HDAC1 suppresses radiotherapy sensitivity in cervical cancer via regulating HIF-1α/VEGF signaling pathway

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ABSTRACT
Cervical cancer (CC) is the fourth most common cancer amongst females worldwide. Histone deacetylase (HDAC) 1 plays a vital role in several tumors. Nevertheless, its potential and mechanism in radiotherapy sensitivity underlying CC remains obscure. Hence, the objective of this research was to probe the potential of HDAC1 in CC radiotherapy sensitivity and its mechanism of action. The expression HDACs and survival analysis of HDAC1 were investigated based on the GEPIA database. Immunohistochemical staining was implemented to detect HDAC1 and Ki-67 expression in tumor tissues. RT-qPCR and Western blot were conducted to assess HDAC1, HIF-1α, VEGFA, along with VEGFR expressions in CC cells and tumor tissues. Cell viability, apoptosis, invasion, migration, along with cell cycle were analyzed by functional assays. Tumor-bearing nude mice model was established, and the tumor weight and volume were determined. HDAC1 was high-expressed in the tumor tissue and CC cells. In vitro, overexpression of HDAC1 suppressed radiotherapy sensitivity in C33A cells, while knockdown of HDAC1 promoted radiotherapy sensitivity in SiHa cells. In vivo, we found that HDAC1 silence hindered tumor growth and cell proliferation and promoted tumor cell apoptosis in nude mice after radiotherapy. In addition, we found that HDAC1 impacted radiotherapy sensitivity by modulating the HIF-1α/VEGF signaling pathway. In conclusion, HDAC1 suppressed the radiotherapy sensitivity of CC mice after radiotherapy. In addition, we found that HDAC1 impacted radiotherapy sensitivity by modulating the HIF-1α/VEGF signaling pathway. In conclusion, HDAC1 suppressed the radiotherapy sensitivity of CC via regulating HIF-1α/VEGF signaling pathway, suggesting that HDAC1 may act as a crucial participant in regulating CC radiosensitivity, which may provide a novel method for treating CC.

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Introduction

The occurrence of cervical cancer (CC) ranks fourth among female malignancies worldwide, which poses a serious threat to women’s health (1). The early clinical manifestations of CC are vaginal bleeding and vaginal discharge. In the late stage, there may be different secondary symptoms such as frequent urination, urgency, constipation, lower limb swelling and pain (2). The main risk factor for CC is persistent infection with high-risk human papilloma virus (HPV) (3). The pathological types of CC are mainly squamous cell carcinoma, adenocarcinoma and adenosquamous carcinoma (4). The treatments of CC mainly include surgery, radiotherapy and chemotherapy (5). Clinically, radiotherapy is the most common treatment for CC, the foremost choice for locally advanced CC, and the major postoperative adjuvant treatment for early CC (6). Due to tumor heterogeneity, some tumor cells have innate radioresistance or multiple fractional irradiations induce acquired radioresistance, resulting in radiotherapy failure in some CC patients. Local uncontrolled and distant metastasis resulting from radiotherapy resistance is a major reason for the poor prognosis of CC patients (7). Hence, it is necessary to reveal the molecular mechanisms underlying CC radiotherapy resistance is necessary, in order to improve the radiosensitivity of CC patients.

Histone deacetylases (HDACs) belong to a kind of proteases that exert critical functions in chromosome structural modification and gene expression regulation. They take part in multiple physiological together with pathological processes of cells, such as regulating DNA transcription, protein synthesis, DNA damage and repair (8). Numerous literatures have displayed that HDACs are implicated in various processes of tumor development, including CC (9). HDAC10 inhibits CC metastasis by inhibiting MMP-2 and -9 expression (10). L-and D-lactate modulates anticancer drug resistance in CC by inhibiting HDAC and HCAR1 activation (11). Cinnamic acid derivatives promote colon and CCs cell death by inhibiting HDACs expression (12). These findings suggested that targeting HDACs may offer a novel approach to CC therapy.

Hypoxia is one of the key features of most solid cancers, CC included (13). Hypoxia can stabilize hypoxia-inducible factor-1α (HIF-1α) (14). HIF-1α stimulates the expression of its target genes such as vascular endothelial growth factor (VEGF), which can induce angiogenesis (15). It is well known that the HIF-1α/VEGF signaling axis is involved in CC (16). In addition, it has been reported that the HIF-1α/
VEGF signaling pathway is involved in the regulation of radiotherapy sensitivity in tumors. For example, nelfinavir exerts a radiosensitizing effect in tumors through decreasing HIF-1α/VEGF expression (17). Bortezomib efficiently radiosensitizes esophageal squamous cell carcinoma cells by decreasing the expression of HIF-1α and VEGF (18). However, the role of HIF-1α/VEGF signaling in the radiotherapy sensitivity of CC remains obscure. Previous literatures have suggested that HDAC1 is involved in modulating the progression of breast cancer (19), colorectal cancer (20) and pediatric liver cancer (21). Besides, on the basis of The Cancer Genome Atlas (TCGA) database, HDAC1 expression was discovered to be increased in most tumors, CC included. Survival curve analysis exhibited that the low expression of HDAC1 harbored a good survival rate, mirroring that HDAC1 played a vital role in promoting CC progression. Furthermore, abnormal expression of HDAC1 in CC is observed. Ali et al. have shown that sulforaphane is involved in modulating several tumor suppressor genes expression by targeting DNMT3B and HDAC1 in CC cells (22). Sixto-López et al. have indicated that HO-AA VPA increases the translocation of HMGB1 levels and inhibits HDAC1 expression in CC cells (23). Nevertheless, the detailed function and mechanism of HDAC1 underlying the radiotherapy resistance of CC are still obscure. Therefore, this research was intended to explore the mechanism of HDAC1 in radiotherapy sensitivity underlying CC, which provided a new molecular target for radiotherapy in CC.

Materials and Methods

Tumor tissues collection and ethics statement

A total of 10 CC tissues and the corresponding adjacent normal tissues were acquired from patients who underwent surgery from 2018 to 2022 at the Affiliated Hospital of Jiangnan University. Tissue samples were preserved at -80 °C. The present research was approved by the Medical Ethics Committee of Jiangnan University and complied with the Declaration of Helsinki. All patients signed informed consent.

Cell culture, transfection, and treatment

Human normal cervical endothelial cells, H8 and CC cell lines (HeLa, SiHa, Caski and C33A), were obtained from Crondabio (Shanghai, China). H8 cells were cultured in minimum Eagle’s medium (MEM, BL306A, Biosharp, China). HeLa and SiHa cells were cultivated in Dulbecco’s modification of Eagle’s medium (DMEM, BL301A, Biosharp, China). Caski and C33A cells were cultivated in RPMI-1640 medium (BL303A, Biosharp, China). All the above mediums were added with 10% fetal bovine serum (FBS) at 37 °C with 5% CO₂. For transfection, the overexpressing HDAC1 plasmid and siRNA of HDAC1 (Invitrogen, USA) were transfected into CC cells using Lipofectamine 2000 Transfection Reagent (Thermo Fisher Scientific, Waltham, USA) as reported before (24). For radiological treatment, the cells or transfected cells were treated by 60-cobalt gamma-ray irradiation with 0, 2, 4, 6, and 8 Gy doses. The sequences of siRNA of HDAC1 as follows: Si-HDAC1-1, Sense: 5'-GCUU-CAUUCAUACUAUCAAAAG-3', antisense: 5'-UU- GUAUGUAGUAUGAAAGCA-3'; Si-HDAC1-2, Sense: 5'- CAGCGAGACUAUCAUAAU-3', antisense: 5'-UUUAAGUGACUAUCGUUG-3'; Si-HDAC1-3, Sense: 5'-CGACUGUUUGAGAACUUAGA-3', antisense: 5'-UUAGGUUCUAAACAGUCCCU-3'.

RNA extraction and qRT-PCR

As described before (25), isolation of total RNA from cells and cervical tissues was implemented using TRizol (BS259A, Biosharp, China). A total of 1 μg RNA was reverse-transcribed into cDNA using a Hifair® II Enzyme Mix Kit (KCD-M1003, Crondabio, China). The RT-PCRs were implemented using the SYBR Green qPCR Master Mix (KCD-M1004, Crondabio, China) by the LightCycler® 480II real-time PCR system (Roche, Switzerland). The β-actin was used as an internal control. The calculation of gene expression was implemented using the 2⁻ΔΔCt method. The primers used in the present study were as follows: β-actin, Forward: 5'-ACGTTGGAACATCCGCAAAG-3'; Reverse: 5'-TGGAAGGTTGACGCGACCC-3'; HDAC1, Forward: 5'-CGCCCCCTCACAAAGGCAATG-3'; Reverse: 5'-CTTCTTTGCTGACTCAGGACA-3'; HIF-1α, Forward: 5'-GAAAGTGCGAAAAGAAGATCCCTG-3'; Reverse: 5'-CTTTATCAAGATGCGAACATCA-3'; VEGFA, Forward: 5'-AGGGCCAGAATCATCACGAGT-3'; Reverse: 5'-AGGGTCTCGATTGGATGACA-3'; VEGFR, Forward: 5'-GGTATCGGAATAGTCATCATC-3'; Reverse: 5'-CATGTTGGTCAC-TAACAGAAGCA-3'.
Transwell assay
As described before (28), 25 µL Matrigel (354234, BD, USA) was covered to the upper chamber of the Transwell plate (3422, Corning, USA), the whole polycarbonate film was covered, and the Matrigel was polymerized into glue at 37 °C for 30 min. Furthermore, CC cells were inoculated into the upper chamber. For SiHa and C33A cells, 8 Gy radiation was performed after transfection for 36 h. After 24 h incubation, non-invading cells were gently removed with a cotton swab. The invaded cells were fixed with 4% polyvinyl alcohol for 30 min, washed twice with phosphate-buffered saline (PBS), and dyed with crystal violet for 10 min. The number of invading cells was determined under a light microscope DMI8 (Leica, Germany).

Scratch test
As described before (29), SiHa and C33A cells in the logarithmic growth period were plated into 6 cm dishes at a density of 2.5 × 10⁴ cells/cm², and the cell density was set to 80%. Before cell radiation, cell scratches were made using 200 µL pipette tips, and the width of each scratch was kept consistent as far as possible. The cell culture solution was sucked out, the pore plate was washed with PBS thrice, and the cell fragments generated by scratches were removed. The migrating cells were determined under a light microscope DMI8 (Leica, Germany) with the same scribing position of each group of cells at 0 and 12 h. The migration distance of each group of cells was analyzed quantitatively with ImageJ software.

Tube formation experiment
As described before (30), SiHa and C33A cells were obtained in the logarithmic growth phase. Afterward, 10 µL refrigerated tip and µ-slide angiogenesis were removed from the freezer, and 10 µL of Matrigel was treated into each well of the µ-slide. After adding the Matrigel, the µ-slide was put into a petri dish of suitable size. Then, the entire petri dish was placed in the incubator to solidify. During gelling, the cells were digested and collected after 12 h, and the cell concentration was adjusted to 2 × 10⁵ cells/mL. Subsequently, the µ-slide was removed from the incubator, and 50 µL of cell suspension was added per well. Afterward, 100 µg/mL RNase A was added into cells for incubation for 30 min. Moreover, 2-(4-Amidinophenyl)-maldehyde, embedded, and then sliced into a 4 µm section. The tissue slice was put into xylene and soaked for 20 min for dewaxing. The slice was dehydrated by gradient ethanol and permeabilized by 0.1% Triton x-100. Afterward, the sections were immersed in an antigen repair solution. Furthermore, primary anti-human Ki67 (ab15580, 1:500, Abcam, UK) was incubated with the sections for 2 h. The slices were incubated with biotin-labeled secondary antibody IgG (ab6721, 1:1000, Abcam, UK) for 20 min, followed by treatment with the streptomycin peroxidase solution for 10 min, stained with diaminobenzidine (DAB) for 8 min, and counterstained with hematoxylin for 3 min at room temperature. The images were observed using a light microscope DMI8 (Leica, Germany) after 8 h.

Flow-cytometric analysis for cell cycle and apoptosis detection
As described before (31), transfected cells (1 × 10⁶) were treated with 0.25% trypsin (1×), followed by washing with 2 mL ice-cold PBS. For the cell cycle, the cells were fixated by 75% ethanol overnight at 4 °C. Then, 1 mL of PBS containing 100 µg/mL RNase A was added into cells for incubation for 30 min. Moreover, 2-(4-Aminophenyl)-6-indolecarbamidine dihydrochloride (DAPI) was added for 30 min. For analysis, a 500 µL single cell suspension was used. For cell apoptosis, the cells apoptosis was detected using the Annexin V-Fluorescein Isothiocyanate (FITC) Apoptosis Detection Kit (KCD-T2007, Cronadbio, China). Briefly, the cells (1 × 10⁶) were resuspended in 100 µL of binding buffer and mixed with 5 µL of Annexin V-FITC and 5 µL propidium iodide (PI). Then the cells were incubated at 37 °C in darkness for 20 min. Cell cycle and cell apoptosis were assessed on a flow cytometer FACS AriaTMIII (BD Biosciences, Franklin Lakes, NJ, USA). Cells percentage “Q2 + Q3” was regarded as the apoptotic rate.

Xenograft experiment in nude mice
Lentiviruses expressing either HDAC1–targeting short hairpin RNA (shRNA, sh-HDAC1) or an NC shRNA (sh-NC) were used to obtain a stable HDAC1 knockdown cell line which was selected with 2 µg/mL puromycin. BALB/c nude mice (6–8 weeks old) were purchased from the Yangzhou University Comparative Medicine Center. Nude mice were kept in the specific pathogen-free (SPF) animal laboratory at 21–23 °C and humidity of 60–65% for one week to ready them for cell inoculation in good condition. Moreover, 75% alcohol was used to sterilize the skin of the injection site in nude mice, and 100% of the skin was injected subcutaneously in nude mice with 100 µL SiHa cell suspension (5 × 10⁶ cells). Each group contained three nude mice. The tumor size was examined every five days. After 30 days of tumor formation, the nude mice were killed by intravenous injection of an overdose of barbiturates, and then the tumors were separated for photographing and weighing. The volumes of the tumor xenografts were calculated by the formula: Tumor volume (mm³) = (length × width²)/2. For radiotherapy, all mice were irradiated with a single dose of 4 Gy when the tumor diameter reached 4 mm, once a week for four weeks; each mouse received a total dose of 16 Gy. All experimental animal procedures were approved by the Animal Research Ethics Committee of the Affiliated Wuxi People’s Hospital of Nanjing Medical University.

Immunohistochemistry (IHC) assay
As described before (32), Clinical CC and adjacent tissues were obtained and embedded and then sliced into 5 µm sections. The tissue slice was put into xylene and soaked for 20 min for dewaxing. The slice was dehydrated by gradient ethanol and permeabilized by 0.1% Triton x-100. Afterward, the sections were immersed in an antigen repair solution. Furthermore, primary anti-human Ki67 (ab15580, 1:500, Abcam, UK) or HDAC1 (ab109411, 1:2500, Abcam, UK) was incubated with the sections for 2 h. The slices were incubated with biotin-labeled secondary antibody IgG (ab6721, 1:1000, Abcam, UK) for 20 min, followed by treatment with the streptomycin peroxidase solution for 10 min, stained with diaminobenzidine (DAB) for 8 min, and counterstained with hematoxylin for 3 min at room temperature. The images were observed using a light microscope DMI8 (Leica, Germany).

TdT-mediated dUTP-biotin nick end-labeling (TUNEL) staining
As described before (33). After tumorigenesis, tumor tissue was harvested from nude mice, fixed with 10% formaldehyde, embedded, and then sliced into a 4 µm section. The sections were put into xylene for 20 min to dewax. The slice was rehydrated by gradient ethanol. Afterward, the sections were treated with 50 µL biotin labeling solution for incubation in the dark for 1 h, followed by treatment with 50 µL streptavidin HRP working solution for incubation for 30 min. After washing, the sections were colored with 50 µL of DAB for 10 min. Next, the sections were incubated with hematoxylin staining for 1 min and turned
blue after differentiation washing. The staining was observed under a light microscope DMI8 (Leica, Germany).

Statistical analysis

Data were analyzed using GraphPad Prism version 7.0 software (GraphPad Software, San Diego, CA, USA). At least three biological repeats were indicated as the mean ± standard deviation (SD). Student's T-test and ANOVA test were adopted to detect the significance of groups. P-value < 0.05 was statistical significance.

Results

HDAC1 is high-expressed in the CC tumor tissue

In order to identify the key HDAC1s that may be implicated in CC development, HDACs expression in the CC tumor tissue was investigated on the basis of the GEPIA database (http://gepia.cancer-pku.cn/index.html). HDAC1, 2 and 8 expression was separately elevated in the tumor tissues compared to normal tissues. Among them, HDAC1 upregulation was the most obvious (Figure 1A). Therefore, HDAC1 was selected for further study. Subsequently, the IHC along with Western blot results suggested that HDAC1 expression was elevated in tumor tissues in comparison with the peritumor tissues (Figures 1B-D). Moreover, we exposed CC cells to different doses of radiation to examine the impacts of radiation on CC cells viability. As shown in Figure 1E, C33A and Caski cells showed reduced cell viability after 2, 4, 6, and 8 Gy irradiation compared to cervical epithelial cells H8, while SiHa and HeLa cells showed no change in cell viability. C33A and SiHa were selected for further study because they were the most sensitive and least sensitive, respectively. RT-qPCR together with Western blot assays displayed that HDAC1 expression was significantly upregulated in the C33A and SiHa cells compared with H8 cells. Meanwhile, HDAC1 expression was prominently promoted in SiHa cells compared to C33A cells (Figure 1F and G). Collectively, HDAC1 might participate in the radiotherapy sensitivity of CC.

Overexpression of HDAC1 suppresses radiotherapy sensitivity in C33A cells

To confirm whether HDAC1 was related to radiotherapy sensitivity, the half-life of HDAC1 in SiHa and C33A cells was detected after radiotherapy. As displayed in Figure 2A, the half-life of HDAC1 was dramatically longer in SiHa cells compared to C33A cells. CCK-8 assay suggested that the lessened cell viability in C33A cells caused by radiotherapy was promoted after overexpression of HDAC1 (Figure 2B). Transwell and scratch experiment assays demonstrated that C33A cells invasion and migration were dramatically reduced after radiotherapy, while overexpression of HDAC1 promoted the invasion and migration of C33A cells (Figure 2C and D). Cell cycle assay illustrated that the G0/G1 cells were increased while S-phase cells were reduced in C33A cells after radiotherapy. However, when HDAC1 was overexpressed, the G0/G1 cells were significantly decreased while S-phase cells were increased (Figure 2E). Flow cytometry assays suggested that radiotherapy-induced increase in C33A cells apoptosis was inhibited upon HDAC1 overexpression (Figure 2F). Tube formation assay demonstrated that the tube-forming potential of C33A cells was dramatically promoted after HDAC1 overexpression, and G: Tube formation assay was used to detect the tube forming ability of C33A with HDAC1 overexpression. * p < 0.05; ** p < 0.001; *** p < 0.0001; radiotherapy group vs normal group; ## p < 0.01; ### p < 0.0001; over-HDAC1 group vs over-NC group.
reduced after radiotherapy, while the cell tube-forming ability of C33A cells was elevated by overexpression of HDAC1 in C33A cells relative to the NC group (Figure 2G). Taken together, HDAC1 elevation suppressed radiotherapy sensitivity in C33A cells. **Knockdown of HDAC1 promotes radiotherapy sensitivity in SiHa cell**

Subsequently, the function of HDAC1 on radiotherapy sensitivity was detected in SiHa cells. As revealed in Figure 3A, in comparison with si-NC, HDAC1 knockdown was obviously declined after transfection of si-HDAC1-1/2/3, and si-HDAC1-2 possessed the highest silencing efficiency, which was selected for subsequent analyses. CCK-8 assay manifested that the inhibited viability of SiHa cells stimulated by radiotherapy was further decreased after the knockdown of HDAC1 (Figure 3B). Transwell and scratch experiment assays showed that the invasion and migration capacities of SiHa cells were dramatically reduced after radiotherapy, and silencing of HDAC1 further reduced the invasion and migration of SiHa cells (Figures 3C and D). Cell cycle assay demonstrated that the G0/G1 cells were increased while S-phase cells were reduced after radiotherapy, and silencing HDAC1 further deepened this phenomenon (Figure 3E). Flow cytometry assays illustrated that the apoptosis in SiHa cells was significantly elevated after radiotherapy. Of note, the down-regulation of HDAC1 further promoted apoptosis in SiHa cells (Figure 3F). The tube formation experiment displayed that the tube-forming capacity of SiHa cells was dramatically lessened after radiotherapy. After HDAC1 knockdown, the tube formation ability of SiHa cells was further reduced (Figure 3G). All above data results implied HDAC1 depletion promoted radiotherapy sensitivity in SiHa cells.

**HDAC1 affects radiotherapy sensitivity by modulating the HIF-1α/VEGF signaling pathway in CC cells**

As reported previously, the HIF-1α/VEGF signaling pathway has a crucial role in various cancers, including CC (34). However, whether HDAC1 affects radiotherapy sensitivity by modulating HIF-1α/VEGF signaling pathway remains undefined. Here, levels of HIF-1α/VEGF signaling pathway-related proteins were assessed. It was revealed that HIF-1α, VEGFA, together with VEGFR mRNA levels were decreased after radiotherapy both in C33A and SiHa cells. Further analysis found that HIF-1α, VEGFA, along with VEGFR expression were dramatically increased with HDAC1 overexpression in C33A cells. Inversely, HIF-1α, VEGFA, as well as VEGFR expression were lessened in SiHa cells with HDAC1 silencing (Figure 4A). Similar results were also verified by Western blot (Figure 4B). Taken together, HDAC1 influenced radiotherapy sensitivity by regulating HIF-1α/VEGF signaling pathway.

**Knockdown of HDAC1 promotes radiotherapy sensitivity in vivo**

In order to further certify the function of HDAC1 in radiotherapy sensitivity for CC, the tumor-bearing nude mice model was established. Tumor volume was significantly decreased at 10 days after tumor-bearing with HDAC1 suppression compared to the NC group with radiotherapy (Figure 5A). Tumor weight in si-HDAC1 group declined compared with the NC group at 30 days after tumorigenesis with radiotherapy (Figure 5B). Immunohistochemical staining analysis showed that Ki-67 expression was reduced upon HDAC1 silence, suggesting that silencing of HDAC1 inhibited tumor cell proliferation (Figure 5C). TUNEL assay showed that the apoptosis was prominently increased with HDAC1 silence (Figure 5D). These outcomes revealed that HDAC1 reduction promoted radiotherapy sensitivity in vivo.

**HDAC1 affects radiotherapy sensitivity by modulating HIF-1α/VEGF signaling pathway in vivo**

Moreover, we investigated whether HIF-1α/VEGF
Development. Several reports have also proved that HDAC1 mirrors that HDAC1 plays a crucial role in tumor development and enhance tumor cell motility (38-40). These data suggest that HDAC1 participates in the regulation of tumor progression. Saad et al. have demonstrated that fucoidan can be a therapeutic molecule for CC by targeting HDAC1 (41). Recently, HDAC1 is screened as a hub gene in the Gene Expression Omnibus (GEO) and TCGA databases as the potential predictor for CC. These outcomes recommend the importance of HDAC1 in CC (42). Consistent with these findings, our study indicated that HDAC1 was high-expressed in the tumor tissue together with C33A and SiHa cells of CC. Nevertheless, few studies have focused on the radiotherapy of CC by targeting HDAC1. In the present research, we established that overexpressed HDAC1 suppressed radiotherapy sensitivity while knockdown of HDAC1 promoted radiotherapy sensitivity in vivo, providing a new strategy for the radiotherapy of CC. Consistently, it has been documented that HDAC1 is a potent factor resulting in decreased sensitivity of laryngeal squamous cell carcinoma in radiotherapy (43).

The occurrence of malignant tumors is linked to the excessive proliferation of cells, and tumor proliferation requires a lot of oxygen consumption (44). Hypoxia of tumor tissue is an important biological feature of malignant tumors (45). Many genes in tumor cells responding to hypoxia are regulated by HIF-1α (46). Literature has shown that HIF-1α is closely related to tumor growth, proliferation, invasion, metastasis, angiogenesis, apoptosis, drug resistance, and other characteristics (47). VEGF plays a key part in tumor angiogenesis, and plays a variety of roles in prognostic markers, imaging targets, targeted therapy combined with radiotherapy after different tumor radiotherapy (48-51). HIF-1α has a central role in regulating the signal transduction pathway of VEGF during hypoxia, increasing the stability of VEGF mRNA and the transcription activity of VEGF. It has been reported that the HIF-1α/VEGF signaling pathway is implicated in the progression of CC (52). Jae-Moon Shin et al has pointed that melittin inhibits CC progression and angiogenesis by inhibiting the HIF-1α/VEGF signaling pathway (53). Some studies have reported the involvement of HIF-1α in radiotherapy response. Furthermore, Koukourakis et al. have verified that HIF-1α together with -2a responds to photodynamic therapy and radiotherapy in early esophageal cancer (54). Wachter et al. have proved that HIF-1α, CA-IX, along with OPN show prognostic significance for CC.

### Discussion

CC has become the fourth most common female malignant tumor affecting women’s health (4). Radiotherapy is the mainstay treatment for CC. However, some patients have poor clinical outcomes due to a lack of radiosensitivity for CC (35). Therefore, it is significant to seek and clarify the mechanism of the key factors of radiotherapy for CC. In the present work, HDAC1 was high-expressed in the tumor tissue and CC cells. Furthermore, we demonstrated that the knockdown of HDAC1 promoted radiotherapy sensitivity in vivo and in vivo via modulating the HIF-1α/VEGF signaling pathway. These findings may provide a therapeutic strategy for boosting CC radiosensitivity.

HDACs harbor a crucial role in chromatin remodeling, gene repression, cell cycle regulation as well as differentiation (36). The abnormal function of HDAC1 in tumor cells can inhibit gene transcription as well as the expression of tumor suppressor genes (37). The high expression of HDAC1 in tumor cells can increase tumor cell proliferation and enhance tumor cell motility (38-40). These data mirrors that HDAC1 plays a crucial role in tumor development. Several reports have also proved that HDAC1 participates in the regulation of CC progression. Saad et al. have demonstrated that fucoidan can be a therapeutic molecule for CC by targeting HDAC1 (41). Recently, HDAC1 is screened as a hub gene in the Gene Expression Omnibus (GEO) and TCGA databases as the potential predictor for CC. These outcomes recommend the importance of HDAC1 in CC (42). Consistent with these findings, our study indicated that HDAC1 was high-expressed in the tumor tissue together with C33A and SiHa cells of CC. Nevertheless, few studies have focused on the radiotherapy of CC by targeting HDAC1. In the present research, we established that overexpressed HDAC1 suppressed radiotherapy sensitivity while knockdown of HDAC1 promoted radiotherapy sensitivity in vivo, providing a new strategy for the radiotherapy of CC. Consistently, it has been documented that HDAC1 is a potent factor resulting in decreased sensitivity of laryngeal squamous cell carcinoma in radiotherapy (43).

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### Figure 5. Knockdown of HDAC1 promotes radiotherapy sensitivity in vivo. A: Representative images for the tumor growth with different treatments and the tumor volume was measured at 10 days after tumor-bearings with HDAC1 suppression after radiotherapy. B: Tumor weight was measured in si-HDAC1 nude mice at 30 days after tumorigenesis with radiotherapy, C: Immunohistochemical staining analysis was used to detect the expression of Ki-67 in tumor tissue with HDAC1 suppression and D: TUNEL assay was used to detect the apoptosis-positive cells in tumor tissue with HDAC1 suppression; scale bar = 100 µm; ***, p < 0.001, RT+SiHa+si-HDAC1 group vs RT+SiHa+si-NC group.

### Figure 6. HDAC1 affects radiotherapy sensitivity by regulating HIF-1α/VEGF signaling pathway in vivo. A: Expression of HIF-1α, VEGFA and VEGFR was detected by RT-qPCR in silencing HDAC1 tumor tissue in bearing nude mice after radiotherapy, and B: Western blot was used to detect the expression of HIF-1α, VEGFA and VEGFR in silencing HDAC1 tumor tissue in bearing nude mice after radiotherapy, and the gray level was calculated by ImageJ software. ***, p < 0.001; ****, p < 0.0001, RT+SiHa+si-HDAC1 group vs RT+SiHa+si-NC group.
in T1-T2 laryngeal carcinoma treated with radiotherapy (55). Recently, Guo et al. have confirmed that baicalein promotes radiosensitivity of esophageal squamous cell carcinoma (ESCC) through regulating HIF-1α, inhibiting ESCC progression (56). These results suggest that HIF-1α/VEGF signaling pathway has an important role in response to radiotherapy. However, there are few studies fixated on radiotherapy for CC by targeting HIF-1α/VEGF signaling pathway. Of note, previous literatures have indicated that HDAC1 activates HIF1α/VEGFA signal pathway in colorectal cancer (57). In the current research, HIF-1α, VEGFA, and VEGFR expression were significantly decreased with HDAC1 knockdown after radiotherapy in vitro and in vivo. All the above data implied that HDAC1 affected radiotherapy sensitivity via modulating the HIF-1α/VEGF signaling pathway.

However, there are some limitations in this study. First, the prognostic value of HDAC1 in CC patients with radiotherapy is unclear. Besides, the cooperation between HDAC1, HIF-1α, and VEGF-A on the molecular mechanism needs to be further elucidated.

In conclusion, we demonstrated that the elevation of HDAC1 contributed to reducing the radiotherapy sensitivity of CC by modulating the HIF-1α/VEGF signaling pathway. These findings may provide new insights to develop effective interventions targeting HDAC1 for CC patients undergoing radiotherapy.

**Interest conflict**
The authors report no conflict of interest.

**Consent for publications**
The author read and proved the final manuscript for publication.

**Availability of data and material**
All data generated during this study are included in this published article.

**Ethics approval and consent to participate**
No human or animals were used in the present research.

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