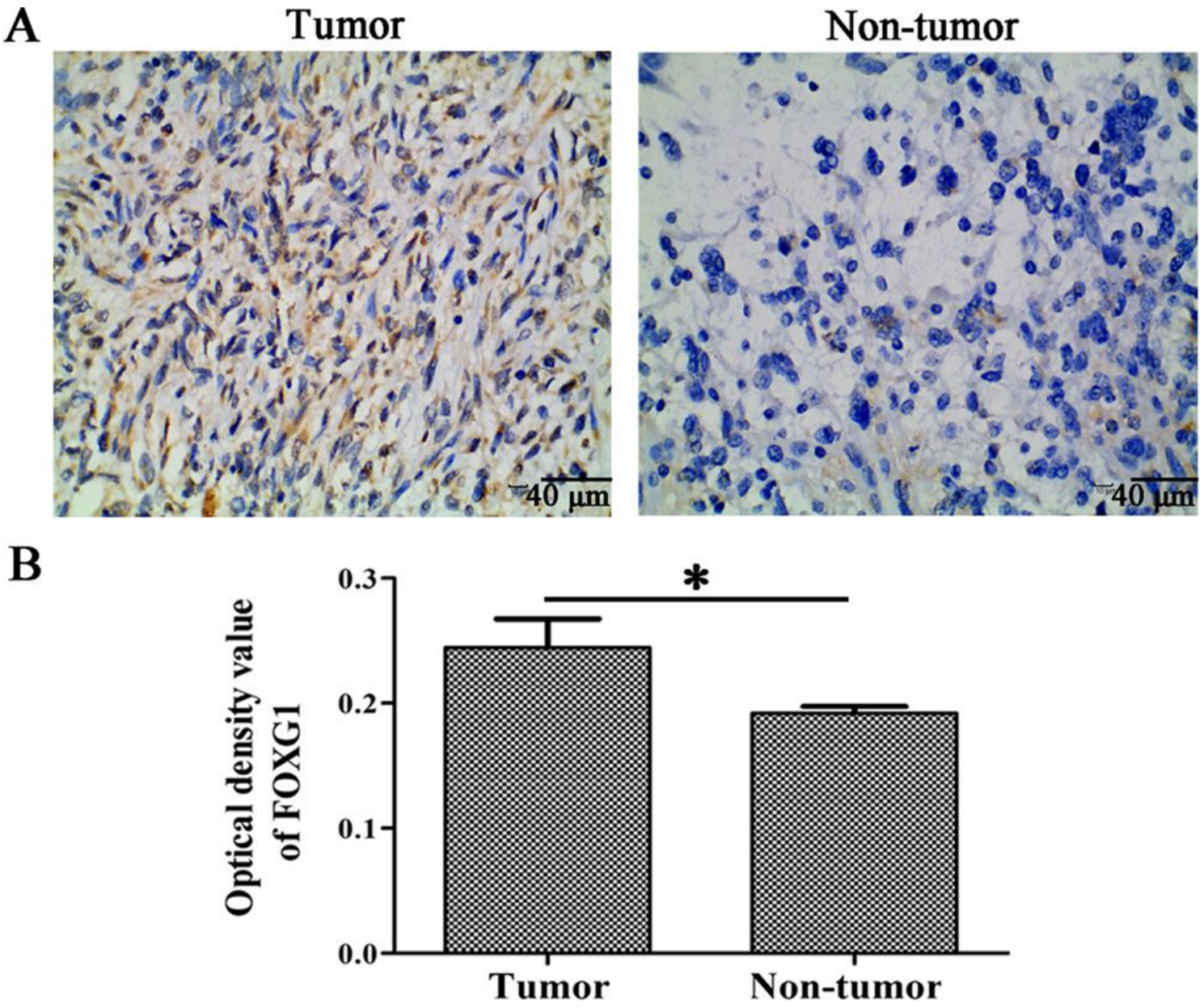

Figure 1

Expression of FOXG1 in glioma tissues. (A) Immunohistochemical staining was performed to detect the expression levels of FOXG1 in glioma tissues and adjacent tissues. (B) The expression levels of FOXG1 were statistically analyzed; tumor, n=19; non-tumor, n=5). The results are presented as the mean ± standard deviation. **P <0.01. FOXG1, forkhead box G1

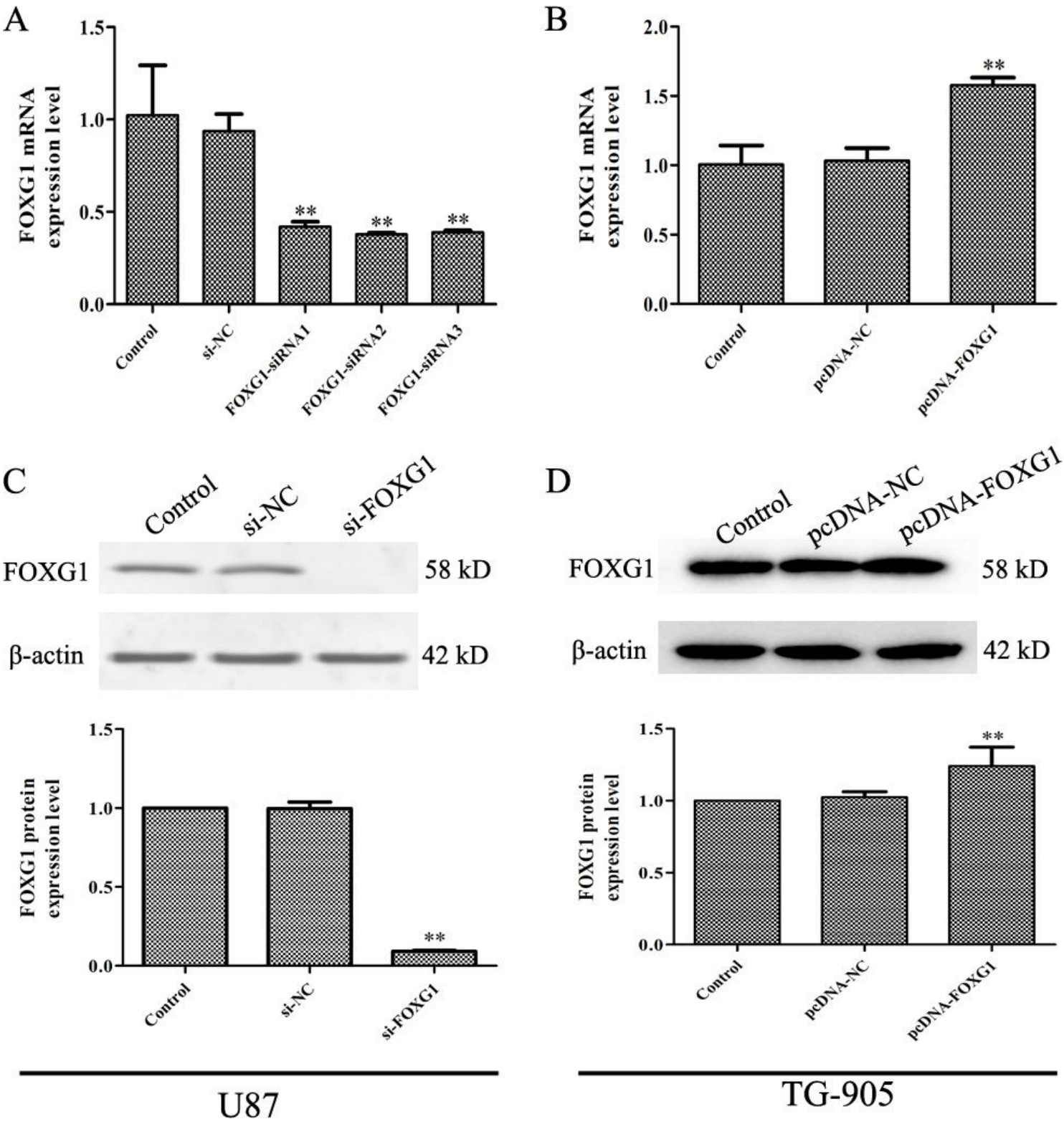

Figure 2

The effect of si-FOXG1 sequence or pcDNA-FOXG1 plasmid on the expression of FOXG1. U87 and TG-905 cells were transfected with si-FOXG1 sequence or pcDNA-FOXG1 plasmid for 24 h, respectively. (A and B) RT-qPCR was applied to detect the expression level of FOXG1 mRNA. (C and D) Western blot was applied to quantify the expression level of FOXG1 protein. The results are shown as the means ± standard

deviation. **P<0.01 vs. control group. FOXG1, forkhead box G1; siRNA, small-interfering RNA; NC, negative control

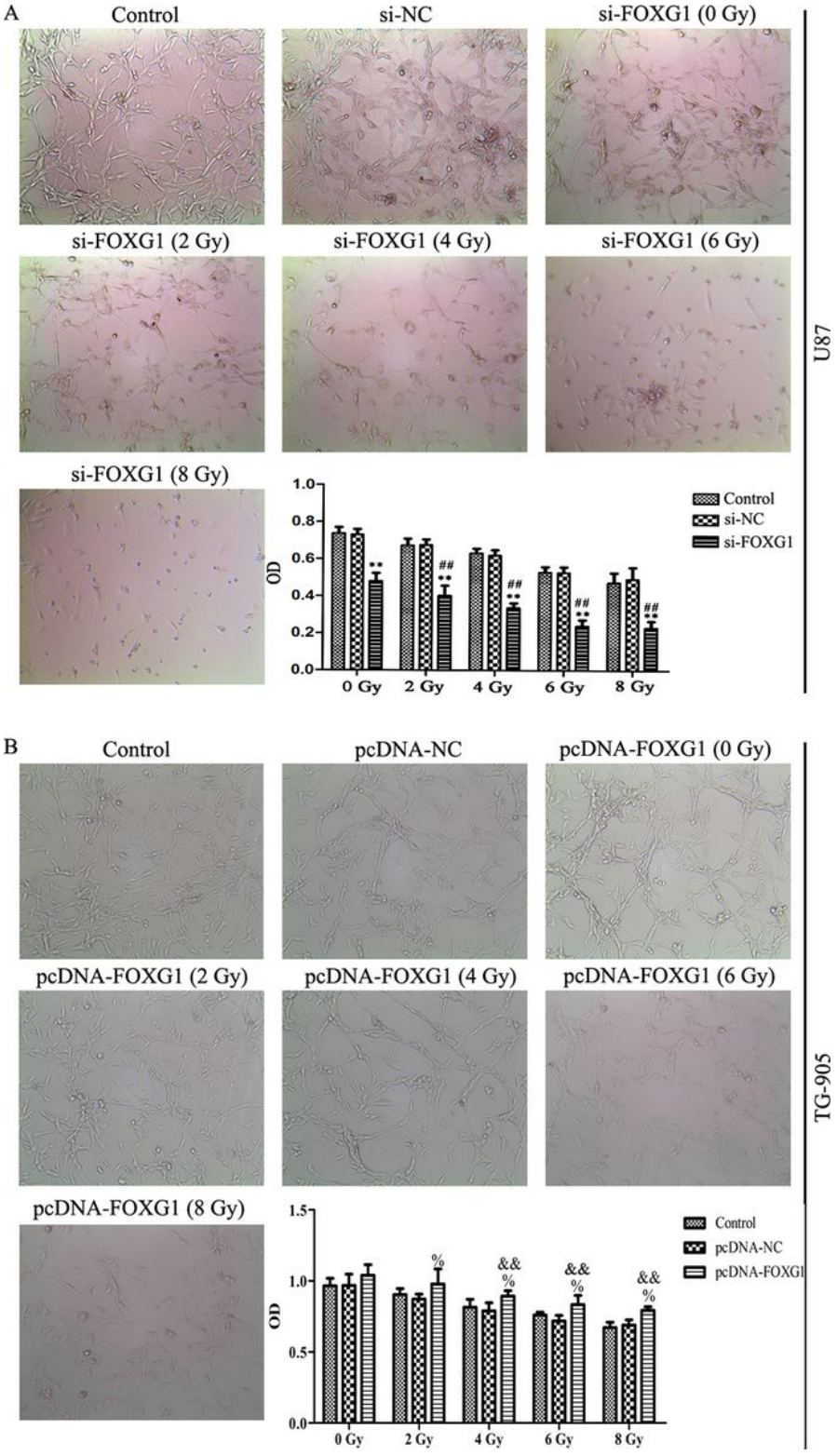

Figure 3

Effect of FOXG1 on the inhibitory effect of cell proliferation induced by X-ray radiation. U87 and TG-905 cells were transfected with si-FOXG1 sequence or pcDNA-FOXG1 plasmid for 6 h, and then the cells subjected to different doses of X-ray radiation (0, 2, 4, 6 and 8 Gy) and were cultured for 24 h. (A) Cell

morphology was microscopically observed, and cell viability was determined using the CCK-8 assay in U87 cells. **P<0.01 vs. control group; ##P<0.01 vs. si-FOXG1 (0 Gy) group. (B) Cell morphology was microscopically observed, and cell viability was determined using the CCK-8 assay in TG-905 cells. %P<0.05 vs. control group; &&P<0.01 vs. pcDNA-FOXG1 (0 Gy) group. FOXG1, forkhead box G; siRNA, small-interfering RNA; NC, negative control.

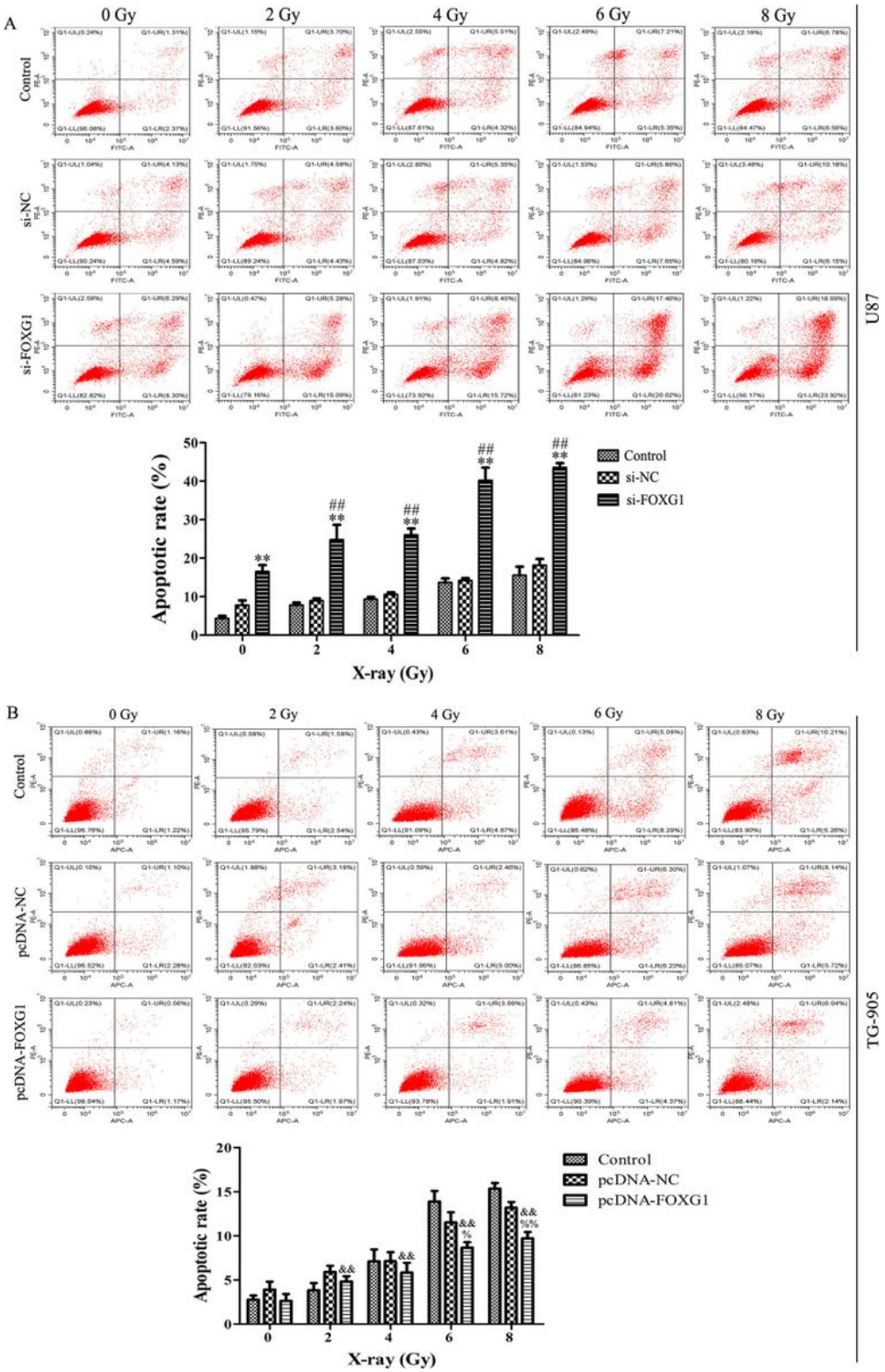

Figure 4

Effect of FOXG1 on apoptosis induced by X-ray radiation. U87 and TG-905 cells were transfected with si-FOXG1 sequence or pcDNA-FOXG1 plasmid for 6 h, and then the cells subjected to different doses of X-ray radiation (0, 2, 4, 6 and 8 Gy) and were cultured for 24 h. (A) Apoptotic cells were stained by Annexin-V/PI, and then detected using flow cytometry in U87 cells. **P<0.01 vs. control group; ##P<0.01 vs. si-FOXG1 (0 Gy) group. (B) Apoptotic cells were stained by Annexin-V/PI, and then detected using flow cytometry in TG-905 cells. %P<0.05 and %%P<0.01 vs. control group; &&P<0.01 vs. pcDNA-FOXG1 (0 Gy) group. FOXG1, forkhead box G1; siRNA, small-interfering RNA; NC, negative control

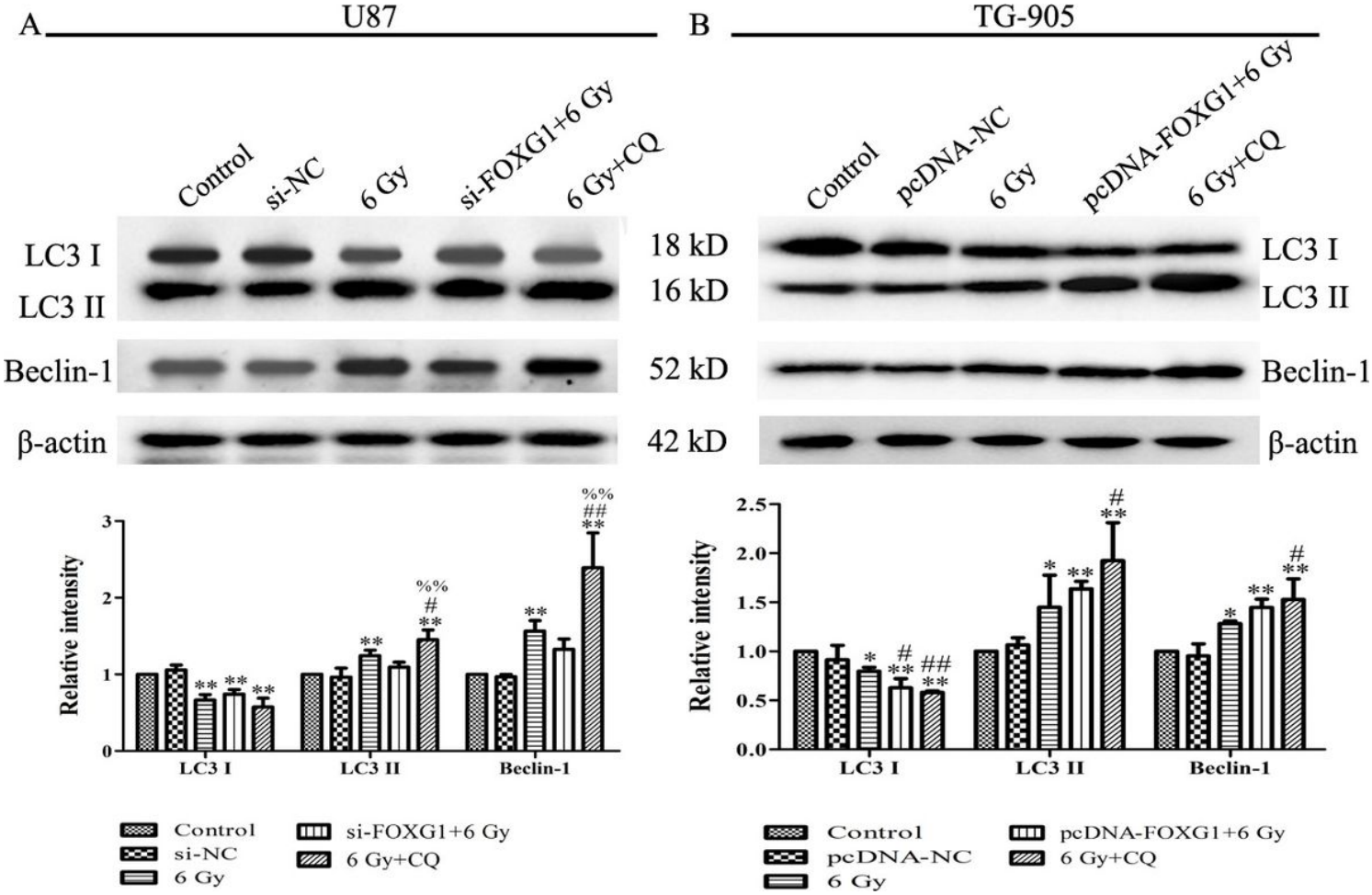

Figure 5

Effect of FOXG1 on the expression of autophagy related proteins induced by X-ray irradiation. (A) U87 cells were transfected with si-FOXG1 sequence for 6 h or pretreatment with CQ for 2 h, and then the cells subjected to different doses of X-ray radiation (6 Gy) and were cultured for 24 h. Autophagy related proteins were examined by Western blot assay. (B) TG-905 cells were transfected with pcDNA-FOXG1 plasmid for 6 h or pretreatment with CQ for 2 h, and then the cells subjected to different doses of X-ray radiation (6 Gy) and were cultured for 24 h. Autophagy related proteins were examined by Western blot assay. *P<0.05 and **P<0.01, compared with the control group; #P<0.05 and ##P<0.01, compared with the 6 Gy group; %%P<0.01, compared with the si-FOXG1+6 Gy group. FOXG1, forkhead box G1; siRNA, small-interfering RNA; CQ, chloroquine.

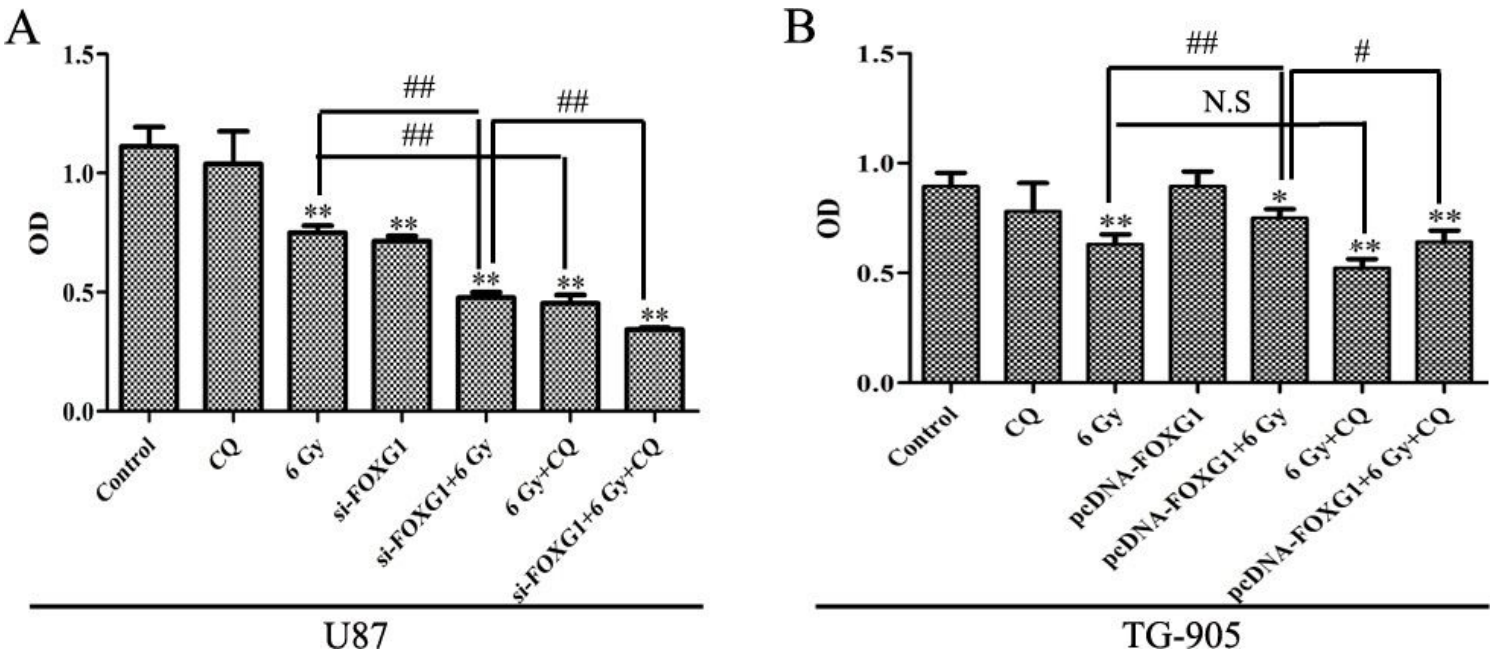

Figure 6

Effect of autophagy on proliferation inhibition induced by X-ray irradiation. U87 or TG-905 cells were transfected with si-FOXG1 sequence or pcDNA-FOXG1 plasmid for 6 h or pretreatment with CQ for 2 h, and then the cells subjected to different doses of X-ray radiation (6 Gy) and were cultured for 24 h. (A) cell viability was determined by CCK-8 assay in U87 cells. (B) cell viability was determined by CCK-8 assay in TG-905 cells. *P<0.05 and **P<0.01, compared with the control group; #P<0.05 and ##P<0.01; FOXG1, forkhead box G1; siRNA, small-interfering RNA; CQ, chloroquine.

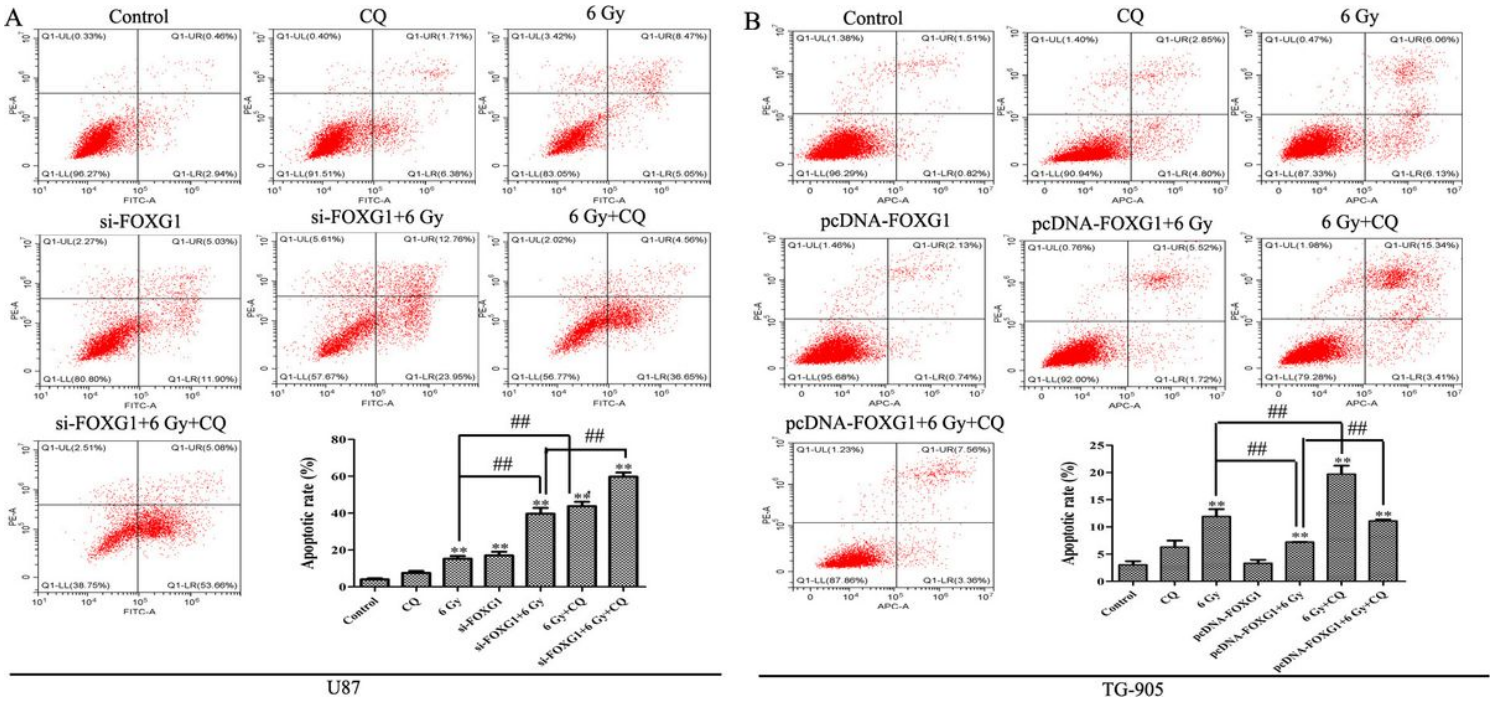

Figure 7

Effect of autophagy on apoptosis induced by X-ray irradiation. U87 or TG-905 cells were transfected with si-FOXG1 sequence or pcDNA-FOXG1 plasmid for 6 h or pretreatment with CQ for 2 h, and then the cells subjected to different doses of X-ray radiation (6 Gy) and were cultured for 24 h. (A) cell apoptosis was determined by flow cytometry in U87 cells. (B) cell apoptosis was determined by flow cytometry in TG-905 cells. **P<0.01, compared with the control group; ##P<0.01; FOXG1, forkhead box G1; siRNA, small-interfering RNA; CQ, chloroquine.