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Research article

Downregulation of circSTX6 suppresses tumor progression while facilitating radiosensitivity in cervical squamous cell carcinoma

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ABSTRACT

Background: cervical squamous cell carcinoma (CSCC) is the second gynecological tumors that seriously threaten women's life quality. Circular RNA (circRNA) is related with cervical cancer carcinogenesis and radiosensitivity.
Aim: To investigate the performance of hsa_circ_0007905 (circSTX6) on regulating cellular activities and radiosensitivity in CSCC.
Methods: The relative expression of circSTX6 in different tissue samples was detected by RT-qPCR. The cellular activity influence of circSTX6 in cervical cancer cells was measured by CCK-8 and Transwell assays. The survival fractions of cancer cells were detected after the radiation treatment to explore the relationship between circSTX6 and radiosensitivity of cervical cancer. The downstream miRNAs were predicted and analyzed. Rescue experiments confirmed their targeting relationship. Bioinformatic analysis was performed to identify the potential targets of miR-203a-3p.
Results: circSTX6 was increased and miR-203a-3p was decreased in cervical cancer tissues and radio-resistant tissues. CircSTX6 expression was related to the patient's survival rates. CircSTX6 absence decreased cervical cancer cell proliferation and invasion while enhancing the sensitivity of cervical cancer cells to radiotherapy by regulating miR-203a-3p. RAB27B may be a target of miR-203a-3p.
Conclusion: circSTX6 may be a clinical prognostic biomarker in CSCC. The absence of circSTX6 inhibits cellular behaviors and increases the sensitivity of cervical cancer cells to radiation by modulating miR-203a-3p/RAB27B axis.

1. Introduction

As a common gynecological tumor in the female reproductive system, cervical squamous cell carcinoma (CSCC) is a serious threat

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to women's health [1]. The vast proportion of cervical cancer cases have occurred in developing countries. Both the incidence and death rates from this cancer have been trending upward annually in China [2]. Nearly all cases of cervical cancer are associated with certain types of HPV infection. With advancement of diagnosis, such as liquid-based thin-layer cytology combined with HPV virus detection, colposcopy, and histopathology, has been widely popularized, which makes the early detection and diagnosis of cervix possible. Enduring infection of high-risk HPV infections contributes to the risk of cervical cancer. The preponderance of cervical cancer cases can be traced back to HPV infections [3]. In the aspect of treatment, cervical cancer patients are mainly treated by surgery, and for advanced cervical cancer, brachytherapy combined with external radiation therapy is an integral part of radiotherapy [4]. Synchronous chemoradiotherapy is the gold-standard treatment approach for individuals with pathological cervical cancer at stage IIB or above. With the improvement of treatment, the survival rate of early cervical cancer patients has increased. However, there are still a considerable number of cases with local recurrence and distant metastasis after treatment in clinical practice. Various factors, such as HPV status and individual differences among patients, will affect the effect of radiotherapy. Studying the regulatory mechanism of radiation resistance and enhancing the sensitivity of cervical cancer to radiotherapy is the key to improving the prognosis of cervical cancer, reducing complications after radiotherapy, and improving the quality of life of cervical cancer.

Circular RNA (circRNA) is aberrantly expressed in various tumors and has the potential to act as biomarkers to participate in the diagnosis and prognosis progression to be novel predictive targets [5]. Some circRNAs can participate in the repair of DNA damage by silencing DNA damage, transmitting damage signals, repairing damaged DNA, activating cell checkpoints, and inducing apoptosis [6]. Hsa_circ_0007905 (circSTX6) was aberrantly overexpressed and involved in tumorigenesis of several cancers, such as pancreatic ductal adenocarcinoma and hepatocellular carcinoma [7,8]. A profile study in cervical cancer indicated that circSTX6 was one of the upregulated circRNAs [9]. However, there is a lack of research that explores the specific function of circSTX6 in HPV-positive CSCC and its potential association with radiation therapy.

Considering that over 99 % of cervical cancer are caused by high-risk HPV infection, in this study, we further delved into the expression of circSTX6 in CSCC and the effect of circSTX6 on cellular activities and radiosensitivity of HPV-positive cervical cancer cells and its potential regulatory mechanism.

2. Materials and methods

2.1. Source of experimental tissue

The tumor tissue specimens were gathered from HPV-positive CSCC patients who were diagnosed in The 2nd Affiliated Hospital of Harbin Medical University from 2020 to 2022. Cervical biopsy was performed in patients with cervical cancer before external-beam radiotherapy to obtain external-beam radiotherapy to obtain tumor tissues before radiotherapy. The dose for external-beam radiotherapy to primary cervical cancer and pelvic lymph nodes was a total of 40–50 Gy (1.8–2.0 Gy daily fractions using 18 or 25 MV photons). After 25 fractions of external-beam radiotherapy, a biopsy was performed again in patients whose tumors did not achieve complete response according to MRI examination to obtain tumor tissues after radiotherapy. All cases were diagnosed as cervical squamous carcinoma by experienced pathologists, and the effectiveness of radiotherapy was evaluated according to the Response Evaluations Criteria for Solid Tumors (RECIST) rules [10]. The grouping criteria were as follows: radiosensitive patients were defined as patients with complete tumor regression after radical radiotherapy and no visible tumor at 6 months after the end of treatment. The radioresistant patients were defined as those with incomplete tumor regression, visible tumor at 6 months after the end of treatment, and residual tumor confirmed by cervical biopsy. According to the evaluation results, the samples were divided into a radiosensitive group (n = 51) and a radioresistant group (n = 58). All participants underwent preoperative clinical staging and provided written informed consent, with the process being ethically reviewed and approved by the Ethics Committee of The 2nd Affiliated Hospital of Harbin Medical University.

2.2. Cell culture and transfection

The cervical epithelial cell line End1/E6E7 and human cervical cancer cells Me180 (HPV68), SiHa (HPV16), and HeLa (HPV18) were purchased from the Cell Bank of the Chinese Academy of Sciences. The culture condition was DMEM medium containing 10%FBS and a 5 % CO_2 cel incubator at 37 °C. The medium was changed every 2–3 days depending on cell growth.

CircSTX6 siRNA (si-circSTX6, 5′-GGACAAUGUGAUGAAGAAACU-3′), siRNA negative control (si-NC, 5′-UCACAACCUCCUAGAAAGAGUAGA-3′), miR-203a-3p mimic (miR-203 mimic, 5′-GUGAAAUGUUUAGGACCACUAG-3′), mimic NC (5′-TCGCCACATGATCGCCTAAGT-3′), miR-203a-3p inhibitor (anti-miR-203, 5′-CUAGUGGUCCUAAACAUUUCAC-3′), and inhibitor NC (anti-NC, 5′-UUCUCCGAACGUGUCACGUTT-3′) were obtained from Genepharma (Suzhou, China). Before transfection, 1.5–2.0 × 10^5 cells/well were seeded in 6-well plates, and a complete culture medium was added to each well. Then, transfection or co-transfection was carried out according to the procedure of Lipofectamine 2000 (Invitrogen, USA).

2.3. Cell radiotherapy

Following transfection, cells from each group were placed in the cell incubator for 24 h. Subsequently, the cells requiring radiotherapy were taken out and irradiated with 4 Gy of 6 MV X-rays (Elekta, Stockholm, Sweden), and then returned to the incubator for another 24 h. The radioactive source distance was 100 cm. After being co-cultured for 48, the cells were used for subsequent experiments.

2.4. RNA isolation and RT-qPCR

The samples of cervical cancer (0.1 g) were collected from liquid nitrogen, loaded into a centrifuge tube, and added Trizol reagent (Invitrogen, USA) to obtain total RNA. The purity and concentration of total RNA were detected by the NanoDrop 2000 analyzer. Total RNA was reverse transcribed into cDNA using PrimeScript qRT-PCR reagent (Takara) according to the reverse transcription kit instructions. Then PCR was performed using SYBR Premix Ex Taq with the condition of 95 °C for 30 s, and 35 cycles of 95 °C for 5 s, 60 °C for 30 s. At the end of amplification, the expression differences of the target circSTX6 were analyzed by the $2^{-\Delta\Delta Ct}$ method. The primers for PCR were as follows: circSTX6, forward, 5′- TCTGTGCAGGCATTAGCTGA-3′ and reverse, 5′- TCATCACATTGTCCAGCCGG-3'; GAPDH, forward, 5′-GCACCGTCAAGGCTGAGAAC-3′ and reverse 5′-TGGTGAAGACGCCAGTGGA-3'.

2.5. CCK-8 proliferative ability detection

After transfection, SiHa and HeLa cells in the logarithmic growth phase in each group were digested, centrifuged, resuspended into a single-cell suspension, and counted. The cell suspension (3000 cells) was seeded in each well of a 96-well plate. At 24, 48, and 72 h after culture, 10 μL CCK-8 solution was added to the cell plate, and the cells were incubated for a further 1 h at 37 °C. The microplate reader was used to measure the absorbance of each well at 450 nm and then was taken for analysis.

After the cells were tightly bound to the bottom wall of the culture flask, 4 Gy of radiation was used to irradiate the cells. Cell proliferation was observed at different time points after irradiation.

2.6. Survival fraction analysis assay

The SiHa and HeLa cells (200, 500, 1000, 5000/well) in logarithmic growth period were inoculated in 6-well plates with 2 ml liquid in each well, and then irradiated with 0, 2, 4, 6, and 8Gy X-ray. The cells were irradiated within 2 h after adherence, fixed, and stained 12 days later. Cell survival fraction was calculated by colony formation assay. The survival fraction (SF) = the number of colonies in the irradiated group/(the number of cells inoculated in the irradiated group × the colony formation rate in the non-irradiated group).

2.7. Transwell invasion assay

Matrigel matric gel and serum-free DMEM were diluted at a ratio of 1:8 and added to the upper chamber of the Transwell chamber (8 μm core size, Corning, USA), and then put into the incubator for condensation to gel 6 h in advance. The transfected cells were resuspended in a serum-free culture medium. The cell suspension was vertically added to the upper chamber and the lower chamber was added to complete the culture medium. Following a 24-h culture period, the invaded cells were stained, and the number of cells per field of view was assessed.

2.8. Dual-luciferase assay

The full length of circSTX6 or RAB27B containing potential binding sites of miR-203a-3p was cloned into pmiR-RB-REPORT luciferase reporter vector (RiboBio, Guangzhou, China) to conduct the wt-circSTX6 or wt- RAB27B vector. The mutant sequences of circSTX6 or RAB27B within miR-203a-3p binding sites were constructed into the vector to conduct mut-circSTX6 or mut-RAB27B. Then the construct was co-transfected with miR-203a-3p mimic or mimic NC with the help of Lipofectamine 2000 for 48 h. The Dual-Luciferase Reporter Assay System (Promega) was used for luciferase assays.

2.9. Bioinformatic analysis

With the help of online TargetScan and miRDB databases, the downstream targets of miR-203a-3p were predicted and overlapped potential target mRNAs utilizing Venn. The GO and KEGG enrichments of these potential targets were analyzed using DAVID tools and visualized utilizing bioinformatics tools with the ggplot2 R package.

2.10. Statistical analysis

Data analysis (presented with mean ± SD) was handled with GraphPad version 9.0 and SPSS version 26.0. Statistical analysis was performed using unpaired *t*-test, Chi-square test, one-way/two-way ANOVA, and Pearson's correlation analysis, and $P < 0.05$ meant a significant difference.

3. Results

3.1. CircSTX6 expression and its clinical significance in cervical cancer

The RT-qPCR results indicated that circSTX6 levels were increased in cervical cancer tissues in contrast to adjacent normal tissues (Fig. 1A). After grouping radiosensitivity and radioresistant patients, the circSTX6 expression before treatment was divided. As shown in Fig. 1A, circSTX6 levels were higher in radioresistant tissue samples than in sensitivity patients' tissue samples. Based on the

medium level of circSTX6 in tumor tissues, the patients were classified into two categories (low and high expression groups). The Chi-square analysis found that high expression of circSTX6 was more common in patients with locally advanced FIGO stage cervical cancer and positive lymph node metastasis (Table 1). In addition, radioresistant patients have high circSTX6 expression (Table 1).

The Kaplan-Meier analysis showed that the survival time after radiotherapy in the group with low circSTX6 expression was higher than that in the group with high circSTX6 expression (Fig. 1B). Further multiple COX regression analysis results displayed that circSTX6 expression was a risk prognostic factor associated with patients' three-year overall survival (Fig. 1C).

3.2. The effect of circSTX6 silencing on cervical cancer cell activities

The circSTX6 expression was compared in cervical cancer cell lines with different radiotherapy sensitivity. RT-qPCR detection showed that the expression level of circSTX6 in cervical cancer cells was higher than that in normal End1/E6E7 cells, and circSTX6 level in Me180 CELLS was lower than that in SiHa and HeLa cells (Fig. 2A). Because circSTX6 was upregulated in SiHa and HeLa cells, circSTX6 siRNA was used to underexpressed circSTX6 in cervical cancer cells. circSTX6 expression was successfully underexpressed in cervical cancer cells (Fig. 2B). The functional assays indicated that the knockdown of circSTX6 repressed cell proliferation capacities (Fig. 2C and **D**) and invasion abilities (Fig. 2E).

3.3. Downregulation of circSTX6 increased radiosensitivity of cervical cancer cells

The correlation between three types of cervical cancer cell lines and radiotherapy sensitivity was investigated. The survival fraction of SiHa and HeLa cells was higher than that of Me180 cells (Fig. 3A). After radiation treatment, the colonies and survival fraction of SiHa and HeLa cells in the si-circSTX6 group decreased (Fig. 3B–E).

3.4. MiR-203a-3p was a potential target of circSTX6

The CircInteractome database was used to predict the potential target of circSTX6. Based on context + score percentile over 90, four miRNAs (miR-1825, miR-203a-3p, miR-370, and miR-580) were obtained. Among the four miRNAs, miR-203a-3p expression has a statistical difference in circSTX6 knockdown HeLa cells (Fig. 4A). Furthermore, the dual-luciferase reporter assay confirmed the targeting relationship between circSTX6 and miR-203a-3p (Fig. 4B). The miR-203a-3p levels were decreased in cervical cancer tissues and especially in radiotherapy-resistant tissues (Fig. 4C). The negative correlation between circSTX6 and miR-203a-3p was observed (Fig. 4D).

3.5. Interference circSTX6 enhances the radiosensitivity of cervical cancer cells by regulating miR-203a-3p

In the present experiment, to further explore whether circSTX6 regulates the tumor cellular behaviors and radiosensitivity of cervical cancer cells through miR-203a-3p, HeLa cells were divided into four groups: cells were treated with si-NC, si-circSTX6, si-circSTX6+anti-NC, or si-circSTX6+anti-miR-203 (Fig. 5A). Inhibition of miR-203a-3p partial diminished the inhibition effect of si-circSTX6 on cell proliferation (Fig. 5B). The survival fraction of HeLa cells transfected with si-STX6 decreased, which was partially reversed by transfection of anti-miR-203a-3p (Fig. 5C and D).

3.6. RAB27B was a potential target of miR-203a-3p

To identify the downstream targets of miR-203a-3p, we utilized the TargetScan and miRDB databases and overlapped 386 potential

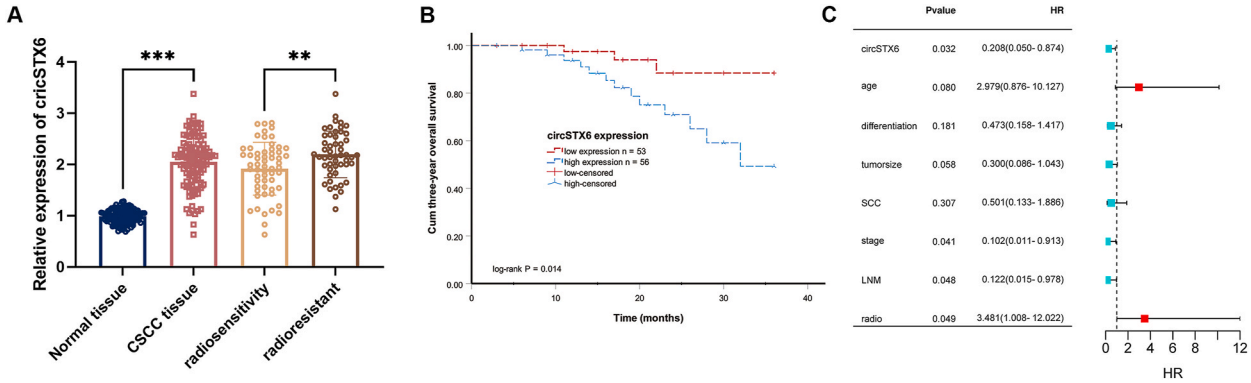

Fig. 1. CircSTX6 expression and its clinical prognostic performance in CSCC. A. Comparison of tissue expression levels of cricSTX6 between CSCC tissues and normal tissues, as well as between radioresistant and sensitive groups. B. Survival analysis of 109 CSCC cases. Log-rank test, $P = 0.014$. C. The prognostic risk factors associated with the patient's prognosis. $^{**}P < 0.01$, $^{***}P < 0.001$.

Table 1
Correlation between circSTX6 expression and clinical parameters and radiosensitivity of cervical cancer patients.

parameters	Case No. (n)	CircSTX6 expression		*P*-value
		Low (53)	High (56)	
Age (years)				0.283
≤50	58	31	27	
>50	51	22	29	
Differentiation				0.131
High-moderate	64	35	29	
Poor	45	18	27	
Tumor size (cm)				0.072
<4	46	27	19	
≥4	63	26	37	
SCC (ng/mL)				0.151
≤3	34	20	14	
>3	75	33	42	
FIGO stage				0.015
IIB	37	24	13	
III-IV	72	29	43	
Lymph node metastasis				0.016
Negative	41	26	15	
Positive	68	27	41	
Radiotherapy				0.041
Sensitivity	59	34	25	
Resistant	50	19	31	
HPV types				0.332
HPV16	57	24	33	
HPV18, 52,58,33	44	24	20	
others	8	5	3	

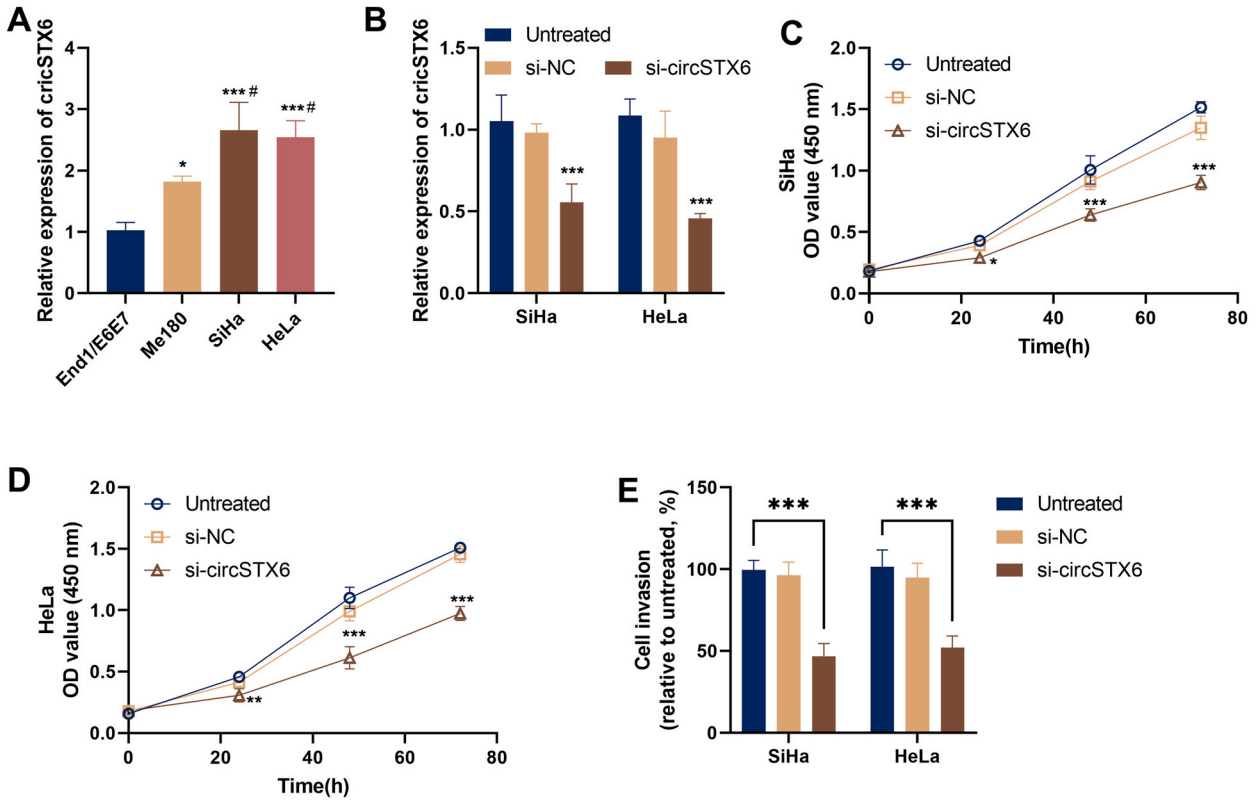

Fig. 2. Effects of circSTX6 absence on cell proliferation and invasion capacities. A. Comparison of circSTX6 expression in cervical cancer cells. B. si-circSTX6 decreased its expression in cervical cancer cells. C and D. circSTX6 absence decreased cancer cell proliferation. E. The invasive abilities were inhibited by circSTX6 knockdown. $^{*}P < 0.05$, $^{***}P < 0.001$ vs End1/E6E7; $^{\#}P < 0.05$.

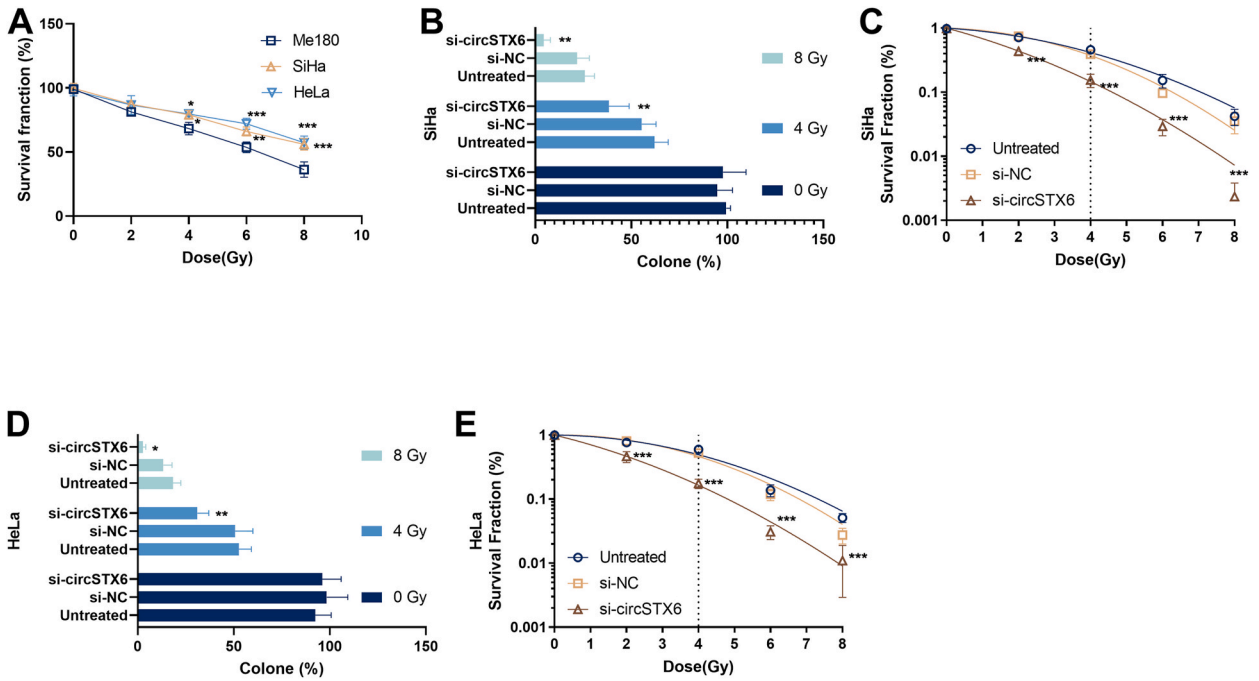

Fig. 3. Silencing of circSTX6 enhanced radiosensitivity of cervical cancer cells. A. Comparison of radiosensitivity of cervical cancer cells. B and C. circSTX6 knockdown enhanced SiHa cell radiosensitivity. D and E. Colone and survival curve of HeLa cells after transfection of si-circSTX6 after radiation. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

targets (Fig. 6A and Supplementary Table 1). The DAVID database was used to perform GO and KEGG enrichment analysis (Supplementary Table 2), and the results with *P* value less than 0.05 were visualized by bioinformatics tools with ggplot2 R package (Fig. 6B and **C**). The GO and KEGG enrichment results include regulation of transcription from RNA polymerase II promoter, protein phosphorylation biological process (BP), chromatin cellular component (CC), and DNA binding molecular function (MF), as well as many crucial pathways, such as TGF-β signaling pathway, EGFR tyrosine kinase inhibitor resistance, insulin signaling pathway, FoxO signaling pathway, and AMPK signaling pathway. Although miR-203a-3p is anticipated to influence a vast array of downstream targets, our study focused on RAB27B (target score 97) due to its association with the progression of cancer, chemoresistance, and radiation [11–13]. The binding sites are displayed in Fig. 6D. Dual-luciferase reporter assay indicated that downregulation of miR-203a-3p could decrease the luciferase activities of HeLa cells cotransfected with wt-RAB27B and miR-203a-3p mimic (Fig. 6E). Moreover, silencing circSTX6 decreased the expression of RAB28B mRNA, while downregulation of miR-203a-3p partially reversed the decreased RAB27B mRNA caused by si-circSTX6 (Fig. 6F).

4. Discussion

In most cases, radiotherapy is the only available treatment for patients with cervical cancer. The efficacy of radiotherapy is highly influenced by the tumor's response to radiation [4,14]. Ever since the integration of radiotherapy into the clinical management of patients with cervical cancer, addressing the issue of radiation tolerance has remained a challenge in treatment strategies. Therefore, improving radiosensitivity and reducing radiation dose may be a better strategy to improve the survival rate of cervical cancer patients. The current investigation revealed that circSTX6 was elevated in cervical cancer tissues and was related to radiosensitivity. Reducing circSTX6 led to a reduction in the proliferation and invasion of cervical cancer cells. In addition, silencing circSTX6 enhanced cancer cell radiosensitivity, particularly regulating the miR-203a-3p/RAB27B axis.

Numerous studies indicated the involvement of ncRNAs in various pathophysiological processes in cancer progression and radiosensitivity [15–17]. For instance, silencing circCCDC66 could improve the radiosensitivity of colon cancer cells by modulating miR-338-3p [18]. Consistent with the expression of circSTX6 in other solid tumors [7,8], the current study demonstrated that circSTX6 was overexpressed in CSCC tissues and correlated with several clinical parameters (FIGO stage and lymph node metastasis). In bladder cancer, increased expression of circSTX6 was related to tumor metastasis and chemoresistance [19]. In this study, patients with radioresistant showed higher circSTX6 levels, revealing that circSTX6 might correlate with the radiosensitivity of CSCC. The stable structure of circRNAs makes them might serve as clinical biomarkers in diseases and cancer [20,21]. Clinical analysis revealed high circSTX6 expression before treatment was related to CSCC patients' three-year overall survival after radiotherapy and might be a risk prognostic factor.

Furthermore, the influence of circSTX6 was investigated in cervical cancer cells. The circSTX6 levels were higher in SiHa and HeLa cells than in normal cervical cells and radiotherapy-sensitive Me180 cells, which suggests that circSTX6 might correlate with

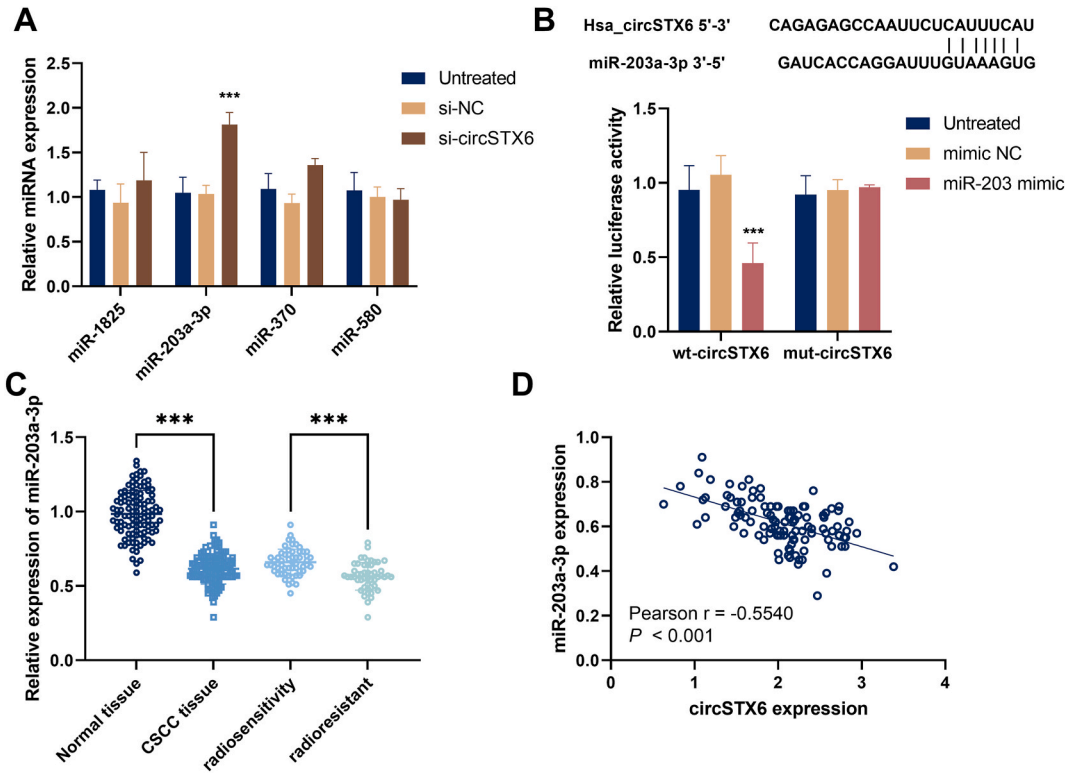

Fig. 4. The potential target miRNAs of circSTX6 using HeLa cells. A. The CircInteractome database predicted the potential miRNAs and detected their expression using RT-qPCR. B. The binding sites and dual-luciferase reporter assay confirmed the targeting relationship between circSTX6 and miR-203a-3p. C. miR-203 expression in different tissue samples. D. A negative correlation was observed between circSTX6 and miR-203a-3p. ***$P < 0.001$.

radiotherapy sensitivity. The data are also in line with previous studies that the Me180 is sensitive to radiation [22]. The cellular experiments indicated that inhibition of circSTX6 decreased cell invasion and proliferation, which suggests that silencing circSTX6 might have an inhibitory role in cervical cancer. Studies have shown that circRNA is a key factor regulating the progression of cervical cancer [23,24]. As a circRNA with obvious carcinogenic activity, circ_0085616 is upregulated in cervical cancer and contributes to tumor progression and radio-resistance by regulating miR-541-3p/ARL2 axis [25]. The results of this study show that circSTX6 absence enhanced cervical cancer cell radiosensitivity and increased the lethality of radiation on cervical cancer, significantly inhibiting the proliferation of cells irradiated by 4 Gy of radiation, revealing that circSTX6 is an important target for the regulation of radiosensitivity of cervical cancer, and its knockout is a potential means to improve the radiotherapy effect of CSCC patients.

CircRNAs could mediate the occurrence and progression of tumors through the sponge effect of downregulating miRNA expression [26,27]. Bioinformatics analysis by querying the CircInteractome database showed that circSTX6 might regulate cervical cancer cellular activities and radiosensitivity through miR-203a-3p. Previous studies demonstrated that miR-203 acts as a radiosensitizer in many tumors, such as gastric cancer, hepatocellular carcinoma, and glioblastoma [28–30]. Previous studies indicated that miR-203a-3p has diagnostic value and represses tumor angiogenesis in cervical cancer [31,32]. The data in this study observed that downregulated miR-203a-3p partially reversed the enhancement of radiosensitivity by si-circSTX6 in HeLa cells, revealing that knockdown of circSTX6 enhanced the radiosensitivity of cervical cancer through upregulation of miR-203a-3p.

Bioinformatic analysis results indicated that the downstream targets of miR-203a-3p were enriched in regulation of transcription from polymerase II promoter, protein phosphorylation, and DNA binding with higher count, which were the main GO enrichment. KEGG enrichment showed that these targets were involved in TGF-β signaling pathway, EGFR tyrosine kinase inhibitor resistance, choline metabolism in cancer, focal adhesion, and other crucial signaling pathways. These pathways are closely related to the progression of tumors, chemoresistance, and radiosensitivity [33–35]. These analyses are exploratory and offer insights into the broader implications of miR-203a-3p′s action. Furthermore, a potential target RAB27B with a high target score was verified to be a potential target of miR-203a-3p. circSTX6 and miR-203a-3p could affect the expression of RAB27B in HeLa cells. These data replied that RAB27B may be a target of miR-203a-3p and circSTX6 may influence the progression and radiosensitivity of CSCC by regulating miR-203a-3p/RAB3B.

By predicting the different responses of patients to radiotherapy treatment, it is of great significance to implement individualized treatment as early as possible according to individual differences, enhance the sensitivity of radiotherapy, reduce the incidence of radiotherapy complications, and finally improve the prognosis of cervical cancer. There are several limitations in the present study.

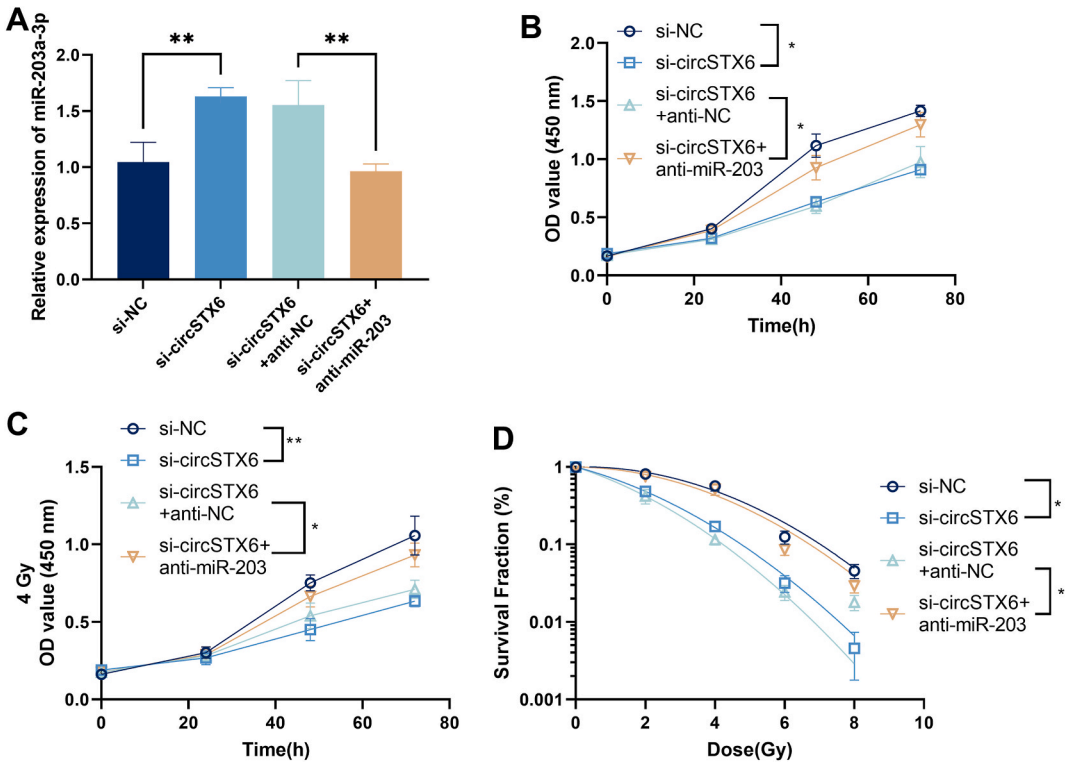

Fig. 5. Interference circSTX6 enhances the radiosensitivity of cervical cancer cells by regulating miR-203a-3p. A. Transfection detection in HeLa cells. B. Inhibition of miR-203a-3p partially diminished the inhibition effect of si-circSTX6 on cell proliferation. C. After 4 Gy of radiation, si-circSTX6 further inhibited cell proliferation while miR-203a-3p absence partially reversed the inhibitory effect of si-circSTX6. D. Survival fraction of HeLa cells after co-transfection. $^{*}P < 0.05$, $^{**}P < 0.01$.

Firstly, we only included HPV-positive cervical cancer cells in this study. Whether silencing circSTX6 could enhance the radiosensitivity remains unclear, which needs to be explored in HPV-negative cervical cancer cells. Besides, a previous study indicated that cancer-initiating cell lines HeLa and SiHa have enhanced cell survival and radioresistance that were due to enhanced expression of ROS defenses and EMT-associated genes [36]. Thus, the detailed mechanism of circSTX6 in progression and radiosensitivity in CSCC remains to be probed in vivo experiments.

There are several limitations in this study. The HPV status of cervical cancer is related to patients' prognosis. This study only enrolled CSCC patients with high-risk HPV types, thus, future studies need to include low-risk HPV type CECC patients to elucidate the differential effects of high-risk and low-risk HPV types on disease prognosis. Besides, the investigation of circSTX6's impact on radio sensitivity in HPV-negative cervical cancer cells remains a critical area requiring exploration, which will be explored in the future.

In conclusion, circSTX6 may have prognostic significance and promoting influence in CSCC. CircSTX6 absence may promote miR-203a-3p/RAB27B axis expression, thus improving the radiosensitivity of cervical cancer cells, enhancing the damage of radiation to cervical cancer cells, and finally exerting anti-proliferation and invasion effects. This study provides novel scientific data to explain the regulatory mechanism of cervical cancer radiosensitivity, which may be conducive to implementing individualized treatment in CSCC.

CRediT authorship contribution statement

Xiaokang Hu: Writing – original draft, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Fan Xing:** Software, Formal analysis, Data curation. **Yue Yin:** Software, Formal analysis, Data curation. **Ning Zhao:** Software, Formal analysis, Data curation. **Lina Xing:** Software, Formal analysis, Data curation. **Guanglu Dong:** Writing – review & editing, Project administration, Methodology, Conceptualization. **Wei Xu:** Writing – review & editing, Project administration, Methodology, Conceptualization.

Ethical approval statement

All patients underwent preoperative clinical staging and signed informed consent, which was approved by the Ethics Committee of The 2nd Affiliated Hospital of Harbin Medical University (Approval Id: 20–0015).

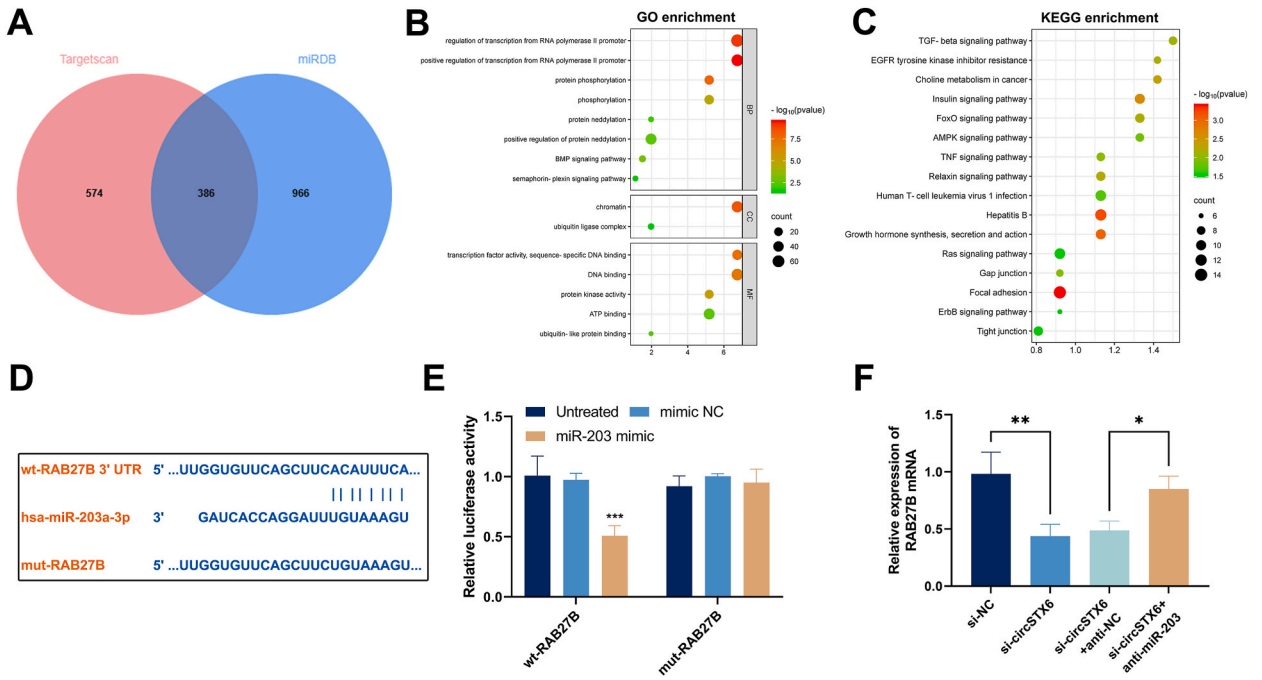

Fig. 6. RAB27B was a potential target of miR-203a-3p. A. TargetScan and miRDB databases were utilized to predict the potential targets of miR-203a-3p. B and C. The bubble diagram of GO and KEGG enrichment. D. The binding sites between miR-203a-3p and RAB27B. E. Dual-luciferase reporter assay was used to verify the targeting relationship between miR-203a-3p and RAB27B. F. The RAB27B mRNA expression was decreased by interfering with circSTX6 expression while was partially reversed by downregulation of miR-203a-3p. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Data availability

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e39262.

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