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Long noncoding RNA LINC00511 involves in breast cancer recurrence and radioresistance by regulating STXBP4 expression *via* miR-185

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Abstract. – OBJECTIVE: The aim of this study was to explore the molecular function of long intergenic noncoding RNA 00511 (LINC00511) and its target proteins in recurrent breast cancer after breast-conserving surgery followed by radiotherapy.

PATIENTS AND METHODS: LINC00511 expression in tissues was measured by quantitative polymerase chain reaction (qPCR). The association between LINC00511 expression and the clinicopathological features of breast cancer was analyzed by Chi-square tests. The impact of LINC00511 on overall survival was evaluated by the log-rank test. MDA-MB-231/MDA-MB-436 cell lines transfected with short hairpin RNA (shRNA) were used to investigate the influence of LINC00511 silencing on tumor growth and radiosensitivity *in vitro* and *in vivo*. A series of experiments including cell apoptosis assay, cell colony formation assay, and mouse xenograft models were applied to test *those* transfected cell lines. MicroRNA (miRNA) targets of LINC00511 were identified by bioinformatics analysis and further validated by dual luciferase reporter assay, qPCR, and Western blot analysis.

RESULTS: LINC00511 expression was significantly increased in breast cancer tissues and correlated with recurrence and poor survival after breast-conserving surgery followed by radiotherapy. LINC00511 knockdown by shRNA restricted cell proliferation, promoted cell apoptosis, and enhanced radiosensitivity *in vitro*, and inhibited tumor growth with an increased response to radiation *in vivo*. In addition, elevated LINC00511 was found to increase syntaxin-binding protein 4 (STXBP4) expression through competitive binding to miR-185, while silencing LINC00511 decreased STXBP4 expression and increased radiosensitivity.

CONCLUSIONS: LINC00511 inhibition impairs its competitive binding to miR-185, resulting in increased STXBP4 expression and improved radiation response in breast cancer. Our study results suggest that the LINC00511/miR-185/STXBP4 axis may be a promising therapeutic target for improving the prognosis of breast cancer.

Key Words:
Breast cancer, Long intergenic noncoding RNA 00511, MiR-185, Syntaxin-binding protein 4.

Introduction

Breast cancer is a leading cause of cancer-related deaths in women worldwide[1]. Radiation therapy plays a vital role in the current postoperative management of lymph node-positive breast cancer[2]. Even though most patients benefit from radiation therapy, some are resistant to treatment due to limited hormone receptors, tumor cells heterogeneity, or mutations[3]. Patients with a high level of hormone receptors are relatively radiosensitive[4]. A variety of novel methods including intensity-modulated radiotherapy have been developed to enhance therapeutic accuracy, but radiation resistance in breast cancer still hinders treatment efficacy[5]. Therefore, investigations into the mechanisms of radiotherapy tolerance will help in optimizing the treatment strategy. Many studies have reported that tumor metastasis and recurrence following radiotherapy tolerance are mediated by long noncoding RNAs (lncRNAs)[6,7]. LncRNAs are transcripts with lengths exceeding 200 bases that do not encode proteins[8] but affect gene expression via inhibition of microRNA (miRNA) function by complementary binding[9]. Usually, oncogenic lncRNAs are overexpressed in tumors and enhance cellular proliferation and metastasis[10]. Restraining these oncogenic lncRNAs can cause apoptosis and cell cycle arrest, thereby controlling cancer growth and promoting sensitivity to treatments[11]. Gao et al[12] have found that lncRNAs are involved in tumorigenesis and regulation of cellular response to chemotherapeutics in breast cancer. Long intergenic noncoding RNA 00511 (LINC00511) is highly expressed in breast cancer and strongly associat-

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ed with stemness and inferior survival of breast cancer patients[13]. Increased LINC00511 can boost proliferation and sphere-formation ability, in addition to upregulating Oct4, Nanog, and Sox2 expression by removal of E2F1 protein repression[13]. However, the biological function and mechanism of LINC00511 in radioresistance of breast cancer are still unknown.

We report the aberrant expression of LINC00511 in radioresistant human breast cancer tissues and outline the underlying functional mechanism that can explain this manifestation. We show that LINC00511 is of pathogenetic importance in breast cancer radiotherapy and also a helpful predictor of therapeutic efficacy. In conclusion, we suggest that LINC00511 may be a potential target to increase radiosensitivity and thereby improve patient survival in breast cancer.

Materials and Methods

Patients, Radiotherapy, Follow-up and Tissue Specimens

A total of 98-breast cancer patients were enrolled between July 2016 and October 2018. All patients received breast-conserving surgery followed by radiotherapy. Tissue specimens were firstly used for histopathological diagnosis, then frozen in -80°C.

The inclusion criteria were as follows: breast cancer confirmed by histology (biopsy), I-II stage suitable for breast-conserving surgery, completive radiotherapy treatment fulfilled. The exclusion criteria for this study were as follows: clinical stage of M1 (the 7th AJCC TNM staging system) proved by bone scan or magnetic resonance imaging, incomplete follow-up data, other conditions that required medical treatment.

A total dose of 40-42.5 Gy radiotherapy was implemented to all these patients (3 Gy per fraction per day, 5 days per week).

A follow-up was administrated to evaluate the overall survival of patients. All patients received a physical examination every 3-6 months, a mammography or ultrasonography per year. This study was approved by the Affiliated Hospital of Southwest Medical University Ethics Committee. We obtained consent from each patient before their treatment.

RNA Extraction and Quantitative Polymerase Chain Reaction (Qpcr)

According to the manufacturer's protocol, either breast cancer tissues or cell lines was homogenized for total RNAs extraction using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA). All extracted RNAs were qualified (A260: A280 ratio $\geq$ 2.0) and quantificationally measured by NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). cDNA synthesis was performed by PrimeScript™ RT kit (TaKaRa Biotechnology, Dalian, Liaoning, China), which is complemented with gDNA Eraser for putative target RNAs following the manufacturer's instruction. The quantitative test of target RNA in tissues and cells was done using TB Green™ Premix Ex Taq™ (Tli RNaseH Plus) (TaKaRa Biotechnology, Dalian, Liaoning, China) in accordance with protocol on the Roche LightCyber 480 System (Roche Molecular Systems, Mannheim, Baden-Wuerttemberg, Germany). The target RNA expression was calculated utilizing $2^{-\Delta\Delta Ct}$ method in a relative way (gene GAPDH as the endogenous control). All primer sequences and oligonucleotides used for transfection (Invitrogen, Carlsbad, CA, USA) were presented in **supplemental Table I**.

Cell Lines and Culture

The breast cancer cell line MDA-MB-231, MDA-MB-436, MDA-MB-361, MCF-7 and breast epithelial cell MCF-10A were purchased from Chinese Academy of Sciences (Shanghai Institute of Cell Biology, Shanghai, China). Cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% (v/v) fetal bovine serum (FBS; Gibco, Waltham, MA, USA) in a circumstance of 5% CO^2 at 37 °C.

Cell Transfection

Short hairpin RNAs (shRNAs) for LINC00511 knockdown were constructed (Invitrogen, Carlsbad, CA, USA) and expressed using pcDNA3.1 vector (Invitrogen, Carlsbad, CA, USA). Breast cancer cells were seeded in six-well plates with 1×10^6 per well, and were transfected with vector after overnight, using Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA) in accordance with protocol. The efficacy of transfection must be verified by PCR.

Apoptosis Assay

The apoptosis analysis of the transfected GC cells (after 48 h culture) was performed Utilizing Annexin V Apoptosis Detection Kit (eBiosciences, Waltham, MA, USA), the apoptosis rate of irradiated cells was tested after every dose. Stained cells

Table I. Relation between LINC00511 expression and clinicopathological features in breast cancer (n = 98).

Variables	Total N	LINC00511 expression		*p*-value
		Low n=49	High n=49	
Age				0.407
≤ 50	38	17	21	
> 50	60	32	28	
Tumor size				0.038*
< 2 cm	60	35	25	
≥ 2 cm	38	14	24	
Lymph node metastasis				0.418
No	52	28	24	
Yes	46	21	25	
ER status				0.225
Positive	48	27	21	
Negative	50	22	28	
PR status				0.675
Positive	62	32	30	
Negative	36	17	19	
HER-2 status				0.203
Positive	34	14	20	
Negative	64	35	29	
Molecular subtype				0.618
Luminal like	50	26	24	
HER-2 positive	26	14	12	
Triple negative	22	9	13	
Recurrence				0.037*
No	80	44	36	
Yes	18	5	13	

Notes: *$p < 0.05$ represents statistical difference.

were analyzed using BD FACS AriaII Flow Cytometry (BD Biosciences, Franklin Lakes, NJ, USA).

Colony Formation Assay

Breast cancer cells were cultured in 96-well plates with 5×10^4 cells/well for 2 weeks, followed by an implant with 500 cells/well for colony formation assay. Then, 4 % paraformaldehyde (5 min) and 1 % crystal violet (10 min) were using to fix and stain the colonies. All colonies were observed by a microscope.

Cells Irradiation

Cells with a concentration of 5×10^3 /well were cultured for 48 h. Then, an exposure to radiation with a gradient dose (0, 2, 4, 6, 8 and 10 Gy) was carried out using a 6-MV X-ray linear accelerator (ELEKTA, Beijing, China). Cells were placed in the incubator, and samples were collected at the indicated time points (0, 1, 12 and 24 h).

Cell Viability Detection

The viability of irradiated cells was assessed by MTT (3-(4,5)-dimethylthiahiazo (-z-yl)-3,5-di-phenytetrazoliumromide) (KeyGENBiotech, Nanjing, Jiangsu, China) following the manufacturer's instructions. The absorbance representing each viability was measured by a spectrophotometer at 490 nm.

Mice Model Experiments

5-week-old nude mice, purchased from the Laboratory Animal Center of Southwest Medical University, were injected subcutaneously with LINC00511 shRNA and control vector-transfected MDA-MB-231/MDA-MB-436 cells, respectively, at the concentration of 5×10^5 /ml (100 ul). The mice (n=5 per group) were kept under specific pathogen-free (SPF) conditions with an atmosphere of 12 h light/dark cycle. After the injection, a rest of two weeks permits the growth for tumor nudes. A treatment of X-ray at 10 Gy was performed to each mouse. The tumor sizes were recorded every week. Six weeks after inoculation, tumor nodes were resected for weight assessments following the sacrifice of mice. All these animal experiments were approved by the Animal Care and Use Committee of the Affiliated Hospital of Southwest Medical University,

following the Institutional Guide for the Care and Use of Laboratory Animals.

Dual-Luciferase Reporter Analysis

The wildtype target lncRNA or the one containing a mutant miRNA- binding area were constructed (Invitrogen, Carlsbad, CA, USA). Both of these lncRNA were cloned with a luciferase gene in reporter vectors (Promega, Madison, WI, USA). The synthetic vectors, Renilla luciferase reporter vector and miRNA mimic were co-transfected into cells using the Lipofectamine 2000 kit (Thermo Fisher Scientific, Waltham, MA, USA) following the protocol provided by the manufacture, 48 h later cells were seeded into 96-well plates. The luciferase activity of Renilla plasmid (as the endogenic control, Promega, Madison, WI, USA) and target gene were assessed via Dual-Luciferase Reporter Assay Kit (Promega, Madison, WI, USA).

Western Blot Assay

A RIPA buffer containing protease inhibitor (Beyotime, Shanghai, China) was used for proteins exaction from cells and tissues. The protein concentrations were measured using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). Electrophoresis on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel (Thermo Fisher Scientific, Waltham, MA, USA) separated kinds of proteins in each sample, followed by transferring to polyvinylidene difluoride (PVDF) membranes (Thermo Fisher Scientific, Waltham, MA, USA). The membranes were incubated with primary antibodies (Anti-STXBP4, 1:2000; β-actin, 1:5000; Abcam, Cambridge, MA, USA) at 4°C overnight after an incubation with phosphate-buffered saline (PBS, 5% dry milk) at room temperature for 1 h. Then, the membrane with blotting was incubated with a secondary antibody conjugated with horseradish peroxidase (HRP) (1:5000 dilution, Santa Cruz, CA, USA). An ECL™ chemi-luminescence detection system (Pierce, Waltham, MA, USA) was used to compare the protein levels reflected by the blotting.

Statistical Analysis

All experiments were conducted independently in triplicate. Data are presented as mean ± standard deviation (SD). Comparison of within groups was performed using independent Student's *t*-test. GraphPad Prism software (La Jolla, CA, USA) was used for statistical analysis.

The binding site prediction between target miRNA and LINC00511 was performed by Starbase 2.0 (http://starbase.sysu.edu.cn/starbase2/index.php). p-value < 0.05 was considered statistically significant.

Results

Increased LINC00511 Expression is Correlated with Breast Cancer Recurrence and Poor Prognosis in Patients with Breast Cancer

LINC00511 expression in breast cancer tumor tissues and adjacent normal tissues was determined by qPCR and compared. LINC00511 was significantly upregulated in cancer tissues (Figure 1A, $p < 0.01$). Further, compared to the breast epithelial cells MCF-10A, LINC00511 expression was significantly higher in MDA-MB-231, MDA-MB-436, MDA-MB-361 and MCF-7 (Figure 1B, $p < 0.01$) cells. Breast cancer patients were divided into two groups according to the median value of LINC00511 level (high expression group, n = 49; low expression group, n = 49; Figure 1C). A correlation analysis was carried out between LINC00511 expression and clinical-pathological features in breast cancer (Table I). Higher LINC00511 was significantly correlated with tumor size ($p = 0.038$) and recurrence after breast-conserving surgery followed by radiotherapy ($p = 0.037$), while no associations were found between increased LINC00511 and age, lymph node metastasis, endocrine receptors, or molecular subtype. Survival analysis demonstrated that increased LINC00511 was strongly associated with inferior overall survival (Figure 1D, $p < 0.05$). Altogether, these results indicate that aberrant LINC00511 may be a significant predictor for recurrence and poor prognosis in patients with breast cancer.

LINC00511 Contributes to Proliferation and Radioresistance of Breast Cancer In Vitro

Based on the analysis with patient samples, we hypothesized that LINC00511 may not only be involved in enhanced proliferation of tumor cells, but also induce radioresistance in breast cancer resulting in recurrence. A pair of oligonucleotides was synthesized as shRNA to silence lncRNA LINC00511 in highly expressing cancer cell lines MDA-MB-231 and MDA-MB-436. The efficacy of transfection, deter-

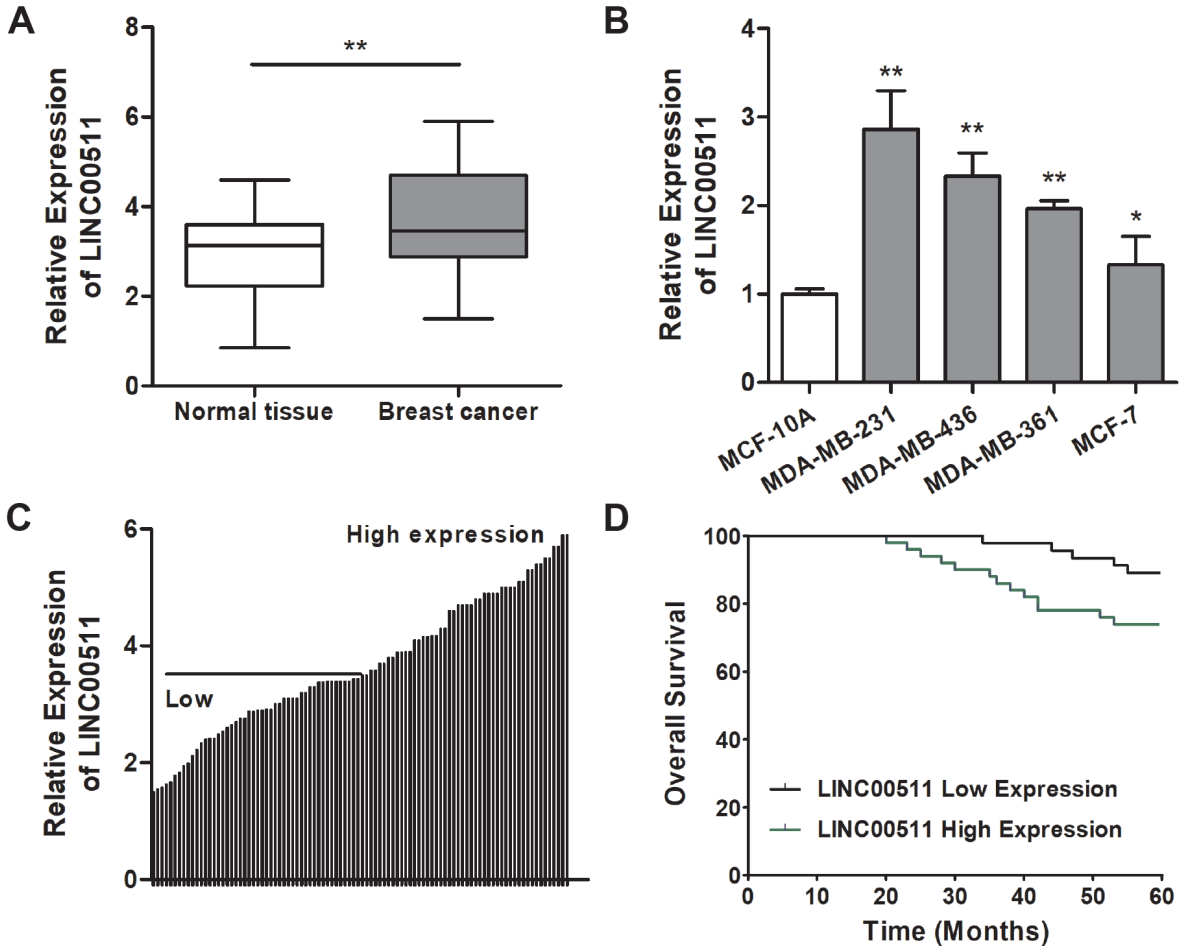

Figure 1. Inclined LINC00511 is related to recurrence and poor prognosis in patients with breast cancer. ***(A)*** Determination of LINC00511 expression in breast cancer tissues and adjacent normal tissues by qPCR. ***(B)*** Comparison of LINC00511 relative expression between breast cancer cells (MDA-MB-231, MDA-MB-436, MDA-MB-361 and MCF-7) and breast epithelial cell MCF-10A by qPCR. ***(C)*** Division of LINC00511 relative level into high expression group (n = 49) and low expression group (n = 49) using median value. ***(D)*** Overall survival analysis of breast cancer patients after breast-conserving surgery followed by radiotherapy between high and low LINC00511 expression. $^{**}p < 0.01$, $^{*}p < 0.05$ compared to the control group.

mined by qPCR, was significant (Figure 2A, $p < 0.01$). LINC00511 knockdown decreased the number of clones (showed by colony formation assay, Figure 2B, $p < 0.01$) and promoted apoptosis (showed by flow cytometry, Figure 2C, $p < 0.01$) in breast cancer cells as compared to the vector control. Cancer cell lines transfected with shRNA were irradiated to investigate the role of LINC00511 in radiosensitivity. Under different doses of irradiation, LINC00511-silenced cells showed significantly reduced cell viability, as evaluated by the MTT assay, in both the breast cancer cell lines compared to that in cells with normal LINC00511 levels (Figure 3A & 3B, $p < 0.05$). Furthermore, apoptotic rates were higher in LINC00511 silenced cells than that in the vector control (Figure 3C and 3D, $p < 0.05$). Altogether, these data indicate that LINC00511 suppression can inhibit cell proliferation and improve radiosensitivity of breast cancer *in vitro*.

LINC00511 Suppression Inhibits Tumor Growth and Improves the Radiosensitivity of Breast Cancer In Vivo

The effect of LINC00511 suppression on radiosensitivity *in vivo* was studied using xenograft mouse models inoculated with MDA-MB-231 or MDA-MB-436 cells stably transfected with shRNA. Both tumor size and tumor weight were significantly repressed in LINC00511 knockdown mice compared to that in the vector control mice (Figure 4). These results suggest that LINC00511 suppression

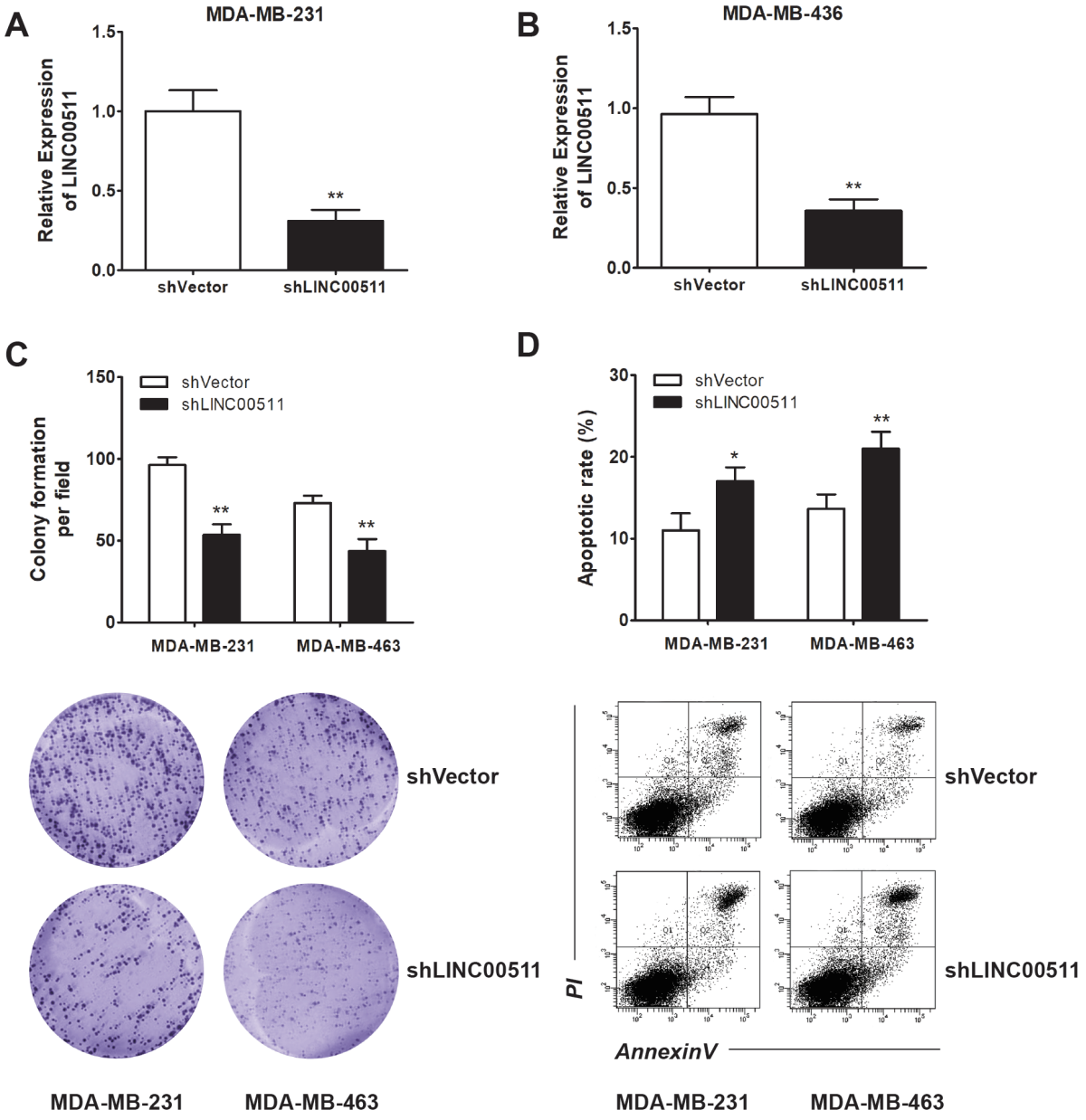

Figure 2. LINC00511 contributes to proliferation of breast cancer *in vitro*. ***(A, B)*** A synthesized shRNA targeting LINC00511 was transfected into MDA-MB-231 ***(A)*** and MDA-MB-436 ***(B)*** for silencing. ***(C)*** Colony formation ability of breast cancer cells with shRNA or control vector transfection. ***(D)*** The apoptotic cells rate of breast cancer cells after LINC00511 knockdown showed by flow cytometry. *$p < 0.05$, **$p < 0.01$ compared to the control group.

in breast cancer can inhibit tumor growth and improve response to radiotherapy *in vivo*.

LINC00511 may Up-Regulate STXBP4 Expression by Competitive Binding to Mir-185

Bioinformatics analysis predicted miR-185 as a potential target of LINC00511 complementary binding (Figure 5A). We used the dual-luciferase reporter assay to confirm the direct interaction between LINC00511 and miR-185. Wild-type LINC00511, but not the mutant, repressed the luciferase activity of miR-185 in breast cancer cells (Figure 5B and C, $p < 0.05$). LINC00511 suppression increased miR-185 expression in both MDA-MB-231 and MDA-MB-436 cells, compared to the control (Figure 5D and E, $p < 0.05$). Thus, our results indicate that miR-185 can be inhibited di-

rectly by complementary binding of LINC00511.

TargetScan predicted syntaxin binding protein 4 (STXBP4) as the possible downstream target of miR-185. Therefore, we investigated the impact of LINC00511 silencing on the expression of STXBP4 in breast cancer cells. In MDA-MB-231 and MDA-MB-436 cells, the transcript levels of STXBP4 were highly downregulated by LINC00511 silencing. Further, under 0 Gy irradiation, the cellular levels of STXBP4 were lower than those under 6 Gy in the vector control group (Figure 6A and B, $p < 0.05$). All PCR results were verified at the protein level by Western blot analysis (Figure 6C and D). Altogether, these results indicate that STXBP4 is the downstream functional protein of LINC00511-miR-185 regulatory axis in breast cancer.

Discussion

Despite extensive research on the molecular mechanisms of radiation tolerance, the leading cause for recurrence of advanced cancer after radical excision, an effective therapeutic strategy, is still lacking. Studying the molecular characteristics underlying the tumor phenotype may result in the discovery of promising therapeutic targets. We investigated the molecular mechanism of LINC00511 in radiotherapy resistance in breast

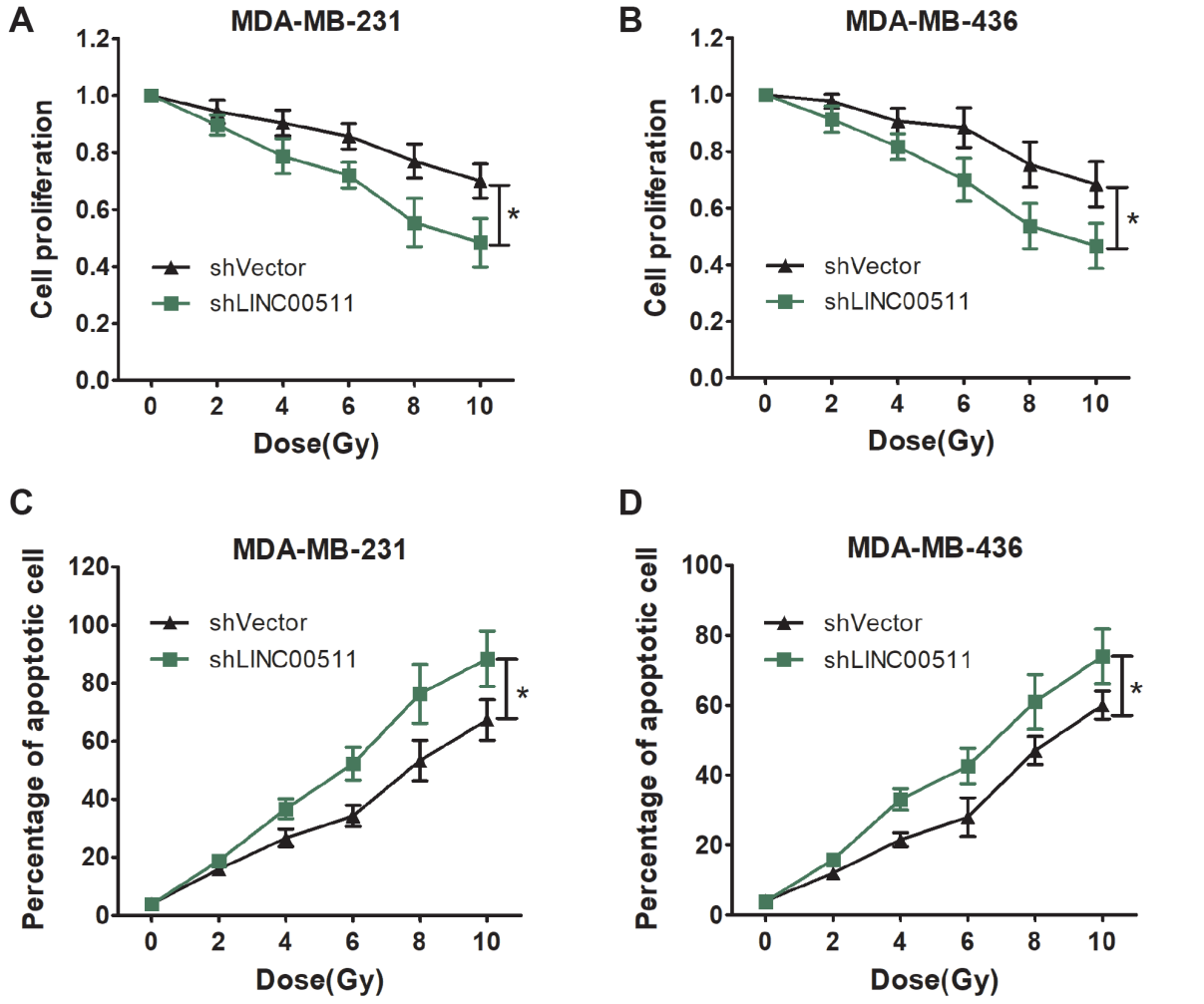

Figure 3. LINC00511 contributes to radioresistance of breast cancer *in vitro*. ***(A, B)*** Cell viability of MDA-MB-231 ***(A)*** and MDA-MB-436 ***(B)*** under varying doses of irradiation measured by MTT assay. ***(C, D)*** The apoptosis analysis of MDA-MB-231 ***(C)*** and MDA-MB-436 ***(D)*** under varying doses of irradiation evaluated by flow cytometry. *$p < 0.05$, **$p < 0.01$ compared to the control group.

cancer to explore potential novel therapeutic interventions during irradiation treatment. Previously, investigators have detected that LINC00511 functions as an oncogene in various cancers[14,15], including breast cancer[16-18]. LINC00511 was predicted to downregulate the Kruppel-like zinc finger protein ZNF217, activating tumorigenesis in breast cancer[16]. Besides, RORγ, a nuclear receptor downstream of LINC00511, was shown to maintain the characteristics of mammary stem cells and regulate metastasis in breast cancer[17]. Further, knockdown of LINC00511 was shown to increase paclitaxel efficacy in breast cancer by decreasing the expression of cyclin-dependent kinase (CDK6)[18]. However, little is known about the role of LINC00511 in breast cancer. In this study, we showed that aberrant expression of LINC00511 is strongly associated with radioresistance and could be an independent predictor of shorter overall survival in breast cancer (Figure 1). Silencing of LINC00511 in breast cancer cells inhibited the proliferation of cell lines *in vitro* and

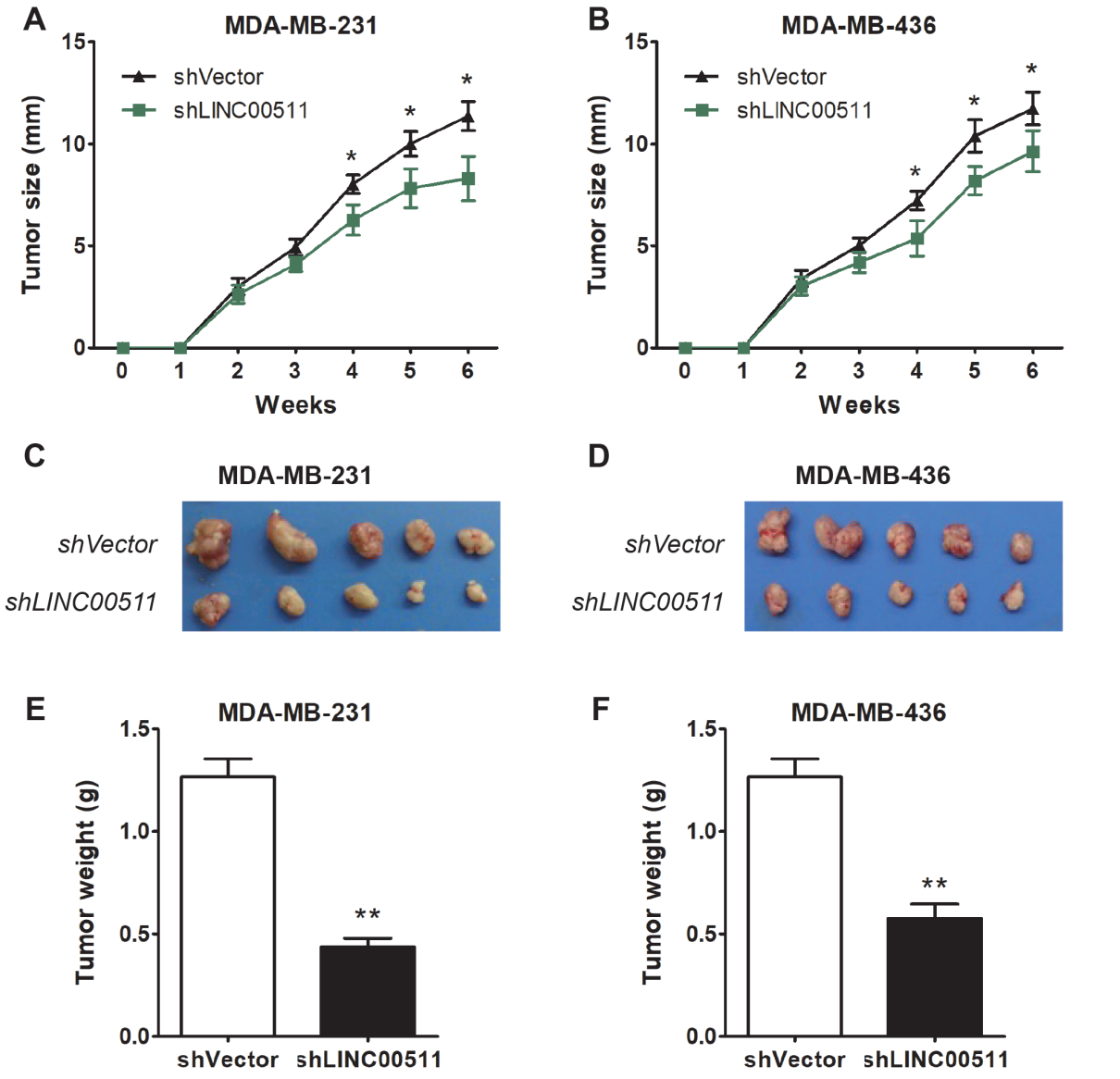

Figure 4. LINC00511 intervention can inhibit tumor growth and improve the radiosensitivity of breast cancer *in vivo*. ***(A, B)*** Tumor size in mice incubated with cancer cells (MDA-MB-231, left A; MDA-MB-436, right B) after LINC00511 knockdown. ***(C, D)*** Tumor growth of MDA-MB-231 ***(C)*** and MDA-MB-436 ***(D)*** in mice. ***(E, F)*** Tumor weight of MDA-MB-231 ***(E)*** and MDA-MB-436 ***(F)*** with LINC00511 knockdown or vector control. *$p < 0.05$, **$p < 0.01$ compared to the control group.

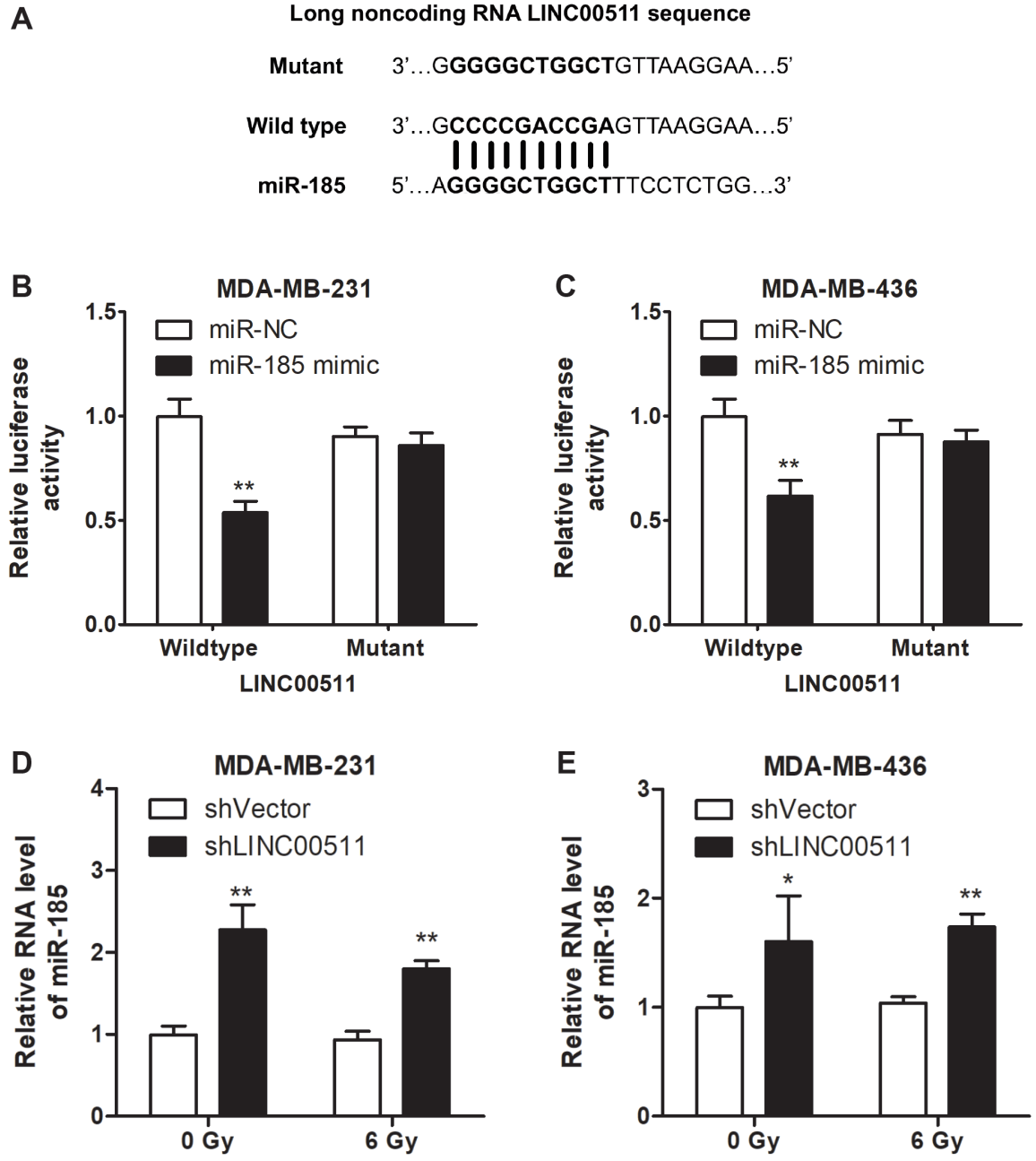

Figure 5. Competitively binding of LINC00511 mediates the miR-185 level. ***(A)*** Bioinformatics suggests the complementary binding sites within LINC00511 and miR-185. ***(B, C)*** The directive combination between LINC00511 and miR-185 in MDA-MB-231 ***(B)*** and MDA-MB-436 ***(C)*** verified by dual luciferase reporter assay. ***(D, E)*** The miR-185 level in MDA-MB-231 ***(D)*** and MDA-MB-436 ***(E)*** transfected with shLINC00511 or vector control under irradiation evaluated by qPCR. $^{**}p < 0.01$ compared to the control group.

tumor growth *in vivo* (Figure 2, 3 and 4). Together, these results indicate that LINC00511 is indeed involved in radiotherapy tolerance and poor prognosis in breast cancer patients.

Binding target microRNA as mRNA sponges is the molecular way lncRNAs mainly function[19]. In our study, bioinformatics analysis predicted the interaction between LINC00511 and miR-185 (Figure 5A). The luciferase activity of miR-185 was repressed by wildtype LINC00511, but not the mutant one, thus verifying the directive binding effect between them (Figure 5B and C). Further-

more, our results revealed that decreased expression of miR-185 in tumor cells could be reversed by LINC00511 knockdown (Figure 5 D and E). Thus, miR-185 is an apparently promising inhibitor of tumor development because of the oncogenic role of LINC00511 and its competitive action to miR-185. Indeed, the tumor-suppressing effect of miR-185 through multiple regulatory paths has already been reported in breast cancer. Upregulation of miR-185 has been reported to not only suppress the proliferation and metastasis of breast cancer by targeting high mobility group AT-hook 2 (HMGA2)[20], but also restrain the stemness of cancer cells by mediating Nanog[13]. Besides, miR-185 was suggested to be a possible indicator of chemotherapy response in breast cancer[21]. Nevertheless, our study represents the first attempt to demonstrate the important role of miR-185 in a radioresistance-associated function in breast cancer, and our observations are in agreement with other studies of miR-185 in radiosensitivity in other cancers [22-25].

Recently, few reports have addressed the role of STXBP4 in at least two kinds of cancers. STXBP4 was shown to promote cell proliferation and cell survival in lung squamous cell carcinoma (LSCC) mediated by platelet-derived growth factor receptor alpha[26]. Moreover, Rokudai et al[27] demonstrated that STXBP4 accelerates tumor progression via the N-terminally truncated isoform of p63 not only in LSCC, but in all human squamous cell carcinomas[28]. In a genome-wide association study, a single nucleotide polymorphism (SNP) at 17q22, a region encoding several enhancer elements that target STXBP4, was found to be causally associated with a high risk of breast cancer[29]. In addition, SNPs localized within STXBP4 were also shown to contribute to the development of breast cancer[30]. Few studies, if at all, have addressed the role of STXBP4 in radiotherapy response in cancer, especially breast cancer. Our study has shown that the LINC00511-miR185 axis increases the intracellular level of STXBP4 in breast cancer cells with a radioresistant feature (Figure 6). Altogether, our

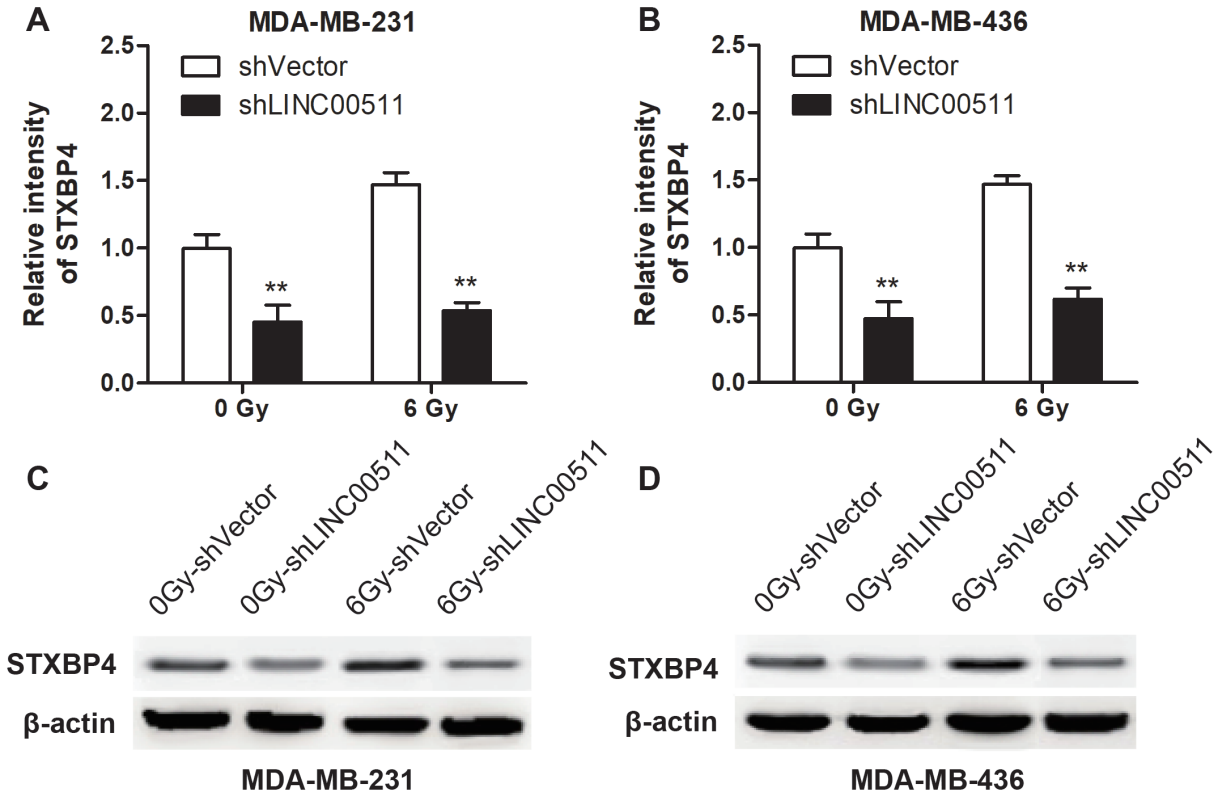

Figure 6. LINC00511 up-regulates STXBP4 expression by miR-185 inhibition. ***(A, B)*** The STXBP4 expression in MDA-MB-231 ***(A)*** and MDA-MB-436 ***(B)*** transfected with shLINC00511 or vector controls under irradiation tested by qPCR. ***(C, D)*** Western blotting showed the STXBP4 protein level in MDA-MB-231 ***(C)*** and MDA-MB-436 ***(D)*** transfected with shLINC00511 or vector controls under irradiation. *$p < 0.05$, **$p < 0.01$ compared to the control group.

results suggest that STXBP4 acts as a modulator of radiation response in a lncRNA-miRNA-dependent manner.

Conclusions

Both *in vitro* and *in vivo*, lncRNA LINC00511 expression promotes cell proliferation and tumor growth whereas silencing it enhances treatment response under radiation. Moreover, competitive binding of LINC00511 to its target miR-185 results in STXBP4 upregulation and increased radiosensitivity. We conclude that the LINC00511/miR-185/STXBP4 axis may be a promising therapeutic target for improving the prognosis of breast cancer.

Conflict of Interests

The Authors declare that they have no conflict of interests.

Data Availability Statement

The datasets used in this study are available from the corresponding author on reasonable request.

Disclosure of Financial Arrangements

The research and manuscript preparation are funded by Yue Chen.

Author Contributions

Guarantor of integrity of the entire study: Yue Chen; Study concepts: Yue Chen; Study design: Lan Liu; Literature research: Yan Zhu; Clinical studies: Yue Feng; Animal experiments: Anmin Liu, Yan Zhu; Molecular assay: Lan Liu Data acquisition: Lan Liu; Statistical analysis: Lan Liu; Manuscript preparation: Lan Liu; Manuscript editing: Lan Liu; Manuscript review: Yue Chen.

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