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RESEARCH ARTICLE

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# LncRNA LINC00210 regulated radiosensitivity of osteosarcoma cells via miR-342-3p/GFRA1 axis

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## Abstract

**Background:** Radiotherapy is an effective strategy for preventing cancer metastasis, including osteosarcoma. However, cancer radioresistance limits the efficiency of radiotherapy. Therefore, it is essential to investigate the mechanism of osteosarcoma radioresistance.

**Methods:** The osteosarcoma tissues and adjacent healthy tissues were collected from 53 osteosarcoma patients. The expression of LINC00210, miR-342-3p, and GFRA1 mRNA were determined using qRT-PCR. Cell viability, cell apoptosis, and cell surviving fraction were determined by MTT assay, flow cytometry, and colony formation assay, respectively. Western blot was performed to detect the protein levels. Luciferase assay was conducted to verify the relationship between LINC00210, miR-342-3p, and GFRA1.

**Results:** LINC00210 and GFRA1 were up-regulated, and miR-342-3p was down-regulated in osteosarcoma tissues and cells. The expression of LINC00210 in osteosarcoma was negatively related to miR-342-3p expression and positively associated with GFRA1. Besides, there was a negative correlation between LINC00210 and GFRA1 expression in osteosarcoma. Also, LINC00210 and GFRA1 were up-regulated, and miR-342-3p was down-regulated in osteosarcoma cells exposed to 4 Gy irradiation treatment. Furthermore, either LINC00210 knockdown or miR-342-3p overexpression enhanced the radiosensitivity of osteosarcoma cells. Moreover, LINC00210 increased GFRA1 expression via sponging miR-342-3p. Additionally, LINC00210 knockdown improved the radiosensitivity of osteosarcoma cells by regulating GFRA1 expression via sponging miR-342-3p.

**Conclusion:** LINC00210 modulated the radiosensitivity of osteosarcoma cells via the miR-342-3p/GFRA1 axis, making LINC00210 a novel target for improving radiotherapy efficiency in osteosarcoma.

**KEYWORDS**

GFRA1, LINC00210, miR-342-3p, osteosarcoma, radiosensitivity

## 1 | INTRODUCTION

Osteosarcoma is the most common malignant tumor in the bone of children and teenagers.[1,2] With the combination of advanced surgical techniques with chemotherapy strategies, the 5-year survival of younger osteosarcoma patients has improved from 20% to 70% in recent years.[1,3] However, the survival rate of osteosarcoma patients remains unsatisfactory due to the distant metastasis.[3,4] In response to metastasis, radiotherapy has emerged as a more efficient therapeutic strategy for osteosarcoma,[5,6] while radioresistance of tumor cells limited the availability of radiotherapy.[6,7] Thus, it is essential to investigate the mechanism of radioresistance for osteosarcoma cells.

Long non-coding RNAs (lncRNAs) are a kind of non-coding RNA that contains more than 200 nucleotides.[8] Accumulating evidence reported that lncRNAs exerted critical roles in cancer metastasis,[9,10] drug resistance,[11,12] and radiosensitivity[13,14] in many cancers, including osteosarcoma. For example, lncRNA TTN-AS1 modulated osteosarcoma cell drug resistance through the miR-134-5p/MBTD1 axis.[15] LncRNA LINC00210 is a vital lncRNA and plays an essential role in many cancers.[16,17] For example, LINC00210 presented aberrant expression in liver cancer, thyroid cancer, and non-small cell lung cancer (NSCLC).[16-18] LINC00210 could promote NSCLC progression by functioning as the sponge of miR-328-5p.[16] Besides, LINC00210 also increased the malignancy of thyroid cancer via regulating miR-195-5p/IGF1R.[18] However, the role of LINC00210 in osteosarcoma remains unclear.

MicroRNAs (miRNAs) are a member of non-coding RNAs that contains about 20 nucleotides.[19] MiRNAs also was proved to modulate cancer progression,[20] drug resistance,[21] and radiosensitivity.[22] For example, miR-122-5p could regulate the radiosensitivity of cervical cancer cells through targeting CDC25A.[23] miR-342-3p is a significant miRNA in cancer regulation.[24,25] miR-342-3p was reported to modulate metabolic reprogramming in triple-negative breast cancer.[24] Besides, miR-342-3p also regulated chemotherapy sensitivity in TBNC.[26] However, the effect of miR-342-3p on osteosarcoma was not established.

GDNF receptor alpha 1 (GFRA1) was a cell surface membrane receptor, and it was considered as an oncogene in some cancer.[27] For example, GFRA1 overexpression was related to the poor overall survival of pancreatic cancer patients and breast cancer patients.[28,29] Besides, GFRA1 could enhance the cisplatin-induced chemoresistance in osteosarcoma cells via promoting cell autophagy.[30] Therefore, GFRA1 was a promising target for the prevention of osteosarcoma chemoresistance, which indicated that GFRA1 might participate in the regulation of osteosarcoma radioresistances.

In the study, to investigate the mechanism of radioresistance for osteosarcoma cells, we investigated the effects of LINC00210, miR-342-3p, and GFRA1 on the regulation mechanism of osteosarcoma radioresistances and the relationship among LINC00210, miR-342-3p, and GFRA1 in osteosarcoma.

## 2 | MATERIALS AND METHODS

### 2.1 | Tissues collection and cell culture

Total of 53 osteosarcoma cancer patients diagnosed in Hunan Provincial People's Hospital were enrolled in the study. The tumor tissues and adjacent normal tissues were obtained from 53 osteosarcoma patients. All patients wrote informed consent. The Ethics Committee of Hunan Provincial People's Hospital approved the study.

### 2.2 | Cell culture

Human osteoblast cell line hFOB1.19 and human osteosarcoma cell lines (SOSP-9607 and U-2OS) were obtained from American Type Culture Collection (ATCC). Human osteosarcoma cell line F5M2 was Shengjing Hospital Affiliated to China Medical University. All cells were cultured in DMEM medium with 10% fetal bovine serum (FBS) (Gibco) at 37°C cell incubator with 5% $CO_2$.

### 2.3 | Irradiation treatment

The osteosarcoma cells were seeded into 6-wells plates and irradiated by a linear accelerator (Varian Medical Systems). The cells were exposed to 0 Gy, 2 Gy, 4 Gy, 6 Gy, 8 Gy, or 10 Gy for different times. After 24 hours, cells were harvested for subsequent experiments.

### 2.4 | Quantitative real-time polymerase chain reaction (qRT-PCR)

RNA was isolated from osteosarcoma tissues and cells using TRIzol reagent (Sigma). The extracted RNA was used to generate cDNA using TaqMan™ Reverse Transcription Reagents and TaqMan™ MicroRNA Reverse Transcription Kit (Thermo Fisher Scientific). Afterward, the qPCR assay was carried out utilizing SYBR™ Green PCR Master Mix and mirVana™ qRT-PCR miRNA Detection Kit (Thermo Fisher Scientific). The sequences of primers were as follows: LINC00210: F, 5′-AACACGTTAGCGGGTTCTCA-3, R: 5′-TCAAAAACCACCGAGGGAGG-3′[31]; miR-342-3p: F, 5′-TCCTCG CTCTCACACAGAAATC-3′, R: 5′-TATGGTTGTTCACGACTCCTTC AC-3′.[32] GFRA1: F: 5′-AGAAGCAGTTTCACCCAG-3′, R: 5′-ATCA TCACCACCACCATC-3′.[33] Data were normalized to the expression of *GAPDH* and *U6*, and calculated by the $2^{-\Delta\Delta Ct}$ method.

### 2.5 | MTT assay

Osteosarcoma cells were seeded into 96-wells plates and maintained in 37°C cell incubator with 5% $CO_2$ for 24 hours, 48 hours,

and 96 hours, respectively. At indicative time points, 20 μL MTT (5 mg/mL, Sigma) was added to the cells in per well. The cells were incubated with MTT for 4 hours, following by adding 150 μL DMSO (Sigma) to solubilize formazan. The absorbance at 490 nm was measured using a microplate reader.

### 2.6 | Flow cytometry

Osteosarcoma cells were harvested and washed with phosphate buffer solution (PBS). After centrifugation, the cells were resuspended in 1× binding buffer. Afterward, the cells were incubated with 5 μL of Annexin V-FITC and 10 μL of PI (BD) for 15 minutes in the dark. The proportion of cell apoptosis was then determined using flow cytometry (BD).

### 2.7 | Colony formation assay

Osteosarcoma cells were inoculated into 6-wells plates with 200 cells per well. The cells were cultured at 37°C cell incubator with 5% $CO_2$. Fourteen days later, the osteosarcoma cells were fixed by 4% paraformaldehyde for 5 minutes and dyed with 1% crystal violet for 30 seconds. The numbers of colonies containing more than 50 cells were determined.

### 2.8 | Cell transfection

The small interfering RNA (siRNA) negative control (si-con), siRNA of LINC00210 (si- LINC00210), miRNA control (miR-con), miR-342-3p overexpression plasmid, and anti-miR-342-3p were synthesized in Sangon Biotech (Sangon Biotech). To perform cell transfection, osteosarcoma cells were seeded into 6-wells plates and cultured for 24 hours at 37°C. The cells were then transfected with si-con, si- LINC00210, miR-con, miR-342-3p overexpression plasmid, or anti-miR-342-3p respectively using Lipofectamine 3000 (Thermo Scientific) according to the instruction of the manufacturer. After 48 hours of transfection, the cells were harvested for subsequent experiments.

### 2.9 | Western blot

Protein extraction was conducted by utilizing the RIPA lysis buffer (Thermo Scientific). The cell lysates were subjected to SDS-PAGE gels and transferred onto the PVDF membrane to separate proteins. After blocked by 4% skim milk for 1 hour, the membrane was probed with anti-Cyclin D1 (1:500), p21 (1:500), Bax (1:500), $Bcl_2$ (1:500), and β-actin (1:500) antibodies (Abcam) at 4°C overnight, following by incubating with Goat Anti-Human IgG H&L (HRP) antibody (Abcam). The blots were then visualized using ECL Western Blotting Substrate (Thermo Fisher Scientific) and quantified by Image J software (National Institutes of Health).

### 2.10 | Luciferase assay

The wild-type and mutant type 3′-untranslated region (3′-UTR) of GFRA1 and wild and mutant type of LINC00210 were constructed into the pGL3 vector (Promega). The wild LINC00210, mutant LINC00210, wild 3′-UTR of GFRA1, mutant 3′-UTR of GFRA1, miR-con, or miR-342-3p overexpression plasmid were transfected into osteosarcoma cells, respectively. The relative luciferase activity was measured using a microplate reader and calculated by normalizing to Renilla luciferase activity after 48 hours of transfection.

### 2.11 | Statistical analysis

Data statistical analysis was conducted by SPSS Statistics (version 22.0, Chicago, IL, USA). All data were presented as mean ± standard deviation (SD). The group differences were determined using the Student's *t* test, one-way ANOVA, and two-way ANOVA. Pearson's correlation analysis determined the correlation. $P < .05$ was identified as statistically significant.

## 3 | RESULTS

### 3.1 | LINC00210 and GFRA1 were up-regulated, and miR-342-3p was down-regulated in osteosarcoma tissue

The expression levels of LINC00210, miR-342-3p, and GFRA1 mRNA in osteosarcoma tissue were determined by qRT-PCR assay. Results showed that LINC00210 was up-regulated in osteosarcoma tissue compared to adjacent healthy tissues ($P < .05$, Figure 1A). Besides, miR-342-3p was down-regulated in osteosarcoma tissue compared to adjacent healthy tissues ($P < .05$, Figure 1B). The mRNA expression of GFRA1 was up-regulated in osteosarcoma tissue compared to adjacent healthy tissues ($P < .05$, Figure 1C). In addition, Pearson's correlation analysis results revealed a negative correlation between LINC00210 and miR-342-3p expression in osteosarcoma ($P < .05$, Figure 1D). There was a positive correlation between LINC00210 and GFRA1 expression in osteosarcoma ($P < .05$, Figure 1E), while a negative correlation between miR-342-3p and GFRA1 expression in osteosarcoma was present ($P < .05$, Figure 1F). Therefore, LINC00210 and GFRA1 were up-regulated, and miR-342-3p was down-regulated in osteosarcoma tissue.

### 3.2 | LINC00210 and GFRA1 were up-regulated, and miR-342-3p was down-regulated in osteosarcoma cells

The above results found that LINC00210 and GFRA1 were up-regulated, and miR-342-3p was down-regulated in osteosarcoma tissue. To further clarify the expression levels of LINC00210, miR-342-3p,

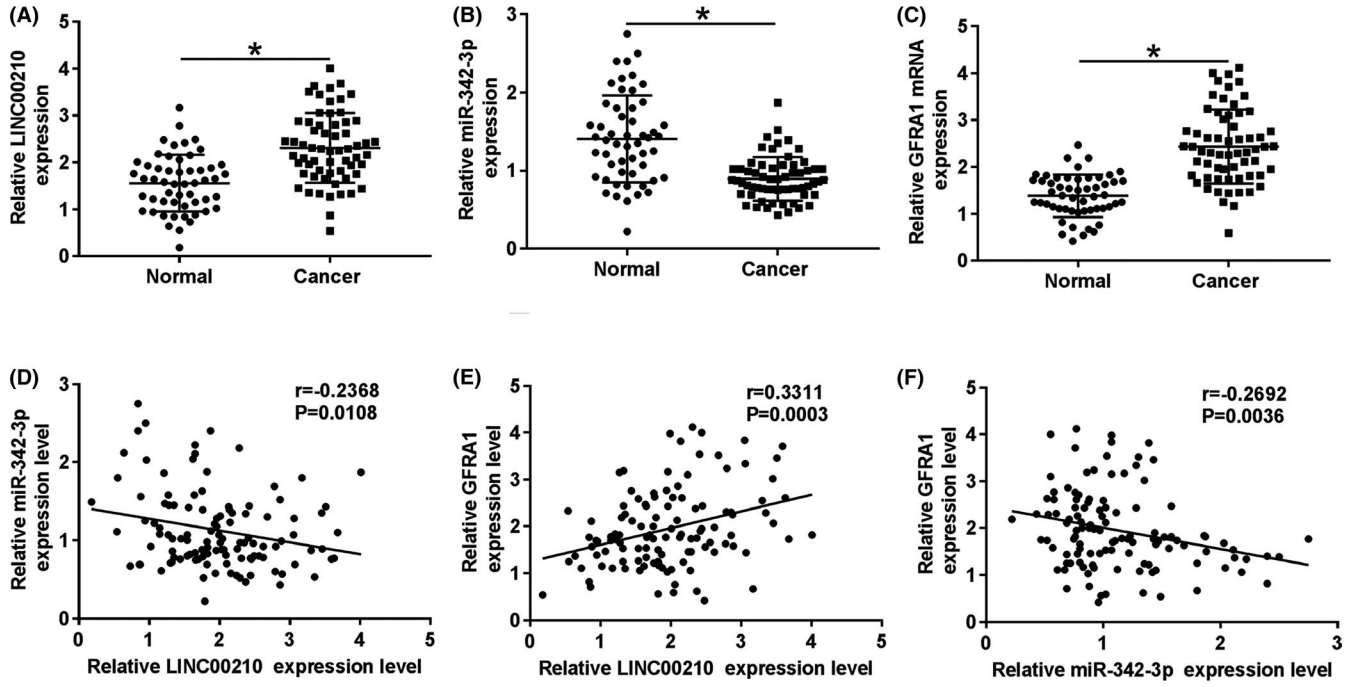


**FIGURE 1** The expression levels of LINC00210, miR-342-3p, and GFRA1 in osteosarcoma tissue. A, The expression of LINC00210 in osteosarcoma tissues and adjacent healthy tissues was determined by qRT-PCR. B, The expression of miR-342-3p in osteosarcoma tissues and adjacent healthy tissues was determined by qRT-PCR. C, The mRNA expression of GFRA1 in osteosarcoma tissues and adjacent healthy tissues was determined by qRT-PCR. D, The relationship between LINC00210 and miR-342-3p expression was analyzed by Pearson's correlation analysis. E, The relationship between LINC00210 and GFRA1 expression was analyzed by Pearson's correlation analysis. F, Pearson's correlation analysis analyzed the relationship of miR-342-3p and GFRA1 expression. *$P < .05$

and GFRA1 in osteosarcoma cells, we performed qRT-PCR assay. Results showed that the expression of LINC00210 was increased in osteosarcoma cell lines SOSP-9607, U-2OS, and F5M2 compared to human osteoblast cell line hFOB1.19 (all $P < .05$, Figure 2A). In addition, miR-342-3p was decreased in SOSP-9607, U-2OS, and F5M2 cells compared to hFOB1.19 cells (all $P < .05$, Figure 2B). The mRNA expression of GFRA1 was also higher in SOSP-9607, U-2OS, and F5M2 cells than that in hFOB1.19 cells ($P < .05$, Figure 2C). Furthermore, the expression levels of LINC00210, miR-342-3p, and GFRA1 in osteosarcoma cells exposed to 4 Gy irradiation treatment were also determined. Results revealed that LINC00210 was up-regulated in osteosarcoma cells after irradiation treatment in a time-dependent manner ($P < .05$, Figure 2D). However, the expression of miR-342-3p was decreased in osteosarcoma cells after irradiation treatment in a time-dependent manner ($P < .05$, Figure 2E). Moreover, the expression level of GFRA1 was increased with the prolonged duration of irradiation treatment ($P < .05$, Figure 2F). Hence, LINC00210 and GFRA1 were up-regulated, and miR-342-3p was down-regulated in osteosarcoma cells with irradiation treatment.

### 3.3 | LINC00210 knockdown enhanced radiosensitivity of osteosarcoma cells

Due to the up-regulation of LINC00210 to irradiation treatment in osteosarcoma cells, we inferred that LINC00210 might influence the radiosensitivity of osteosarcoma cells. Therefore, the LINC00210 knockdown was conducted to investigate the effect of LINC00210 on osteosarcoma cells. The knockdown efficiency of si-LINC00210 was confirmed by qRT-PCR assay in SOSP-9607 cells ($P < .05$, Figure 3A). The SOSP-9607 cells transfected with si-LINC00210 were exposed to 4 Gy irradiation treatment, and then the cell viability was determined. MTT results showed that both LINC00210 knockdown and irradiation treatment significantly decreased cell viability, respectively (all $P < .05$, Figure 3B), while LINC00210 knockdown further decreased the cell viability of SOSP-9607 cells exposed to irradiation treatment ($P < .05$, Figure 3B). In addition, both LINC00210 knockdown and irradiation treatment significantly induced cell apoptosis, respectively (all $P < .05$, Figure 3C). However, the combination of LINC00210 knockdown and irradiation treatment further induced cell apoptosis ($P < .05$, Figure 3C). LINC00210 knockdown or irradiation treatment alone also inhibited the levels of Cyclin D1 and Bcl-2 and increased the levels of p21 and Bax (all $P < .05$, Figure 3D). The combination of LINC00210 knockdown and irradiation treatment further inhibited the levels of Cyclin D1 and Bcl-2 and increased the levels of p21 and Bax (all $P < .05$, Figure 3D). Moreover, colony formation assay results revealed that LINC00210 knockdown also markedly inhibited the surviving fraction of SOSP-9607 cells exposed to irradiation treatment (Figure 3E). Thus, these results indicated that the LINC00210 knockdown improved the radiosensitivity of osteosarcoma cells.

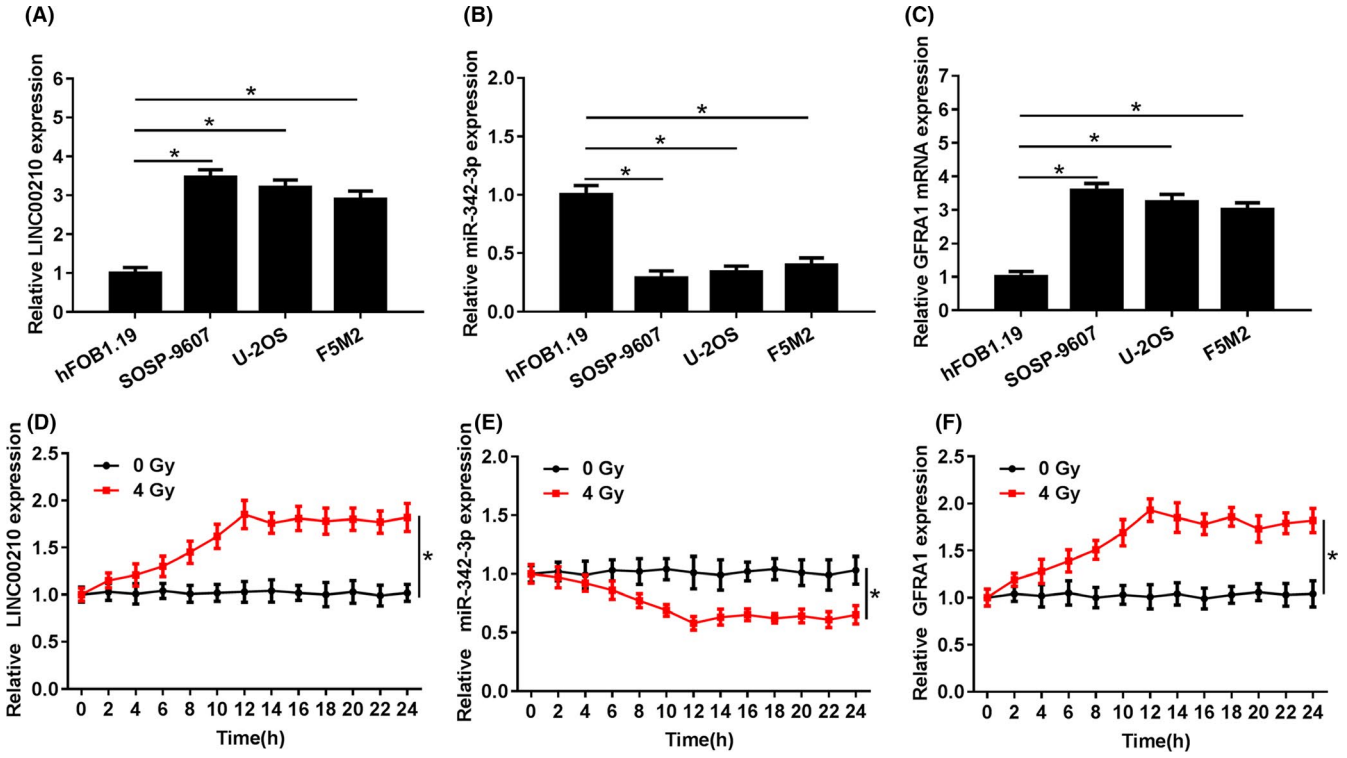


**FIGURE 2** The expression levels of LINC00210, miR-342-3p, and GFRA1 in osteosarcoma cells. A, The expression of LINC00210 in human osteoblast cell line hFOB1.19 and osteosarcoma cell lines SOSP-9607, U-2OS, and F5M2 was determined by qRT-PCR. B, The expression of miR-342-3p in human osteoblast cell line hFOB1.19 and osteosarcoma cell lines SOSP-9607, U-2OS, and F5M2 was determined by qRT-PCR. C, The mRNA expression of GFRA1 in human osteoblast cell line hFOB1.19 and osteosarcoma cell lines SOSP-9607, U-2OS, and F5M2 was determined by qRT-PCR. D, The expression of LINC00210 in SOSP-9607 cells exposed to 4 Gy irradiation treatment was detected by qRT-PCR. E, The expression of miR-342-3p in SOSP-9607 cells exposed to 4 Gy irradiation treatment was detected by qRT-PCR. F. The mRNA expression of GFRA1 in SOSP-9607 cells exposed to 4 Gy irradiation treatment was detected by qRT-PCR. *$P < .05$

### 3.4 | miR-342-3p overexpression enhanced radiosensitivity of osteosarcoma cells

Based on the down-regulation of miR-342-3p to irradiation treatment in osteosarcoma cells, miR-342-3p might also affect the radiosensitivity of osteosarcoma cells. Hence, miR-342-3p overexpression plasmid was transfected to SOSP-9607 cells to increase the expression of miR-342-3p, which was confirmed by qRT-PCR assay ($P < .05$, Figure 4A). The cell viability of SOSP-9607 with miR-342-3p overexpression and irradiation treatment was detected. Results revealed that either miR-342-3p overexpression or irradiation treatment suppressed cell viability (all $P < .05$, Figure 4B). The combination of miR-342-3p overexpression and irradiation treatment suppressed cell viability further inhibited cell viability ($P < .05$, Figure 4B). Besides, both miR-342-3p overexpression and irradiation treatment significantly promoted cell apoptosis, respectively (all $P < .05$, Figure 4C), while miR-342-3p overexpression further increased cell apoptosis of SOSP-9607 cells exposed to irradiation treatment ($P < .05$, Figure 4C). In addition, miR-342-3p overexpression or irradiation treatment alone significantly inhibited the levels of Cyclin D1 and Bcl-2 and increased the levels of p21 and Bax (all $P < .05$, Figure 4D). However, the combination of miR-342-3p overexpression and irradiation treatment further inhibited the levels of Cyclin D1 and Bcl-2 and increased the levels of p21 and Bax ($P < .05$, Figure 4D). Furthermore, miR-342-3p overexpression also markedly inhibited the surviving fraction of SOSP-9607 cells exposed to irradiation treatment (Figure 4E). These results indicated that miR-342-3p overexpression improved the radiosensitivity of osteosarcoma cells.

### 3.5 | LINC00210 increased GFRA1 expression via targeting miR-342-3p

LncRNAs usually modulated gene expression through functioning as the miRNA sponges. Bioinformatics analyses revealed that LINC00210 might bind to miR-342-3p, and miR-342-2p might target to GFRA1. The putative binding site of LINC00210 and miR-342-3p was presented in Figure 5A. The potential binding site of miR-342-3p and GFRA1 was shown in Figure 5B. To further explore the relationship between LINC00210, miR-342-3p, and GFRA1, the expression levels of miR-342-3p and LINC00210 were changed by cell transfection. The expression of miR-342-3p was significantly increased by transfecting of miR-342-3p overexpression plasmid ($P < .05$), while it was inhibited by anti-miR-342-3p ($P < .05$, Figure 5C). The expression of LINC00210 was markedly increased by transfecting of pcDNA-LINC00210 ($P < .05$) and decreased after transfection of

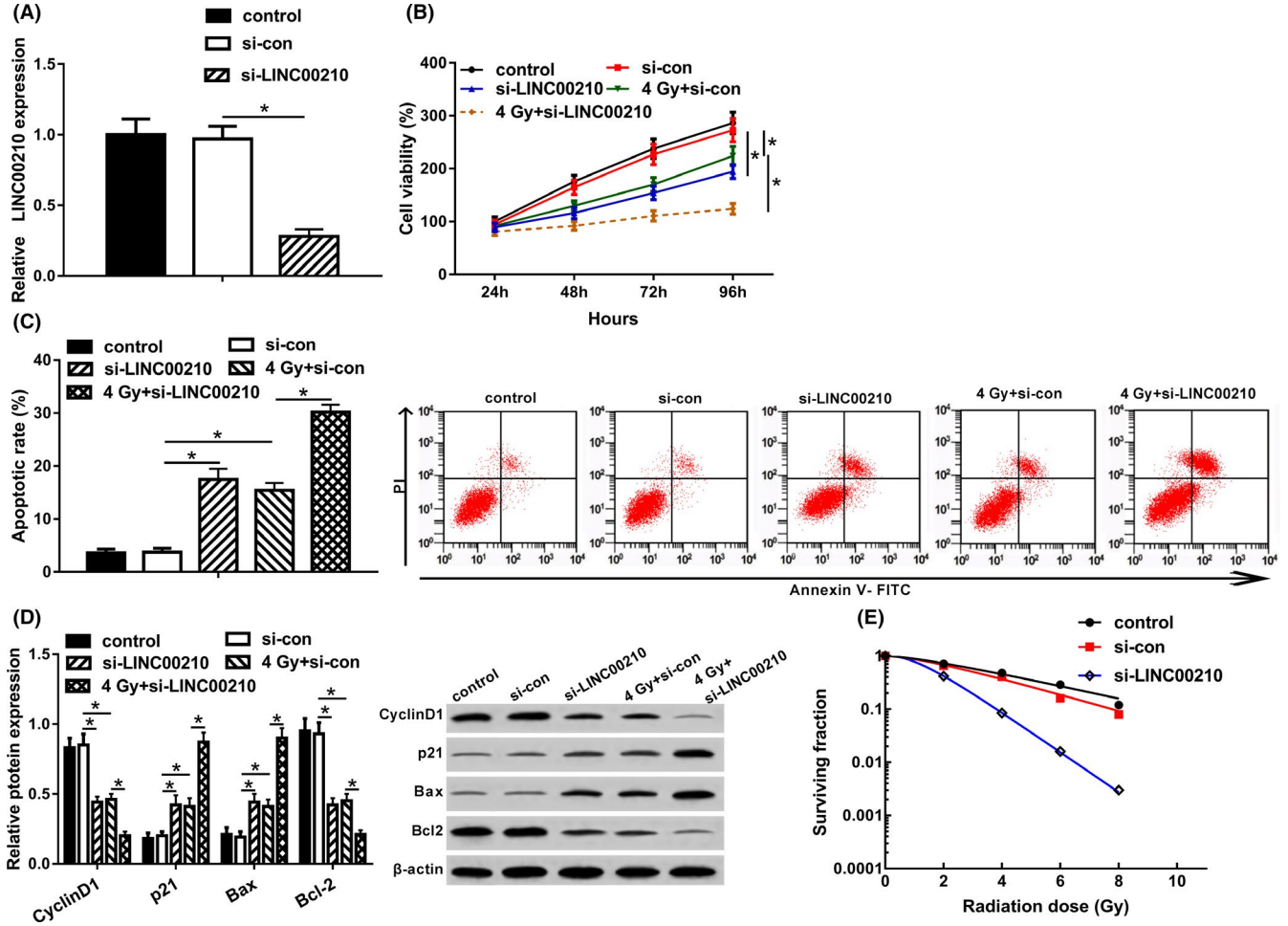


**FIGURE 3** Effect of LINC00210 knockdown on radiosensitivity of osteosarcoma cells. A, The expression of LINC00210 in SOSP-9607 cells transfected with si-LINC00210 was detected using qRT-PCR. B, MTT assay was used to detect cell viability of SOSP-9607 cells transfected with si-LINC00210 and exposed to 4 Gy irradiation treatment. C, The cell apoptosis of SOSP-9607 cells transfected with si-LINC00210 and exposed to 4 Gy irradiation treatment was determined using Flow cytometry. D, Western blot was used to determine the levels of Cyclin D1, Bcl-2, p21, and Bax in SOSP-9607 cells transfected with si-LINC00210 and exposed to 4 Gy irradiation treatment. E, The surviving fraction of SOSP-9607 cells transfected with si-LINC00210 and exposed to 4 Gy irradiation treatment was determined using colony formation assay. *$P < .05$

si-LINC00210 ($P < .05$, Figure 5D). Luciferase assay revealed that miR-342-3p overexpression significantly decreased the luciferase activity of SOSP-9607 cells transfected with wild type of LINC00210 ($P < .05$), while had no significant effects on luciferase activity of cells transfected with the mutant type of LINC00210 (Figure 5E). In addition, miR-342-3p overexpression significantly decreased the luciferase activity of SOSP-9607 cells transfected with wild type of GFRA1 3′-UTR region ($P < .05$), while had no significant effects on luciferase activity of cells transfected with the mutant type of GFRA1 3′-UTR region (Figure 5F). Furthermore, LINC00210 overexpression significantly inhibited the expression of miR-342-3p ($P < .05$), while the LINC00210 knockdown dramatically increased the expression of miR-342-3p ($P < .05$, Figure 5G). Moreover, LINC00210 knockdown significantly inhibited the mRNA expression of GFRA1 ($P < .05$), but was reversed by down-regulation of miR-342-3p ($P < .05$, Figure 5H). Therefore, these results indicated that LINC00210 increased GFRA1 expression via targeting miR-342-3p.

### 3.6 | LINC00210 regulated radiosensitivity of osteosarcoma cells via miR-342-3p/GFRA1 axis

To further investigate the modulation mechanism of LINC00210 on the radiosensitivity of osteosarcoma cells, SOSP-9607 cells were transfected with si-LINC00210, anti-miR-342-3p, or GFRA1 plasmid and exposed to 4 Gy irradiation treatment. The mRNA expression of GFRA1 was inhibited by LINC00210 ($P < .05$), which was reversed by anti-miR-342-3p or GFRA1 plasmid (all $P < .05$, Figure 6A). MTT assay results showed that LINC00210 knockdown significantly inhibited cell viability of SOSP-9607 cells exposed to irradiation treatment ($P < .05$), which were abolished by miR-342-3p down-regulation or GFRA1 up-regulation (all $P < .05$, Figure 6B). Besides, the induction effects of LINC00210 knockdown on cell apoptosis of SOSP-9607 cells exposed to irradiation treatment were reversed by miR-342-3p down-regulation or GFRA1 up-regulation (all $P < .05$, Figure 6C). In addition, LINC00210 knockdown inhibited

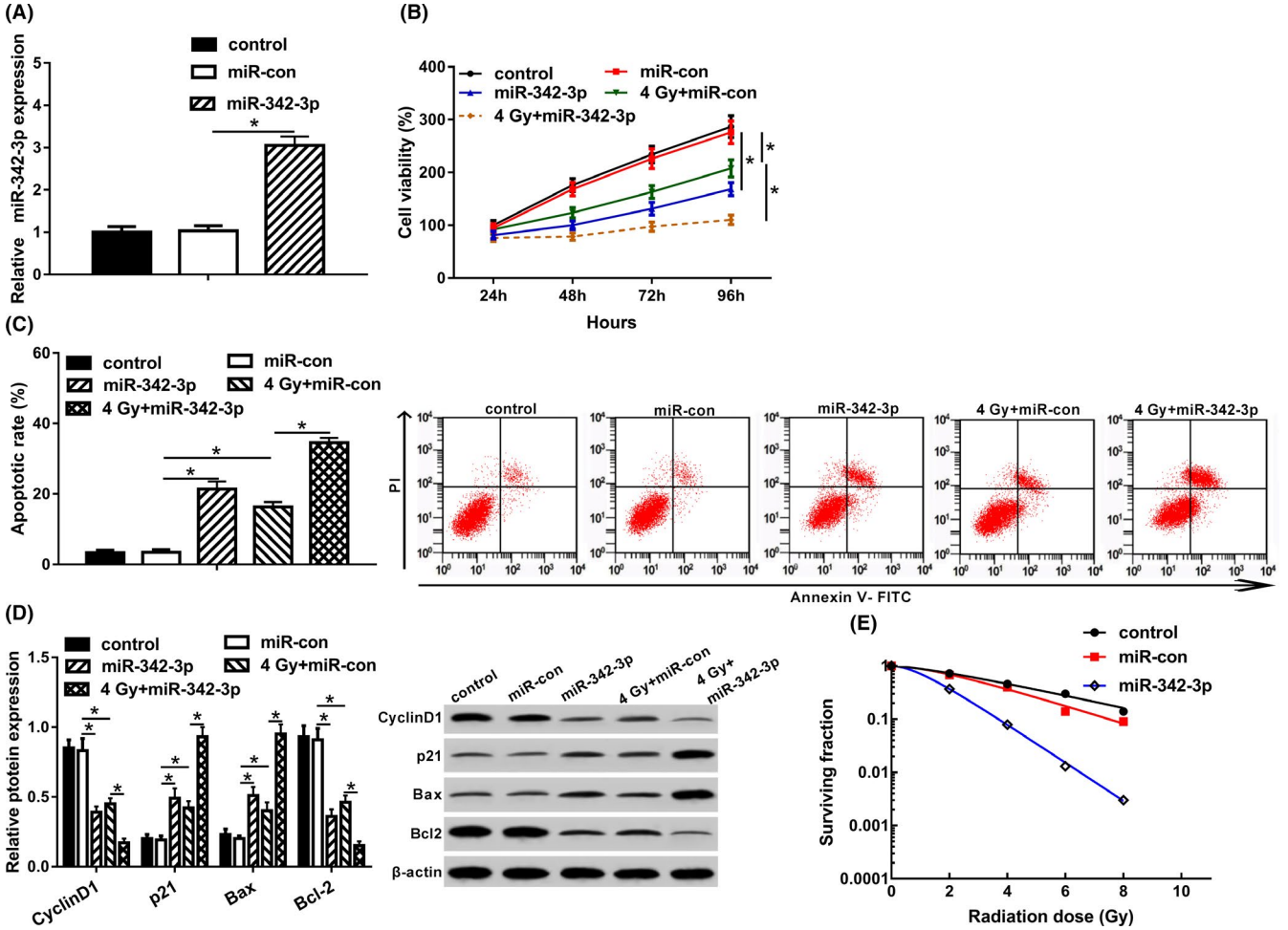


**FIGURE 4** Effect of miR-342-3p overexpression on radiosensitivity of osteosarcoma cells. A, The expression of miR-342-3p in SOSP-9607 cells transfected with miR-342-3p overexpression plasmid was detected using qRT-PCR. B, MTT assay was used to detect cell viability of SOSP-9607 cells transfected with miR-342-3p overexpression plasmid and exposed to 4 Gy irradiation treatment. C, The cell apoptosis of SOSP-9607 cells transfected with miR-342-3p overexpression plasmid and exposed to 4 Gy irradiation treatment was determined using Flow cytometry. D, Western blot was used to determine the levels of Cyclin D1, Bcl-2, p21, and Bax in SOSP-9607 cells transfected with miR-342-3p overexpression plasmid and exposed to 4 Gy irradiation treatment. E, The surviving fraction of SOSP-9607 cells transfected with miR-342-3p overexpression plasmid and exposed to 4 Gy irradiation treatment was determined using colony formation assay. $^*P < .05$

the levels of Cyclin D1 and Bcl-2 and increased the levels of p21 and Bax of SOSP-9607 cells exposed to irradiation treatment (all $P < .05$, Figure 6D). However, the effects of LINC00210 knockdown on the expression levels of Cyclin D1, Bcl-2, p21, and Bax were abolished by miR-342-3p down-regulation or GFRA1 up-regulation ($P < .05$, Figure 6D). Furthermore, LINC00210 knockdown significantly inhibited the surviving fraction of SOSP-9607 cells exposed to irradiation treatment but was reversed by miR-342-3p down-regulation or GFRA1 up-regulation (Figure 6E). These results indicated that LINC00210 regulated the radiosensitivity of osteosarcoma cells via the miR-342-3p/GFRA1 axis.

## 4 | DISCUSSION

Osteosarcoma is one of the most common malignant tumors in bone, accompanied by a high metastasis rate.[3] Radiotherapy is an effective strategy for preventing cancer metastasis.[6] However, the radioresistance of cancer cells limits the efficiency of radiotherapy, including osteosarcoma. Therefore, it is crucial to explore the mechanism of osteosarcoma radioresistance and improve the efficiency of radiotherapy for osteosarcoma.

LncRNAs were proved to modulate cancer progression and radiosensitivity[13,14] in many cancers, including osteosarcoma. In the study, we explored the role of LINC00210 in osteosarcoma radioresistance. Results revealed that LINC00210 was up-regulated in osteosarcoma tissues and cells, which was consistent with previous reports.[17] The expression of LINC00210 was increased in NSCLC,[16] liver cancer,[17] and thyroid cancer.[18] Besides, the expression of LINC00210 was further increased in osteosarcoma cells with irradiation treatment, which indicated that LINC00210 might involve in the modulation of osteosarcoma radioresistance. Hence, the effects of LINC00210 on osteosarcoma radioresistance were investigated. The results indicated that the LINC00210 knockdown enhanced

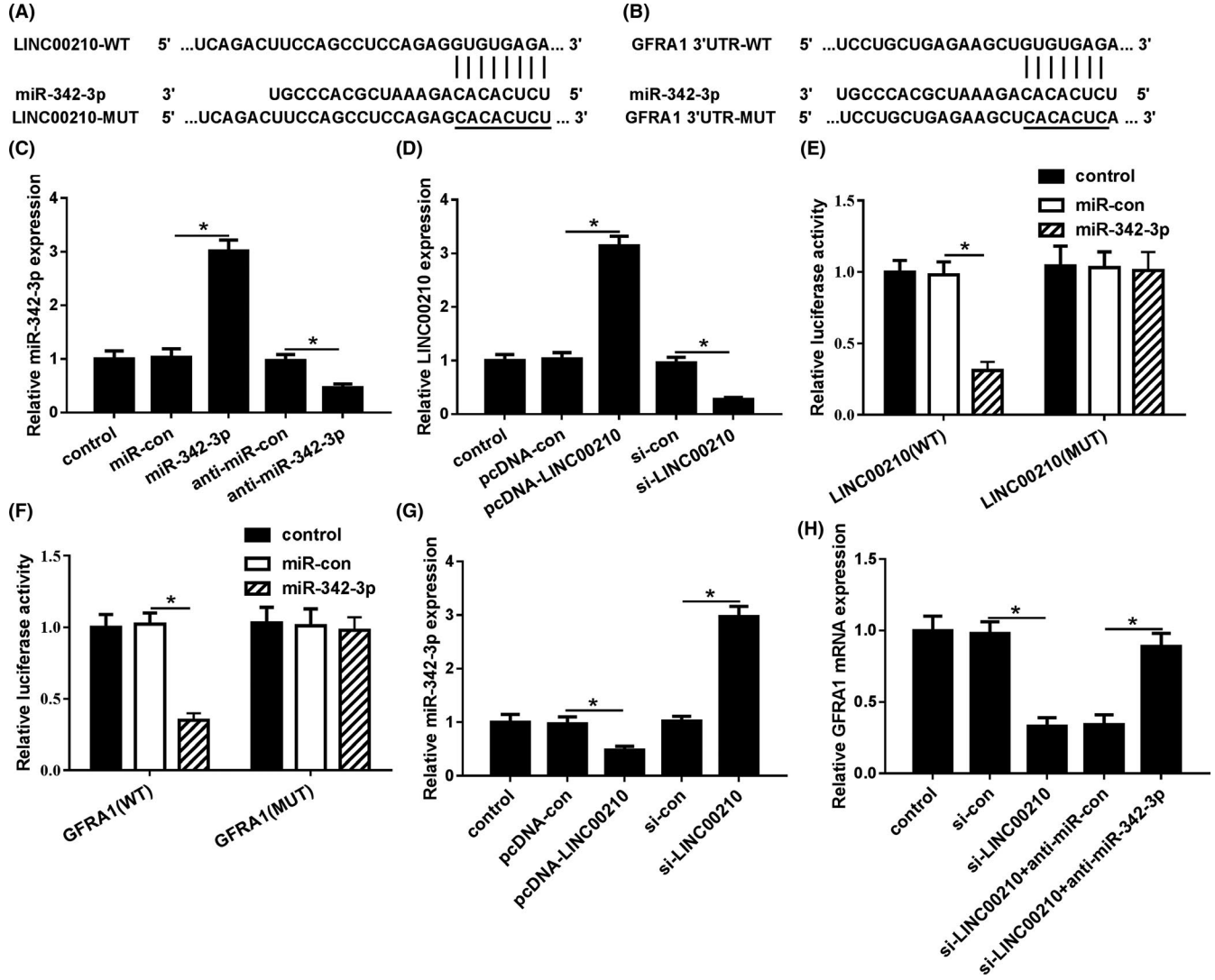


**FIGURE 5** LINC00210 increased GFRA1 expression via targeting miR-342-3p. A, The putative binding site of LINC00210 and miR-342-3p was presented. B, The putative binding site of miR-342-3p and GFRA1 was shown. C, The expression of miR-342-3p in SOSP-9607 cells transfected with miR-342-3p overexpression plasmid or anti-miR-342-3p was determined using qRT-PCR. D, The expression of LINC00210 in SOSP-9607 cells transfected with pcDNA-LINC00210 or si-LINC00210 was determined using qRT-PCR. E, Luciferase assay was used to detect the relationship between LINC00210 and miR-342-3p. F, Luciferase assay was used to detect the relationship of miR-342-3p and GFRA1. G, The expression of miR-342-3p in SOSP-9607 cells transfected with pcDNA-LINC00210 or si-LINC00210 was determined using qRT-PCR. H, The mRNA expression of GFRA1 in SOSP-9607 cells transfected with si-LINC00210 and anti-miR-342-3p was determined using qRT-PCR. *$P < .05$

the radiosensitivity of osteosarcoma cells. At present, there was no study reported the role of LINC00210 in cancer radiosensitivity.

MiRNAs also participated in the regulation process of cancer progression and radiosensitivity. The expression of miR-342-3p was demonstrated to down-regulate in cancer tissues and exerted vital roles in cancer regulation.[24,34] In the present study, miR-342-3p was found to decrease in osteosarcoma tissues and cells. In addition, irradiation treatment inhibited the expression of miR-342-3p. In TBNC, miR-342-3p was proved to regulate the chemotherapy sensitivity of cancer cells.[26] Therefore, we supposed that miR-342-3p could modulate radioresistance in osteosarcoma. The results agreed with the hypothesis that miR-342-3p overexpression enhanced the radiosensitivity of osteosarcoma cells.

LncRNAs usually modulated gene expression through functioning as the miRNA sponges.[35] To investigate the mechanism of LINC00210 on osteosarcoma radioresistance, we explored the relationship between LINC00210 and miR-342-3p. Results revealed a positive correlation between LINC00210 and GFRA1 expression in osteosarcoma tissues. Besides, we found that LINC00210 could bind to miR-342-3p. In addition, GFRA1, a novel target for osteosarcoma chemoresistance prevention, was presented up-regulation in osteosarcoma tissues and cells. Irradiation treatment also increased the expression of GFRA1. Furthermore, GFRA1 was proved to a target of miR-342-3p, and LINC00210 could regulate the expression of GFRA1 via binding to miR-342-3p. Therefore, we concluded that LINC00210 increased GFRA1 expression by targeting miR-342-3p. These results were an

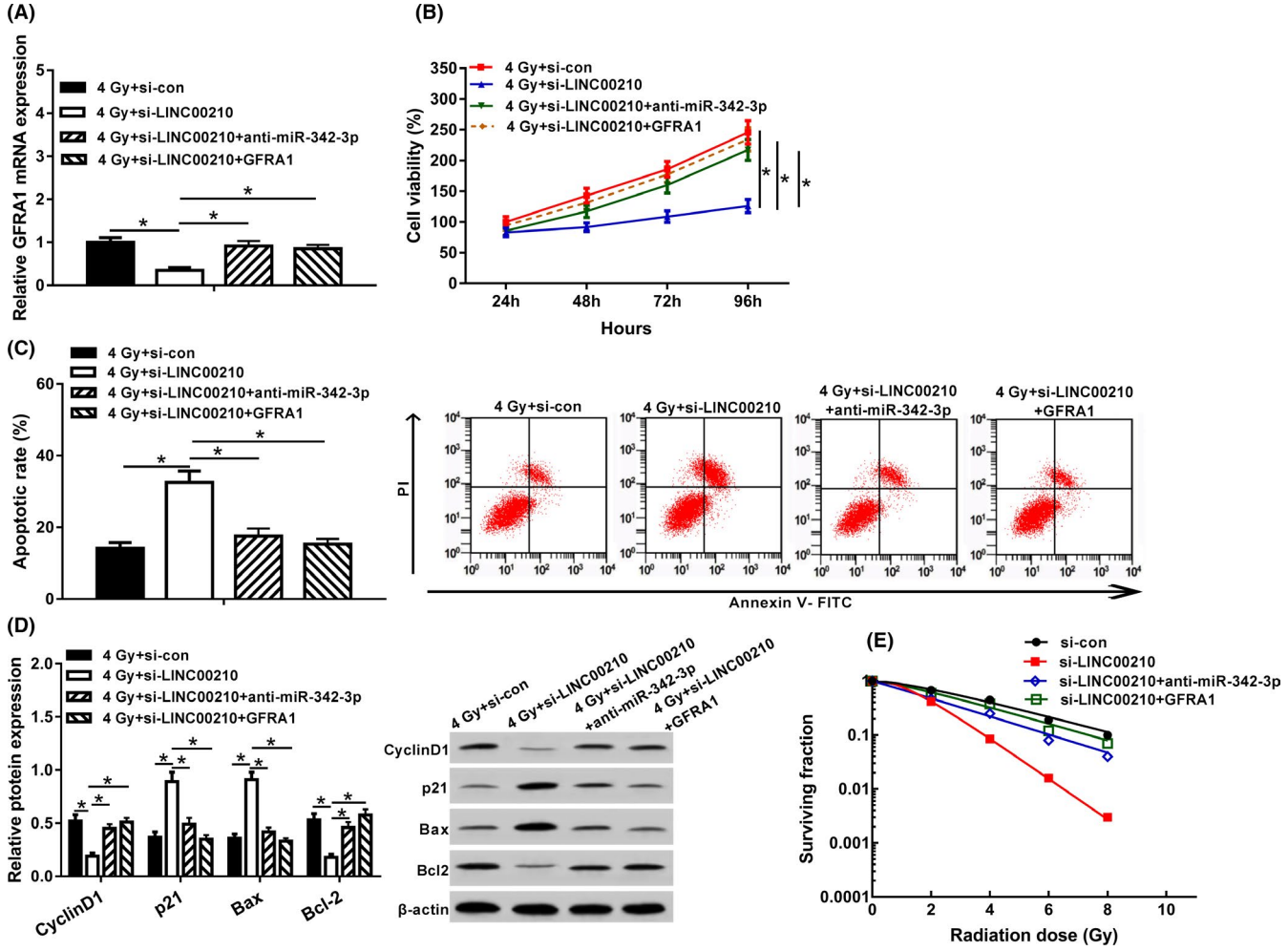


**FIGURE 6** LINC00210 regulated radiosensitivity of osteosarcoma cells via the miR-342-3p/GFRA1 axis. SOSP-9607 cells transfected with si-LINC00210, anti-miR-342-3p, or GFRA1 plasmid and exposed to 4 Gy irradiation treatment. A, The mRNA expression of GFRA1 in SOSP-9607 cells was determined using qRT-PCR. B, MTT assays were used to determine the cell viability of SOSP-9607 cells. C, Flow cytometry was used to detect cell apoptosis of SOSP-9607 cells. D, Western blot was used to determine the levels of Cyclin D1, Bcl-2, p21, and Bax in SOSP-9607 cells. E, The surviving fraction of SOSP-9607 cells was determined using colony formation assay. *$P < .05$

agreement with the previous study. LINC00210 was found to function as a sponge of miR-328-5p in nasopharyngeal carcinoma.[31] In thyroid cancer, LINC00210 could regulate the expression of IGF1R via sponging miR-195-5p.[18] Additionally, GFRA1 modulated osteosarcoma chemoresistance,[30] indicating that GFRA1 might involve in osteosarcoma radioresistances regulation. Thus, we speculated that LINC00210 might improve osteosarcoma radioresistances by regulating GFRA1 expression via binding to miR-342-3p. Therefore, the role of the LINC00210/ miR-342-3p/GFRA1 axis in osteosarcoma radioresistance was determined. Results indicated that LINC00210 knockdown enhanced the radiosensitivity of osteosarcoma cells by regulating the expression of GFRA1 via sponging miR-342-3p. Based on the literature, the role of LINC00210/miR-342-3p/GFRA1 axis in cancer radioresistance was not reported in cancers. Hence, the current study for the first time proved that LINC00210/ miR-342-3p/GFRA1 axis contributes to osteosarcoma radioresistance. The LINC00210/miR-342-3p/GFRA1 axis might be novel therapeutic targets to improve radiotherapy efficiency for osteosarcoma.

## 5 | CONCLUSION

The study demonstrated that LINC00210 regulated GFRA1 expression via sponging miR-342-3p and modulated radiosensitivity of osteosarcoma cells via miR-342-3p/GFRA1 axis. These findings provided novel therapeutic targets to improve radiotherapy efficiency for osteosarcoma.

### AUTHOR CONTRIBUTIONS

Bin Sheng and Zhi-jun Wang designed the research; Pan He and Yong-qiang Xu conducted the research; Pan He, Zhi-jun Wang, and Yong-qiang Xu conducted statistical analysis; Pan He and Yong-qiang Xu wrote the article; Zhi-jun Wang and Sheng Bin revised the article.

### ETHICAL APPROVAL

The experiments involved in human participants were performed according to the declaration of Helsinki.

## DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article.

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