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Overexpression of XIST facilitates cell proliferation, invasion and suppresses cell apoptosis by reducing radio-sensitivity of glioma cells *via* miR-329-3p/CREB1 axis

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Abstract. – OBJECTIVE: Glioma is a malignant brain cancer capable of spreading to the microenvironment. Long non-coding RNA (lncRNA) X inactive specific transcript (XIST) was recognized as a significant regulator in many cancers. However, the molecular mechanism of XIST in glioma cell radio-sensitivity requires further exploration.

PATIENTS AND METHODS: The expression of XIST, microRNA (miR)-329-3p and cyclic AMP response element-binding protein 1 (CREB1) was evaluated by quantitative Real-time polymerase chain reaction (qRT-PCR). Cell viability and apoptosis were examined by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) and flow cytometry, respectively. Transwell assay was performed to detect cell invasion. Protein expression of gamma-H2AX (γ-H2AX) and CREB1 was determined by Western blot. The correlation between miR-329-3p and XIST or CREB1 was determined by dual-luciferase reporter assay. Animal models were established by subcutaneously injecting U251 cells transfected with sh-XIST and sh-NC.

RESULTS: XIST and CREB1 were overexpressed whereas miR-329-3p was low-expressed in glioma tumors and cells compared with the normal counterparts. XIST knockdown inhibited cell proliferation, invasion and induced cell apoptosis by enhancing cell sensitivity to X-ray radiation in glioma. Then, we discovered that miR-329-3p directly interacted with XIST or CREB1 in glioma. In addition, miR-329-3p inhibitor abolished XIST silencing-induced regulatory effects on cell proliferation, apoptosis, invasion, and radio-sensitivity. Meanwhile, miR-329-3p inhibitor counteracted CREB1 silencing-induced inhibition on cell progression and facilitation on radio-sensitivity in glioma. Moreover, we found that XIST could increase CREB1 expression by sponging miR-329-3p. Animal experiments revealed that XIST silencing restrained tumor growth *in vivo*.

CONCLUSIONS: XIST accelerates cell proliferation, invasion and inhibits cell apoptosis by repressing radio-sensitivity of glioma via enhancing CREB1 expression through sponging miR-329-3p, representing prospective methods for glioma treatment.

Key Words:
XIST, MiR-329-3p, CREB1, Radio-sensitivity, Glioma.

Introduction

Glioma is an aggressive brain cancer that could infiltrate to the healthy brain tissues by proliferation and invasion[1]. Despite conventional treatment methods such as surgical resection, chemotherapy and radiotherapy, the median survival of glioma patients is approximately 12 months[2-4]. Recurrence and radio-resistance are the major obstacles for the treatment of glioma. Gene regulation was intensively involved in radio-resistance of cancer cells[5]. For example, miR-153-3p improved radiotherapy outcomes of glioma patients by promoting radio-sensitivity of glioma cells via interacting with BCL2[6]. Therefore, it is imperative to

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investigate the molecular mechanism of glioma cell radio-resistance from genetic perspective.

Long non-coding RNAs (lncRNAs) are critical modulators of many physiological processes, such as cell cycle, survival, migration, epithelial to mesenchymal transition (EMT) inflammation, autophagy and apoptosis[7-9]. LncRNA X-inactive specific transcript (XIST), which mapped on chromosome Xq13.2, was frequently diagnosed in various cancers, such as non-small cell lung cancer, renal cell carcinoma and glioblastoma[10-12]. XIST indicated poor prognosis of thyroid cancer and overexpression of XIST facilitated cell viability and tumor growth by the activation of MET-PI3K-AKT pathway[13]. Consistently, XIST acted as an oncogene to accelerate cell viability and metastasis in pancreatic cancer by promoting TGF-β2 expression via competitively binding to miR-141-3p[14]. By contrast, the depletion of XIST attenuated cell cycle, growth and stimulated cell apoptosis in retinoblastoma by sponging miR-124 to repress STAT3 expression[15]. However, the functional role of XIST in glioma cell proliferation and radio-sensitivity is still poorly understood.

MicroRNAs (miRNAs) refer to small non-coding RNA with 17-28 endogenous nucleotides in length[16]. MiRNAs were able to negatively regulate gene expression by interacting with the messenger RNA and further modulate cell behavior, such as cell metabolism, metastasis, differentiation, chemo-resistance and radio-resistance in cancers[17-19]. Ectopic expression of miRNA was reported to participate in oncogenesis and cell progression in cancers. Of note, miR-494 targeted SIRT3 and further contributed to tumor growth by strengthening EMT of hepatocellular carcinoma through activating TGF-β/SMAD pathway[20]. Similarly, miR-411 functioned as tumor promoter to accelerate cell growth, migration and restrict cell apoptosis in lung cancer by directly repressing SPRY4 and TXNIP expression[21]. In addition, miR-329-3p/TP73-AS1/ARF1 axis was implicated in cell proliferation and migration of cervical cancer cells[22]. However, the regulatory effects of miR-329-3p on glioma cell progression and radio-sensitivity are still unclear.

We assumed that lncRNA XIST could improve radiotherapy outcomes of glioma patients by increasing cell sensitivity to X-ray via sponging miR-329-3p and regulating the expression of the target gene cyclic AMP response element-binding protein 1 (CREB1). The expression of XIST, miR-329-3p and CREB1 was measured to clarify the potential role of the genes. Animal experiments were performed to reveal the function of XIST in glioma.

Patients and Methods

Patient Samples

Glioma patients (n=30) and health controls (n=30) were recruited from the Fifth Affiliated Hospital of Sun Yat-Sen University (Zhuhai, Guangdong, China). All the participants were informed and signed informed consent. Glioma tumor tissues and normal health tissues were collected from the participants by surgery. Experimental protocols were approved by the Ethics Committee of The Fifth Affiliated Hospital of Sun Yat-Sen University (Zhuhai, Guangdong, China).

Cell Culture and Treatment

U251 and A172 cells were purchased from Chinese Academy of Sciences (Shanghai, China) while normal human astrocytes (NHA) cells were purchased from Lonza (Alpharetta, GA, USA). The cells were cultured in complete Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, Rockville, MD, USA). The cells were plated in 96-well plates and incubated overnight. Then, the cells were fixed on Faxitron Cabinet X-ray System (Faxitron, Tucson, AZ, USA) for irradiation (2 Gy, 4 Gy, 6 Gy, 8 Gy).

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Glioma tumors, normal tissues and the cells were lysed by TRIzol reagent (Invitrogen, Carlsbad, CA, USA) to obtain total RNA. The cDNA for XIST, miR-329-3p and CREB1 was synthesized by All-in-One™ Kit (FulenGen, Guangzhou, China). QRT-PCR was conducted by SYBR green (Applied Biosystems, Foster City, CA, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and U6 were exploited as internal references. The primers for XIST, miR-329-3p, CREB1, GAPDH and U6 were as follows: XIST, (Forward, 5'-ATTCCAACGCACTATCAACCAACC-3'; Reverse, 5'-CCAAGGTCTGCTATATCATTACC-3'); miR-329-3p, (Forward, 5'-CACACCTGGTTAACCTC-3'; Reverse, 5'-GAACATGTCTGCGTATCTC-3'); CREB1 (Forward, 5'-CTTTTCTCCGGAACACAGATTTC-3'; Reverse, 5'-GATTTGCCAAGTGGGAGGGA-3'); GAPDH, (Forward, 5'-AG-

GTCGGTGTGAACGGATTTG-3'; Reverse, 5'-GGGGTCGTTGATGGCAACA-3'); U6, (Forward, 5'-ACCCTGAGAAATACCCTCACAT-3'; Reverse, 5'-GACGACTGAGCCCCTGATG-3').

Cell Transfection

Small interfering RNA (siRNA) targeting XIST (si-XIST), siRNA targeting CREB1 (si-CREB1), small harboring RNA (shRNA) targeting XIST (sh-XIST), siRNA negative control (si-NC), shRNA negative control (sh-NC), pcDNA, XIST overexpression vectors were synthesized by Genepharma (Shanghai, China). MiR-329-3p, miR-329-3p inhibitor (anti-miR-329-3p), negative control (miR-NC) and negative control inhibitor (anti-miR-NC) were purchased from RIBOBIO (Guangzhou, China). Cell transfection was performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

3-(4,5-Dimethyl-2-Thiazolyl)-2,5-Diphenyl-2-H-Tetrazolium Bromide (MTT) Assay

U251 and A172 cells were plated in 96-well plates. After incubation for 24 h, 48 h and 72 h, the cells were added with 10 μL MTT (Beyotime, Shanghai, China) for 4 h. Next, the reaction was terminated using dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, MO, USA). Lastly, the optical density (OD) value at 490 nm was measured by a spectrophotometer.

Flow Cytometry

U251 and A172 cells were placed in 24-well plates and incubated for 48 h. Then, the cells were collected, resuspended and stained by fluorescein isothiocyanate tagged Annexin V (Annexin V-FITC)/propidium iodide (PI) detection kit (Invitrogen). Finally, cell apoptosis was analyzed by a flow cytometer.

Transwell Assay

Cell invasion was examined by transwell assay (Corning, Corning, NY, USA). In brief, the upper chamber of transwell was treated with Matrigel (Sigma-Aldrich) and then U251 and A172 cells were plated in it. After invading for 48 h, the cells at the lower chamber were stained with 0.1% crystal violet (Sigma-Aldrich). Cell invasion was captured by a microscope.

Western Blot

Protein gamma-H2AX (γ-H2AX) and cyclic AMP response element-binding protein 1 (CREB1) were extracted from U251 and A172 cells. Total protein was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After blocking by 5% nonfat milk, the membranes were incubated with primary antibodies against γ-H2AX, CREB1, GAPDH (Abcam, Cambridge, MA, USA) and horseradish peroxidase (HRP)-conjugated secondary antibody (Sangon, Shanghai, China).

Dual-Luciferase Reporter Assay

Wild type XIST (WT-XIST) and mutant type XIST (MUT-XIST) luciferase vectors were constructed. At the same time, wild type CREB1 (CREB1 3'UTR-WT) and mutant type CREB1 (CREB1 3'UTR-MUT) luciferase vectors were constructed. The vectors were co-transfected in U251 and A172 cells with miR-329-3p or miR-NC to construct dual-luciferase reporter system. Luciferase activities were determined using a luminometer.

Animal Models

Male nude mice (5-week old) were purchased from Jinan Pengyue Animal Breeding Center (Jinan, China). The mice were randomly divided into 2 groups: sh-NC group (n=6) and sh-XIST group (n=6). U251 cells stably transfected with sh-NC and sh-XIST were subcutaneously injected in the mice to construct animal models. All the animal experiment protocols were approved by the National Animal Care and Ethics Institution and Ethics Committee of The Fifth Affiliated Hospital of Sun Yat-Sen University (Zhuhai, Guangdong, China).

Statistical Analysis

All the data were presented as means ± standard deviation (SD). Statistical analysis was performed by GraphPad Prism 7 (San Diego, CA, USA). The correlation between miR-329-3p and XIST or CREB1 was analyzed by Pearson's correlation coefficient. p value less than 0.05 ($p<0.05$) was considered statistically significant.

Results

Up-Regulation of XIST and Down-Regulation of MiR-329-3p in Glioma

Initially, we detected the expression of XIST and miR-329-3p to explore the interrelation between them in glioma. As illustrated in Figure

1A-B, XIST was overexpressed in glioma tissues and cells (U251, A172) compared with normal tissues and cells (NHA). On the contrary, miR-329-3p expression was down-regulated in tumors and cells compared with the normal counterparts (Figure 1C-D). By calculating using Pearson's correlation coefficient, we discovered that XIST was negatively correlated with miR-329-3p (r=-0.6239, $p<0.001$) (Figure 1E). These data demonstrated that XIST might function as an oncogene in glioma.

Elimination of XIST Suppressed Cell Proliferation, Invasion and Promoted Cell Apoptosis and Radio-Sensitivity in Glioma

The function of XIST in glioma cell progression and radio-sensitivity was assessed in cells transfected with si-NC and si-XIST. Apparently, XIST expression was decreased in U251 and A172 cells after XIST knockdown, suggesting the high transfection efficiency (Figure 2A). More importantly, XIST knockdown reduced cell proliferation (Figure 2B-C) and induced cell apoptosis (Figure 2D) in glioma. Consistent with MTT results, transwell assay results revealed that cell invasion was attenuated by XIST knockdown (Figure 2E). In addition, cell sensitivity to the X-ray radiation was improved by XIST silencing, especially in 6 Gy and 8Gy groups (Figure 2F-G). In the meantime, protein expression of γ-H2AX was boosted in cells transfected with si-XIST and treated with 6 Gy X-ray radiation (Figure 2H). Taken together, XIST depletion enhanced radio-sensitivity and reduced cell progression in glioma.

XIST Regulated Cell Viability, Apoptosis, Invasion and Radio-Sensitivity in Glioma by Sponging miR-329-3p

Bioinformatics analysis and prediction by starBase exhibited that XIST could specifically bind to miR-329-3p (Figure 3A). Decreased luciferase activity in U251 and A172 cells co-transfected with WT-XIST and miR-329-3p confirmed the interaction between XIST and miR-329-3p (Figure 3B-C). To certify the transfection efficiency, qRT-PCR was conducted. As displayed in Figure 3D, XIST expression was evidently increased in XIST transfection cells and decreased in si-XIST transfection cells. Conversely, miR-329-3p expression in cells was enhanced by XIST knockdown and reduced by XIST transfection (Figure 3E). The result showed that the promotion of XIST silencing on miR-329-3p expression was inversed by miR-329-3p inhibitor (Figure 3F). What's more, miR-329-3p inhibitor counteracted XIST silencing-me-

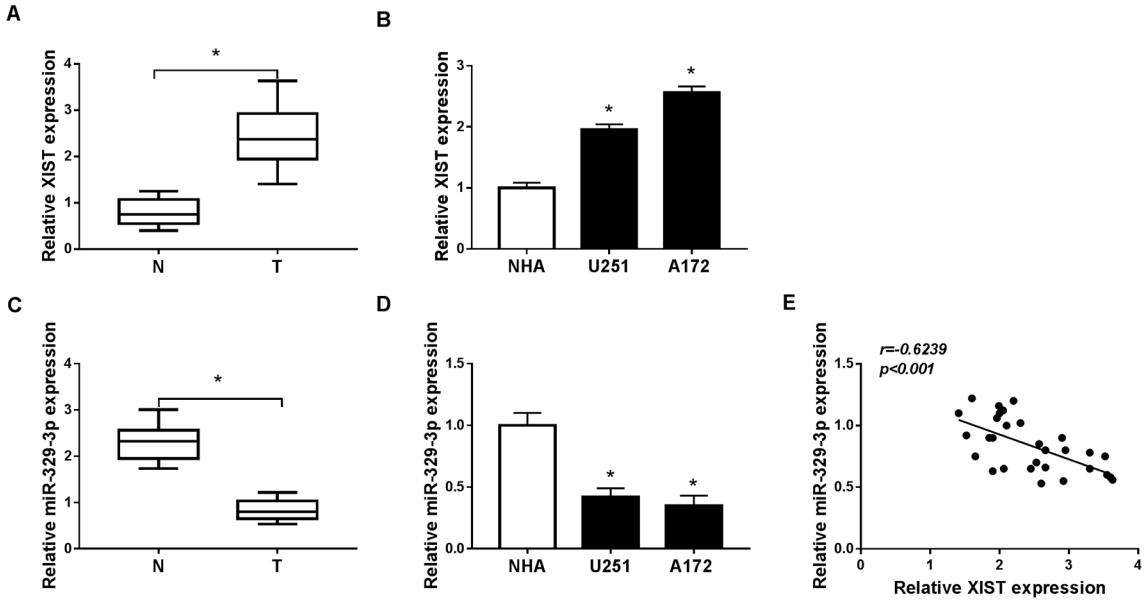

Figure 1. XIST was overexpressed while miR-329-3p was low-expressed in glioma. (**A-B)** XIST expression in glioma tumors and cells (U251, A172) compared with normal tissues and cells (NHA) was measured by qRT-PCR. (**C-D)** The expression of miR-329-3p in glioma tumors and cells compared with the normal counterparts. (**E)** The correlation between XIST and miR-329-3p was analyzed by Pearson's correlation coefficient (r=-0.6239, $p<0.001$). *$p<0.05$.

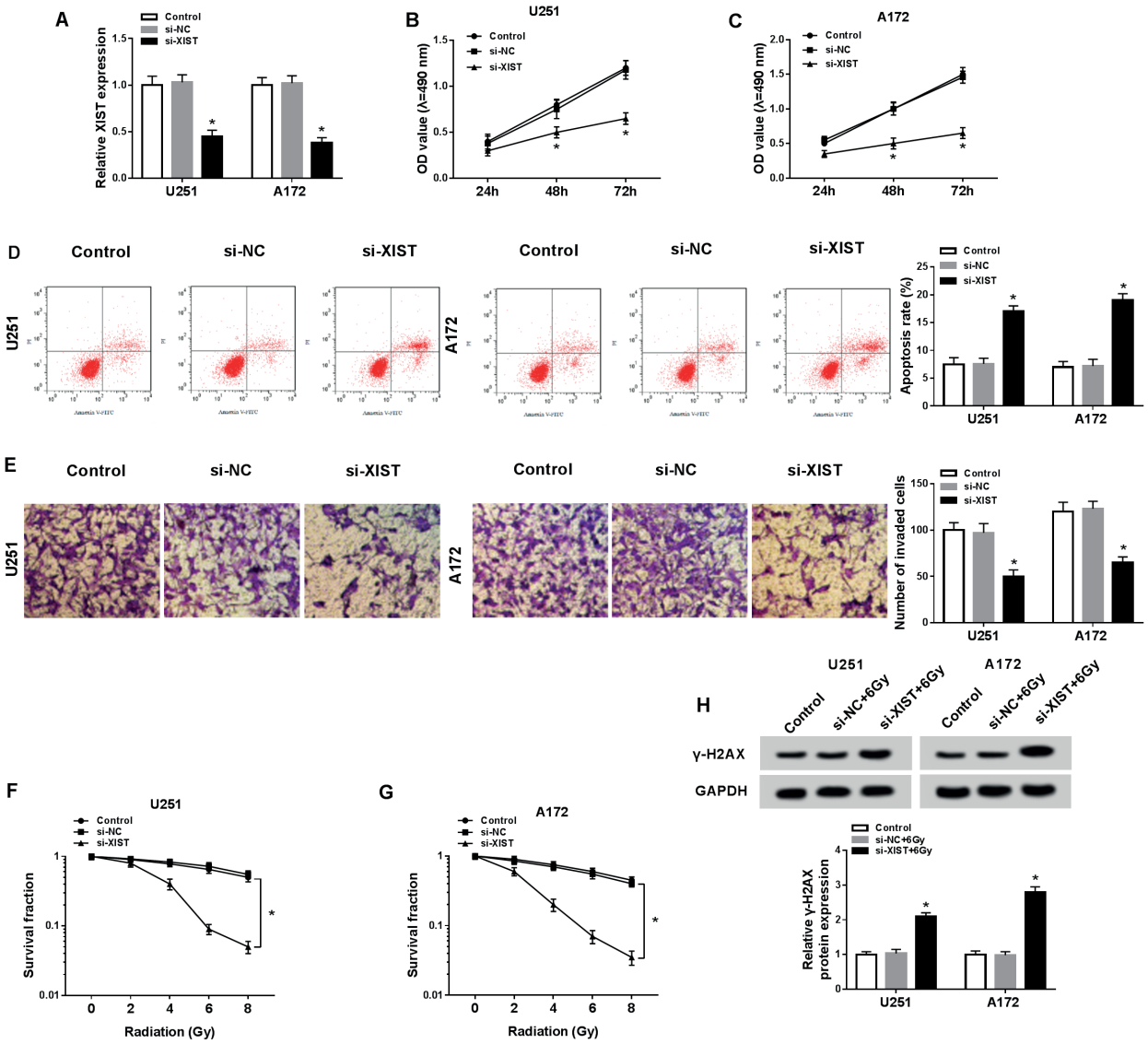

Figure 2. XIST knockdown inhibited cell viability, invasion as well as improved cell apoptosis and radio-sensitivity in glioma. U251 and A172 cells were transfected with si-NC and si-XIST. **(A)** XIST expression in transfected U251 and A172 cells was detected by qRT-PCR. **(B-C)** Cell viability was measured by MTT assay. **(D)** Cell apoptosis was detected by flow cytometry. **(E)** Cell invasion was evaluated by transwell assay (Magnification: 100×). **(F-G)** Survival fraction of U251 and A172 cells treated with radiation. **(H)** Protein expression of γ-H2AX in U251 and A172 cells treated with radiation was analyzed by Western blot. * $p<0.05$.

diated inhibition of cell proliferation (Figure 3G-H), invasion (Figure 3J) and acceleration on cell apoptosis (Figure 3I) in glioma. In addition, the survival fraction of cells underwent X-ray radiation treatment was inhibited by XIST silencing and the inhibitive effects were reversed by miR-329-3p inhibitor (Figure 3K-L). Meanwhile, protein expression of γ-H2AX was strengthened by XIST silencing and weakened by miR-329-3p inhibitor (Figure 3M-N). Therefore, XIST could sponge miR-329-3p and further regulate cell progression and radio-sensitivity in glioma.

CREB1 Was a Target of MiR-329-3p

As predicted by starBase, there were potential binding sites between CREB1 and miR-329-3p (Figure 4A). Luciferase activity of miR-329-3p in cells was inhibited by CREB1 3'UTR-WT com-

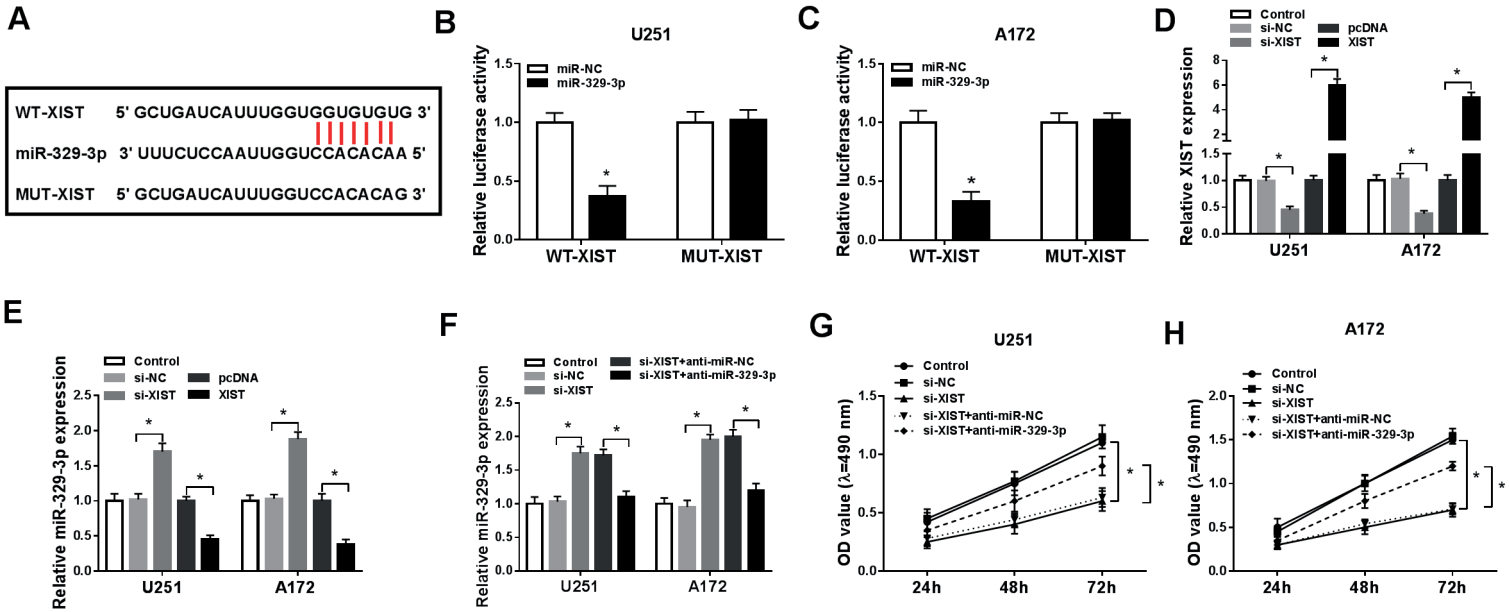

Figure 3. XIST interacted with miR-329-3p to regulate cell viability, apoptosis, invasion and radio-sensitivity in glioma. U251 and A172 cells were transfected with si-NC, si-XIST, si-XIST+anti-miR-NC and si-XIST+anti-miR-329-3p. **(A)** The potential binding sites between XIST and miR-329-3p were determined by starBase. **(B-C)** Luciferase activity of U251 and A172 cells co-transfected with WT-XIST or MUT-XIST and miR-329-3p or miR-NC was determined by dual-luciferase reporter assay. **(D-E)** The expression of XIST and miR-329-3p in cells transfected with si-NC, si-XIST, pcDNA and XIST was examined by qRT-PCR. **(F)** The expression of miR-329-3p in cells was measured by qRT-PCR. **(G-H)** Cell viability was measured by MTT assay. **(I)** Cell apoptosis was analyzed by flow cytometry. **(J)** Cell invasion was determined by transwell assay.

Figure continued

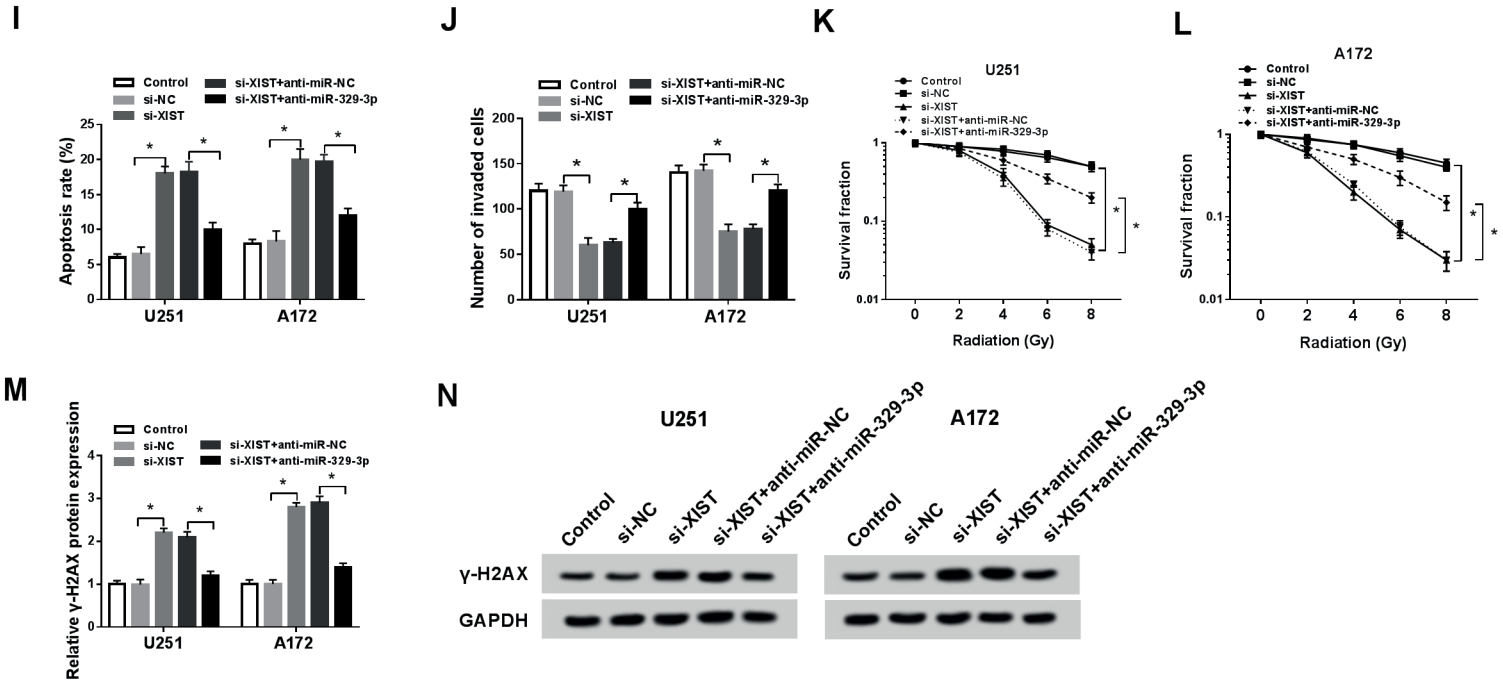

Figure 3. (Continued). **(K-L)** Survival fraction of transfected cells treated with radiation. **(M-N)** Protein expression of γ-H2AX in transfected cells treated with radiation. * $p<0.05$.

pared with CREB1 3'UTR-MUT, demonstrating miR-329-3p directly interacted with CREB1 (Figure 4B-C). Next, the cells were transfected with anti-miR-NC, anti-miR-329-3p, miR-NC, miR-329-3p and the transfection efficiency was determined by qRT-PCR (Figure 4D). In addition, CREB1 protein expression was elevated by miR-329-3p inhibitor and declined by miR-329-3p, revealing that miR-329-3p could regulate CREB1 expression (Figure 4E). Essentially, the expression of CREB1 mRNA and protein was extremely higher in glioma tumors (Figure 4F-G) and cells (Figure 4H-I) compared with the normal ones. We also discovered that CREB1 was negatively correlated with miR-329-3p in glioma ($r=-0.4914$, $p<0.001$) (Figure 4J). The results demonstrated that CREB1 acted as a target of miR-329-3p.

Suppression of miR-329-3p Abrogated CREB1 Silencing-Induced Regulatory Effects on Cell Proliferation, Invasion and Promoted Cell Apoptosis and Radio-Sensitivity in Glioma

The regulatory effects of miR-329-3p/CREB1 axis on the radio-sensitivity of glioma cells were investigated. Declined expression of CREB1 mRNA and protein in cells transfected with si-CREB1 indicated that that cell transfection was carried out successfully (Figure 5A-B). MTT and transwell results revealed that miR-329-3p inhibitor rescued the suppression of cell proliferation (Figure 5C-D) and invasion (Figure 5F) induced by CREB1 depletion. By contrast, cell apoptosis was facilitated by CREB1 knockdown and the facilitation was inversed by miR-329-3p inhibitor (Figure 5E). To explore the influence of X-ray radiation on glioma cells, transfected cells were treated with X-ray radiation (2 Gy, 4 Gy, 6 Gy, 8 Gy). As displayed in Figure 5G-H, miR-329-3p inhibitor alleviated CREB1 knockdown-induced suppression on glioma survival fraction. In the meantime, γ-H2AX protein expression was boosted by CREB1 knockdown and inhibited by miR-329-3p inhibitor (Figure 5I-J). Altogether, miR-329-3p targeted CREB1 to modulate cell proliferation, apoptosis, invasion and radio-sensitivity in glioma.

XIST Sponged miR-329-3p to Improve CREB1 Expression

The molecular mechanism of XIST in glioma cell development and sensitivity to radiation was further explored. Significantly, miR-329-3p inhibitor attenuated XIST knockdown-mediated repression on CREB1 protein expression (Figure 6A-B). Hence, we considered that XIST could regulate CREB1 expression by interacting with miR-329-3p in glioma.

Elimination of XIST Hindered Tumor Growth

To investigate the impact of XIST on tumor growth, xenograft mice were established by subcutaneously injecting U251 cells stably transfected with sh-NC and sh-XIST. As displayed in Figure 7A-B, tumor growth was attenuated by XIST knockdown. In addition, the expression of XIST, CREB1 was down-regulated while miR-329-3p was up-regulated in tumors collected from sh-XIST transfection mice compared with sh-NC group (Figure 7C). Consistently, the expression of protein CREB1 was inhibited by XIST silencing (Figure 7D). In short, XIST deficiency attenuated tumor growth *in vivo* by regulating miR-329-3p/CREB1 axis.

Discussion

Mounting researches revealed that XIST was a crucial biomarker for the prognosis of a variety of cancers, such as osteosarcoma, pancreatic cancer and glioma[23-25]. Dysregulation of XIST was recognized as the pathogenesis of different cancers. However, the role of XIST in cancer cell proliferation is still controversial. XIST acted as tumor promoter to accelerate the aggressiveness of colorectal cancer[26] by promoting cell proliferation and EMT through targeting miR-486-5p to improve neuropilin-2 expression. The abundance of XIST contributed to cell growth, colony formation and migration in laryngeal squamous cell carcinoma[27] by promoting EZH2 expression through interacting with miR-124-3p. Similarly, overexpression of XIST was able to sponge miR-429 and further accelerate tumorigenicity and angiogenesis of glioma[28]. On the contrary, XIST served as tumor suppressor to alleviate the malignancy of ovarian cancer by restraining cell invasion and tumor growth through inhibiting hsa-miR-214-3p[29]. Whether XIST functions as a tumor promoter or suppressor in glioma is still largely obscured.

LncRNAs exerted their regulatory function by interacting with the specific miRNAs. Hence, miRNAs played significant roles in multiple cancers by acting as oncogene or suppressor. MiR-329-3p acted as a tumor suppressor and reduced

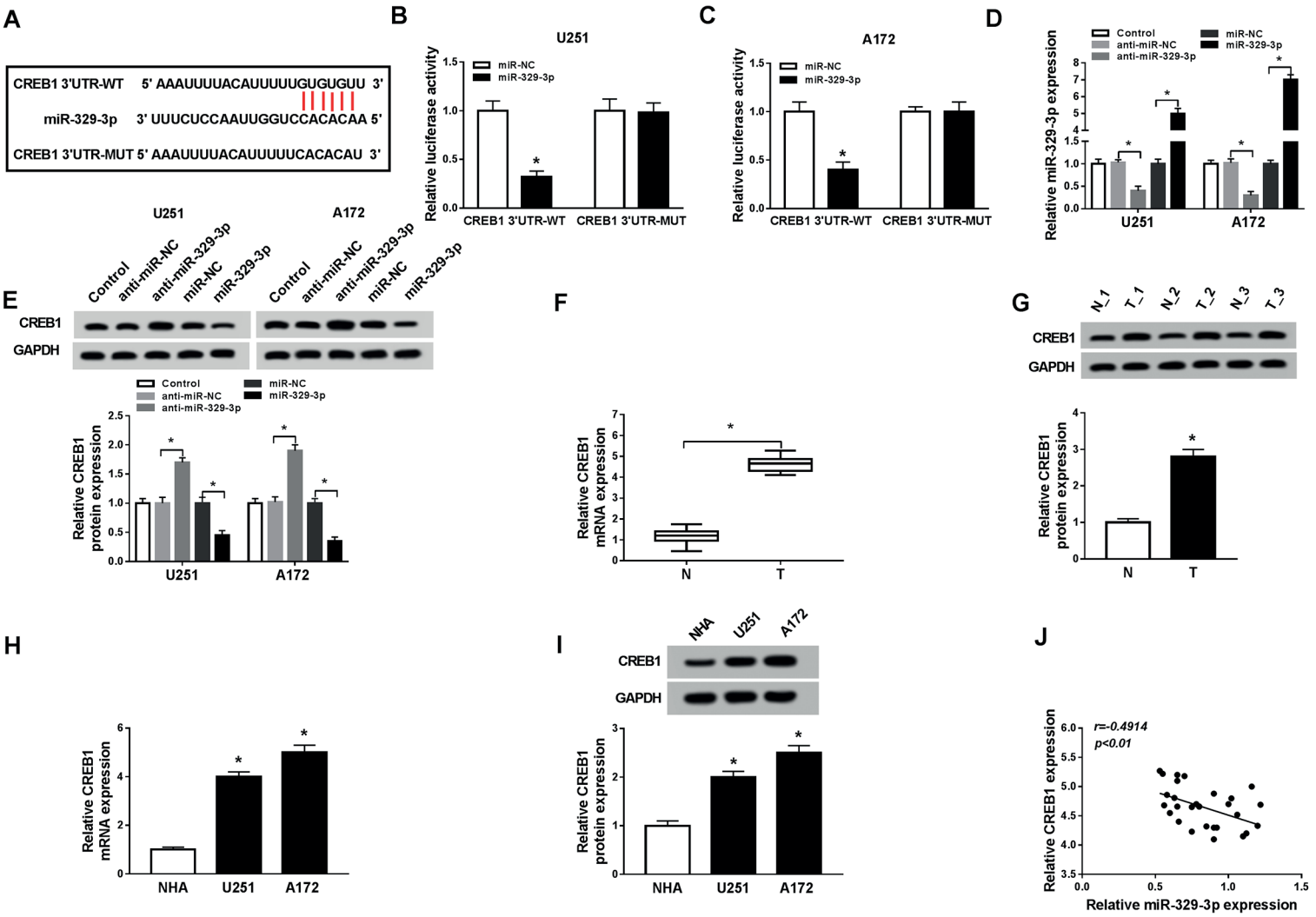

Figure 4. The interaction between miR-329-3p and CREB1 was evaluated. **(A)** The potential binding sites between CREB1 and miR-329-3p were predicted by starBase. **(B-C)** Luciferase activity of U251 and A172 cells co-transfected with CREB1 3'UTR-WT or CREB1 3'UTR-MUT and miR-329-3p or miR-NC was examined by dual-luciferase reporter assay. **(D)** The expression of miR-329-3p in cells transfected with anti-miR-NC, anti-miR-329-3p, miR-NC and miR-329-3p was detected by qRT-PCR. **(E)** CREB1 protein expression in transfected cells was evaluated by Western blot. **(F-G)** CREB1 mRNA and protein expression in glioma tumors compared with normal tissues. **(H-I)** CREB1 mRNA and protein expression in glioma cells compared with NHA cells. **(J)** The linear relationship between CREB1 and miR-329-3p was analyzed (r=-0.4914, $p<0.001$). * $p<0.05$.

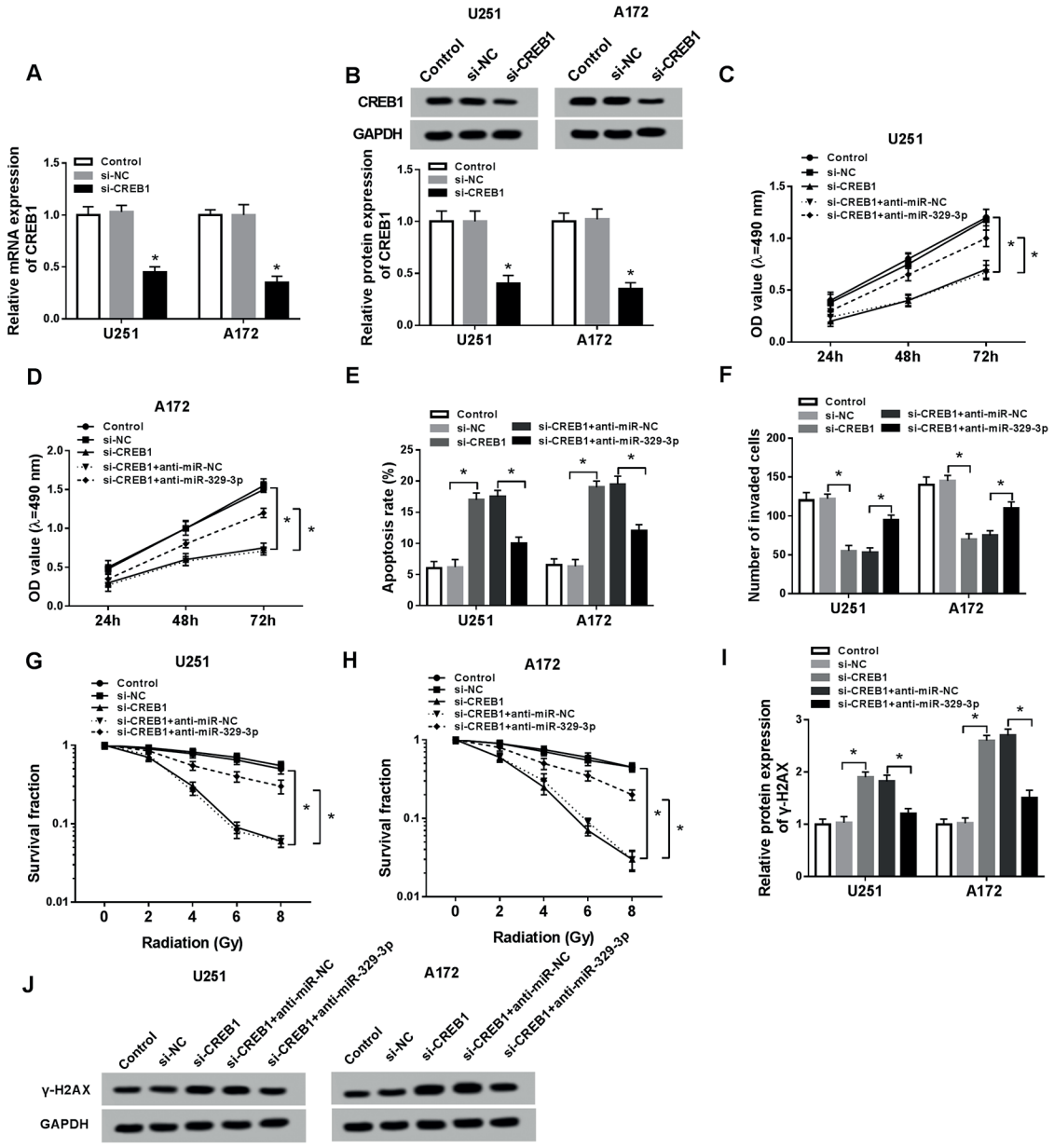

Figure 5. MiR-329-3p inhibitor attenuated CREB1 knockdown-induced repression on cell proliferation, invasion and promotion on cell apoptosis and radio-sensitivity in glioma. U251 and A172 cells were transfected with si-NC, si-CREB1, si-CREB1+anti-miR-NC and si-CREB1+anti-miR-329-3p. **(A-B)** CREB1 mRNA and protein expression in cells transfected with si-NC and si-CREB1 were determined by RT-PCR and Western blot. **(C-D)** MTT was performed to evaluate cell viability. **(E)** Flow cytometry was used to assess cell apoptosis. **(F)** Transwell assay was conducted to detect cell invasion. **(G-H)** Survival fraction of transfected cells treated with radiation. **(I-J)** Protein expression of γ-H2AX in transfected cells treated with radiation. * $p<0.05$.

cell proliferation and metastasis in cervical cancer by regulating MAPK1 expression[30]. Consistently, miR-384 targeted LIMK1 and exerted anti-tumor effects by weakening cell proliferation, invasion and inducing cell apoptosis through LIMK1/cofilin pathway in esophageal squamous cell carcinoma[31]. In addition, miR-485-5p was reported to repress cell cycle, proliferation, colony formation, metastasis and EMT in hepatocellular carcinoma via interacting with EMMPRIN[32]. By comparison, miR-4262 was an oncogene to expedite cell growth in cutaneous melanoma by inac-

tivating EGFR and promoting p21 expression[33]. Therefore, XIST might participate in cell development and radio-sensitivity by interacting with miR-329-3p in glioma.

LncRNAs have been confirmed to play important roles acting as competing endogenous RNAs (ceRNAs) to regulate downstream gene expression by targeting different miRNAs in tumor progression [34]. A significant number of researches revealed that the regulatory ceRNA networks of lncRNA XIST, miRNAs and mRNAs are involved in the pathogenesis of various cancers. Knockdown of XIST impeded cell proliferation and metastasis by regulating CBLL1 mediated by miR-212-3p in non-small cell lung cancer cells[35]. XIST regulated the progression of hepatocellular carcinoma via miR-497-5p/PDCD4 axis[36]. However, the lncRNA-associated ceRNA regulatory networks of XIST in glioma are rare.

In this study, a lncRNA-miRNA-mRNA ceRNA network was built to demonstrate the function of XIST in glioma. We initially investigated the potential role of XIST in glioma by qRT-PCR. The expression of XIST and CREB1 was up-regulated while miR-329-3p was down-regulated in glioma, implying the promotive role of XIST and suppressive role of miR-329-3p in glioma. Loss-of-function experiment exhibited that XIST elimination reduced cell viability, invasion as well as inducing cell apoptosis and radio-sensitivity of glioma cells, confirming the oncogenic role of XIST. Subsequently, we discovered that XIST was a sponge whereas CREB1 was a target of miR-329-3p in glioma to regulate cell be-

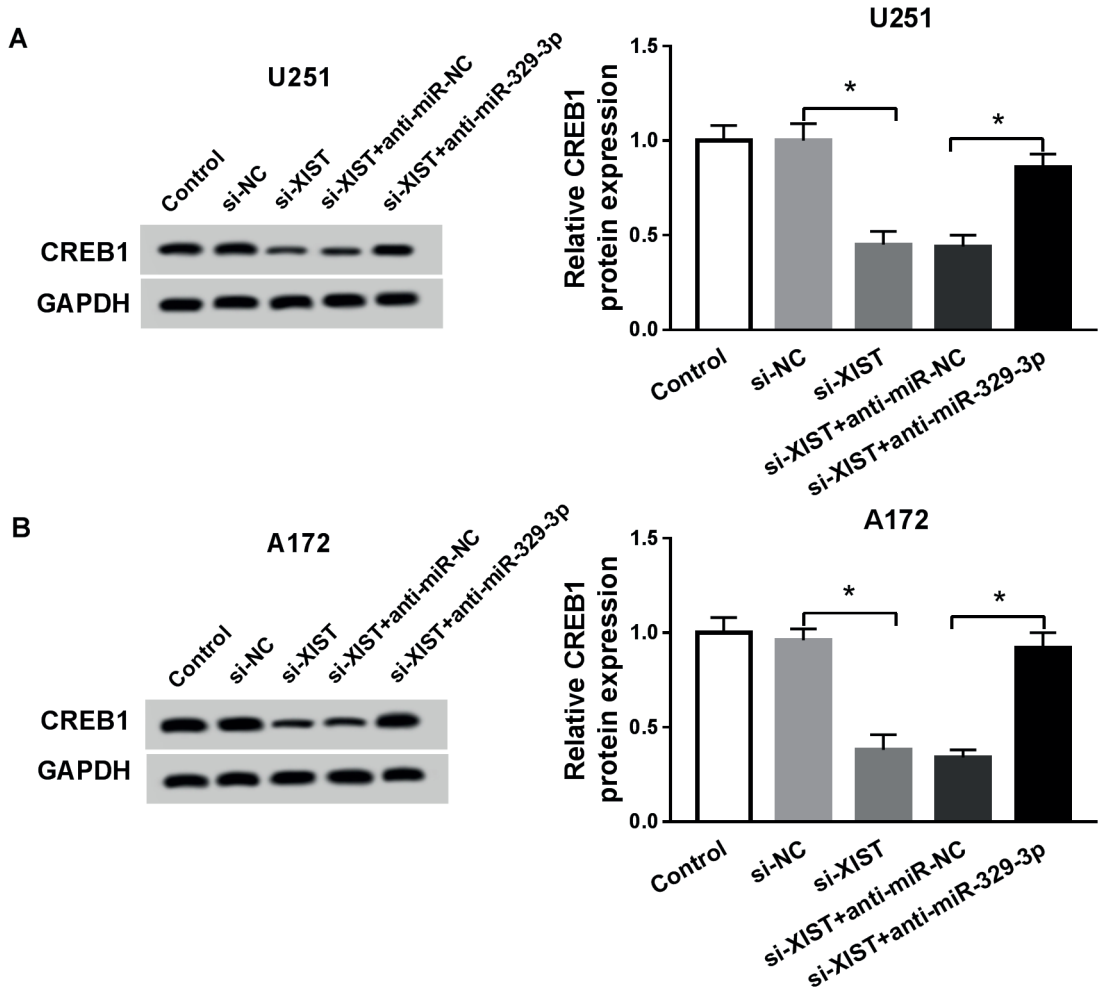

Figure 6. XIST enhanced CREB1 expression by sponging miR-329-3p. **(A-B)** CREB1 protein expression in cells transfected with si-NC, si-XIST, si-XIST+anti-miR-NC and si-XIST+anti-miR-329-3p was detected by Western blot. * $p<0.05$.

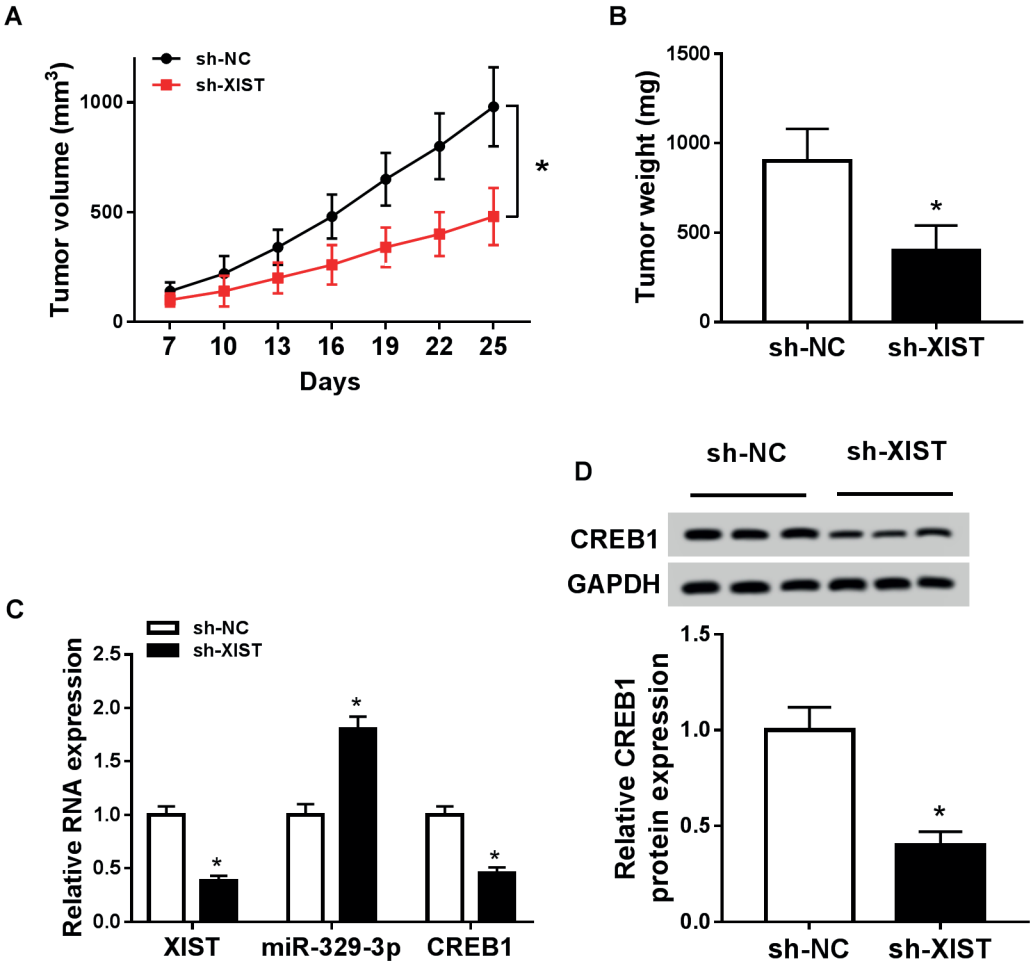

Figure 7. XIST knockdown retarded tumor growth *in vivo*. **(A-B)** Tumor volume and weight of the xenograft mice were measured. **(C)** The expression of XIST, miR-329-3p and CREB1 in tumors was analyzed by qRT-PCR. **(D)** Protein expression of CREB1 in tumors was determined by Western blot. * $p<0.05$.

havior. Thus, a ceRNA network associated with lncRNA XIST was successfully constructed. The following functional experiments further verified the lncRNA-miRNA-mRNA ceRNA regulatory network. For example, miR-329-3p inhibitor neutralized XIST silencing-mediated suppression on cell proliferation, invasion and promotion on cell apoptosis and radio-sensitivity in glioma. Similarly, miR-329-3p could target CREB1 and further regulate cell progression and sensitivity to X-ray radiation in glioma. Significantly, our result revealed that XIST was able to promote tumor growth *in vivo* by facilitating CREB1 expression via absorbing miR-329-3p in glioma.

Conclusions

In brieg, the results of this study revealed that lncRNA XIST could inhibit radio-sensitivity of glioma cells by sponging miR-329-3p and enhancing CREB1 expression, thereby accelerating the malignancy of glioma. This work might enhance the understanding of the biological mechanisms of ceRNAs and provide potential alternative targeted radiotherapy of glioma.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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