Downregulation of long non-coding RNA UCA1 enhances the radiosensitivity and inhibits migration via suppression of epithelial-mesenchymal transition in colorectal cancer cells

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Abstract. Colorectal cancer (CRC) is the third most commonly diagnosed cancer and common cause of cancer-related deaths. Radiotherapy has become a routine treatment for CRC. However, radioresistance affects therapeutic efficacy. Long non-coding RNA urothelial carcinoma associated 1 (UCA1) has been demonstrated to be overexpressed in several tumors and predicts a poor prognosis. In the present study, we revealed that lncRNA-UCA1 was overexpressed in colorectal cancer tissue and colon cancer cells when compared to normal tissue and cells. Quantitative real-time PCR revealed that the expression of UCA1 was significantly higher in CRC tissues after chemoradiotherapy. Downregulation of UCA1 enhanced the radiosensitivity of CCL244 cells via inhibition of the colony formation, proliferation and promotion of radiation-induced apoptosis and G2/M arrest. Moreover, downregulation of UCA1 suppressed the epithelial-mesenchymal transition (EMT) in CCL244 cells.

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Introduction

Colorectal cancer (CRC) is the third most malignant tumor and common cause of death in both men and women worldwidely (1). Radiotherapy (RT), chemotherapy and surgical resection are the conventional treatment options of colorectal cancer (2). In recent years, radiotherapy has generally become a routine treatment for colorectal cancer (3). Compared to standardized total mesorectal excision (TME) alone, preoperative radiotherapy can produce a higher survival rate (4). However, radiation resistance can lead to tumor recurrence and poor prognosis. Consequently, overcoming tumor radioresistance and promoting radiosensitivity are of vital importance for the treatment and prognosis of CRC.

Non-coding RNAs (ncRNAs) are functional RNA molecules that cannot be translated into proteins, compared to protein-coding RNAs (5). These ncRNAs are divided into short ncRNAs and long non-coding RNAs (lncRNAs) according to their length (6). As a member of ncRNA family, lncRNAs are defined as an RNA transcript of more than 200 nucleotides (nt) (7,8). In the last decade, a number of lncRNAs have been revealed to play an important role in the regulation of tumors. Recently, it was revealed that MALAT1 promoted CRC tumor development via its target protein AKAP-9 (9). Upregulation of HOTAIR drove malignancy in gastrointestinal stromal tumors (10). High expression of lncRNA DANCR (anti-differentiation ncRNA) was associated with poor prognosis for both OS and DFS in CRC (11). lncRNA-UCA1 has been demonstrated to play an oncogenic role in breast cancer in part through the suppression of p27 and by influencing cell proliferation, apoptosis and cell cycle distribution of CRC cells (12,13). In addition, UCA1 had been reported in many other cancers such as tongue squamous cell carcinomas, bladder cancer, melanoma and non-small cell lung cancer (14-19).

Epithelial-mesenchymal transition (EMT) is a process characterized by loss of cell-cell adhesion and gain of migratory traits (20). The EMT process has been reported in many types of cancer such as colorectal cancer (20,21). EMT is controlled by many transcriptional regulators and non-coding RNAs (22). lncRNA H19 modulated EMT by acting as a competing endogenous RNA in CRC (20) knockdown of SPRY4-IT1 and MALAT1 inhibited EMT phenotype in glioma cells and cervical cancer cells (23,24). To date, there is no study concerning the influence of UCA1 on EMT.

Although UCA1 has been revealed to play an important role in tumor regulation, whether it modulated the radiosensitivity of CRC cells was still unknown. In the present study, we aimed to explore the impact of UCA1 silencing on EMT and radiosensitivity of CRC.

Materials and methods

Collection of clinical samples. CRC tissues and adjacent normal tissues from 32 CRC patients were collected immediately after surgical resection from The Second Affiliated Hospital of Soochow University (Suzhou, China). Fresh colorectal cancer tissues and the paired adjacent normal tissues with a distance of 10 cm from the border of the tumor tissues were stored at -80˚C. None of the patients had undergone any treatment before surgical resection. In addition, four pairs of CRC tissues before and after radiotherapy were collected from The Second Affiliated Hospital of Soochow. The present study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Soochow University. Signed informed consent was obtained from all participants. The diagnosis of CRC was based on the World Health Organization criteria. The clinical stage of CRC was evaluated based on the TNM classification system. The data included age, sex, tumor size, stage, differentiation levels, invasion level such as lymphatic invasion, perineural invasion and vascular invasion. Tables I and II reveal the clinical characteristics of these patients. In addition, the radiotherapy effect was evaluated according to the WHO standards: CR, tumor completely disappeared; PR, >50% tumor shrinkage; SD, not >50% tumor shrinkage; PD, tumor volume increased.

Cell culture and treatment. Human CRC-derived cell lines HCT116, CCL244, SW480, LoVo and normal epithelial cells FHC were purchased from the Shanghai Institute of Cell Biology (Shanghai, China) and stored in the laboratory of the School for Radiological and Interdisciplinary Sciences, Soochow University (Suzhou, China). All the cells were cultured in high-glucose Dulbecco's modified Eagle's medium (DMEM; HyClone Laboratories; GE Healthcare, Chicago, IL, USA) supplemented with 10% fetal bovine serum (FBS; Biological Industries, Kibbutz Beit Haemek, Israel), 100 U/ml penicillin G and 100 mg/ml streptomycin (HyClone Laboratories; GE Healthcare) and maintained at 37ºC in a humidified atmosphere with 5% CO_2. Cells were exposed to a single dose of X-ray irradiation from a linear accelerator (Rad Source Technologies, Inc., Suwanee, GA, USA) at a dose rate of 1.15 Gy/min or sham-irradiated.

Table I. Clinical characteristics of the CRC patients.

Clinical parameters	Numbers
Cases	32
Age (years)	
<65	17
≥65	15
Tumor size (cm)	
Small size (<5)	14
Large size (≥5)	18
Sex	
Male	16
Female	16
Invasion levels	
Mucosa	2
Submucosa	3
Muscle	6
Serosa	21
TNM stage	
Stage 1-2	10
Stage 3-4	22
Lymph node metastasis	
Positive	15
Negative	17
Grade of tumors	
Low grade	8
Intermediate grade	22
High grade	2
Vascular invasion	
Positive	12
Negative	20
Perineural invasion	
Positive	11
Negative	21

Construction and transfection of siRNAs. The siRNA sequences silencing human UCA1 (si-UCA1) were designed and synthesized by Shanghai GenePharma, Co., Ltd. (Shanghai, China). The negative control siRNA was also provided by Shanghai GenePharma Co., Ltd. The siRNA sequences are presented in Table III. CCL244 cells were transiently transfected using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA). After transfection for 4 h, the serum-free medium was replaced by a medium containing 10% FBS. After transfection for 48 h, the cells were harvested for further experiments. Quantitative real-time PCR was used to determine the efficiency of transfection.

RNA extraction and real-time RT-PCR. Total RNA was extracted from cells and tissues using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.). The reverse transcription of mRNAs was performed using a Reverse

Table II. Clinical characteristics of the CRC patients who received radiotherapy.

Sample number	Treatment protocols	Radiotherapy effect	Distant metastasis
1	6MvX-ray IMRT DT5000cGy/200cGy/25f mFOLFOX6	PR	NO
2	6MvX-ray 3DCRT Dt4500cGy/180Gy/25f FOLFOX	PR	NO
3	6MvX-ray IMRT DT5000cGy/200cGy/25f XELOX	SD	NO
4	6MvX-ray 3DCRT Dt4500cGy/180Gy/25f XELOX	SD	NO

CR, tumor completely disappeared; PR, >50% tumor shrinkage; SD, not >50% tumor shrinkage; PD, tumor volume increased.

Table III. Target sequences of UCA1 siRNAs and negative control (NC) siRNA.

siRNAs	Target sequences
si-UCA1-1 sense	5'-GAGCCGAUCAGACAAACAATT-3'
si-UCA1-1 antisense	5'-UUGUUUGUCUGAUCGGCUCTT-3'
si-UCA1-2 sense	5'-GGGCUUGGGACAUUUCACUTT-3'
si-UCA1-2 antisense	5'-AGUGAAAUGUCCCAAGCCCTT-3'
si-UCA1-3 sense	5'-GGGAAUACUAUUCGUAUGATT-3'
si-UCA1-3 antisense	5'-UCAUACGAAUAGUAUUCCCTT-3'
NC sense	5'-UUCUCCGAACGUGUCACGUTT-3'
NC antisense	5'-ACGUGACACGUUCGGAGAATT-3'

Table IV. Primer sequences for qRT-PCR analysis.

Gene	Forward sequence	Reverse sequence
UCA1	5'-GCCCCTTGGACCATCACA-3'	5'-GACGGCAGTTGGTGTGCTAT-3'
GAPDH	5'-CATGAGAAGTATGACAACAGCCT-3'	5'-AGTCCTTCCACGATACCAAAGT-3'

Transcription kit (Roche, Basel, Switzerland). The real-time PCR assay was carried out with a SYBR-Green kit (Tiangen Biotech Co., Beijing, China) on an ABI 7500 system (Applied Biosystems, Foster City, CA, USA). The thermocycling conditions were as follows: Initial denaturation: At 94˚C for 3 min 30 cycles: At 94˚C for 30 sec, at 55˚C for 30 sec, and at 72˚C for 1 min; final extension: At 72˚C for 5 min. The GAPDH genes were used for the detection of normalization of each sample. The relative expression of UCA1 was calculated using the $2^{-\Delta\Delta Ct}$ method. The primers of UCA1 and GAPDH were designed by Shanghai GenePharma Co., Ltd. The primer sequences are presented in Table IV.

Cell proliferation assays. An MTT assay was used to examine cell proliferation. After transfection, the cells were seeded in a 96-well plate at 2,000 cells/well and cultured in normal medium. The cells were subjected to 8-Gy irradiation after 24 h. After 48 and 72 h, the cells were incubated in 0.5 mg/ml MTT at 37°C for 4 h and lysed in dimethyl sulfoxide (DMSO) at room temperature for 10 min. Then, viability was assessed by obtaining the optical density (OD) values using a microplate reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA) at a wavelength of 490 nm.

Colony-formation assay. After transfection, the cells were inoculated into 6-well plates at 300, 500, 3,000 and 8,000 cells/well and exposed to X-rays with different irradiation doses of 0, 2, 4, 6 and 8 Gy after 24 h. Following 14 days of incubation, the colonies were fixed with 4% paraformaldehyde and stained with Giemsa. Colonies with a minimum of 50 cells were counted. The radiation sensitivity enhancement ratio (SER) was measured according to the multi-target single hit model.

Cell apoptosis assays. Apoptosis was assessed using Annexin V/propidium iodide (PI) double-staining following the manufacturer's instructions (BD Biosciences, San Jose, CA, USA). After transfection with siRNA and NC-siRNA for 24 h, the cells were treated with 8 Gy X-ray irradiation. Cells were washed twice with cold PBS and resuspended in 1X binding buffer at a concentration of $1x10^6$ cells/ml. Then, 100 μl of the solution ($1x10^5$ cells) was transferred into a 2-ml culture tube. Next, the cells were incubated for 15 min in the dark following the addition of 5 μl FITC-Annexin V and 5 μl of PI. Subsequently, 400 μl of 1X binding buffer was added into each tube. Flow cytometric analysis was performed with the BD FACSDiva™ software (BD Bioscienes, San Jose, CA, USA) within 1 h.

Cell cycle distribution analysis. After transfection, the cells were subjected to 6-Gy irradiation. Twenty-four hours later, the cells were harvested by trypsin digestion, pelleted by centrifugation, washed with ice-cold PBS, and then fixed with 75% cold ethanol overnight. The staining solution containing PI (50 mg/ml) (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) and DNA-free RNAs (20 mg/ml) were added 30 min before the detection. The fraction of the population in each phase of the cell cycle was determined as a function of the DNA content using a coulter flow cytometer (Beckman Coulter, Inc., Brea, CA, USA).

Cell wound healing assay. lncRNA-UCA1-silenced and negative control cells with or without 6-Gy irradiation were seeded in 6-well plates and incubated overnight. Wounds were created by scratching cell monolayers with a sterile 200-μl plastic pipette tip. The cells were further incubated in a hypoxic environment for 24 h and images were monitored using phase-contrast microscopy (Nikon Corp., Tokyo, Japan). The analysis of the cell migration assay was performed using the ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Protein extraction and western blot analysis. Cells ($1x10^5$ cells/dish) in 6-well plates were incubated in DMEM containing 10% FBS to 70% confluence. The cells were transfected with siRNA for 24 h and then were sham-irradiated or exposed to 6-Gy X-ray irradiation. The cells were then harvested in lysis buffer with 1 mM phenylmethylsulfonyl fluoride (PMSF). Subsequently, the cells were allowed to swell on ice for 30 min. The homogenates were centrifuged for 15 min at 12,000 x g and the supernatant was used as the cytosolic extract. Proteins were separated using 10% sodium dodecyl sulfate poly-acrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes The membranes were blocked with 5% non-fat dried milk in TBS containing 1% Tween-20 (TBST) for 2 h at room temperature, followed by incubation with the appropriate primary antibodies caspase-3 (cat. no. sc-7272), Bcl-2 (sc-509; both from Santa Cruz Biotechnology, Inc., Santa Cruz, CA), MMP2 (cat. no. ab92536), MMP9 (cat. no. ab76003), ZEB1 (cat. no. ab124512) and vimentin (cat. no. ab137321; all from Abcam, Cambridge, MA, USA) at a 1:1,000 dilution for 2 h, and either horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse antibodies (cat. nos. A0208 and A0216, respectively; both from Beyotime Institute of Biotechnology, Nantong, China) for 1 h. β-actin (Beyotime Institute of Biotechnology) was used as the loading control. Antibody-bound proteins were detected using an ECL Stable Peroxide solution (PointBio, Shanghai, China). All protein bands were visualized using a FluroChem MI imaging system (Alpha Innotech, Santa Clara, CA, USA) at the room temperature.

Statistical analysis. All experiments were performed at least three times (n=3). Data were expressed as the means ± SEM. Student's t-test was used to analyze the data when only two groups were compared or one-way analysis of variance (ANOVA) when more than two groups were compared. Differences were considered statistically significant when $P<0.05$. SPSS Statistical software (version 19.0; IBM Corp., Armonk, NY, USA) was utilized for statistical analysis.

Results

UCA1 levels are upregulated in CRC tissues and cell lines. Thirty-two pairs of human CRC and adjacent normal tissues were collected to investigate the expression level of UCA1 using real-time PCR. As shown in Fig. 1A and C, UCA1 was overexpressed in CRC tissues compared to paired adjacent normal tissues ($P<0.001$). In addition, our results demonstrated that UCA1 was highly expressed in human CRC-derived cell lines HCT116, CCL244, SW480 and LoVo, in contrast with the normal epithelial cells FHC (Fig. 1B). These results indicated that UCA1 was significantly upregulated in CRC tumor tissues and cell lines. CCL244 cells were selected to perform the following experiments since the ascending order of these four CRC cell lines in terms of radiosensitivity was demonstrated to be CCL244, SW480, LoVo and HCT116 (25).

Expression of UCA1 in two CRC cell lines and four clinical samples after irradiation compared to unirradiated cells. CCL244 and HCT116 cells were seeded in 6-well plates and then exposed to 8-Gy X-ray irradiation. After 48 h, total RNA was extracted and then a real-time PCR assay was used to detect the expression of UCA1. As shown in Fig. 2A and B, the expression level was higher in the CCL244 cells with irradiation than the cells without irradiation ($P=0.01$). However, there was no statistically significant difference in HCT116 cells ($P=0.84$). Similarly, Fig. 2C indicated that the expression of UCA1 was upregulated in four clinical samples after radiotherapy.

UCA1 regulates the radiosensitivity of CCL-244 cells. The results of Fig. 2 demonstrated that UCA1 was upregulated in CCL244 cells following X-ray irradiation. Thus, we conjectured that UCA1 may be involved in the regulation of radiosensitivity of CRC cells. Three different siRNA vectors were transfected into CCL244 cells and the efficiency of transfection was verified by real-time PCR. siRNA-2 exhibited the highest efficiency (57%) of transfection compared to the other two siRNAs (66 and 62%) in Fig. 3A. Thus, siRNA-2 was then used for further experiments. siRNA and NC-siRNA were transfected into CCL244 cells and then the cells were exposed to 8-Gy X-ray irradiation after transfection for

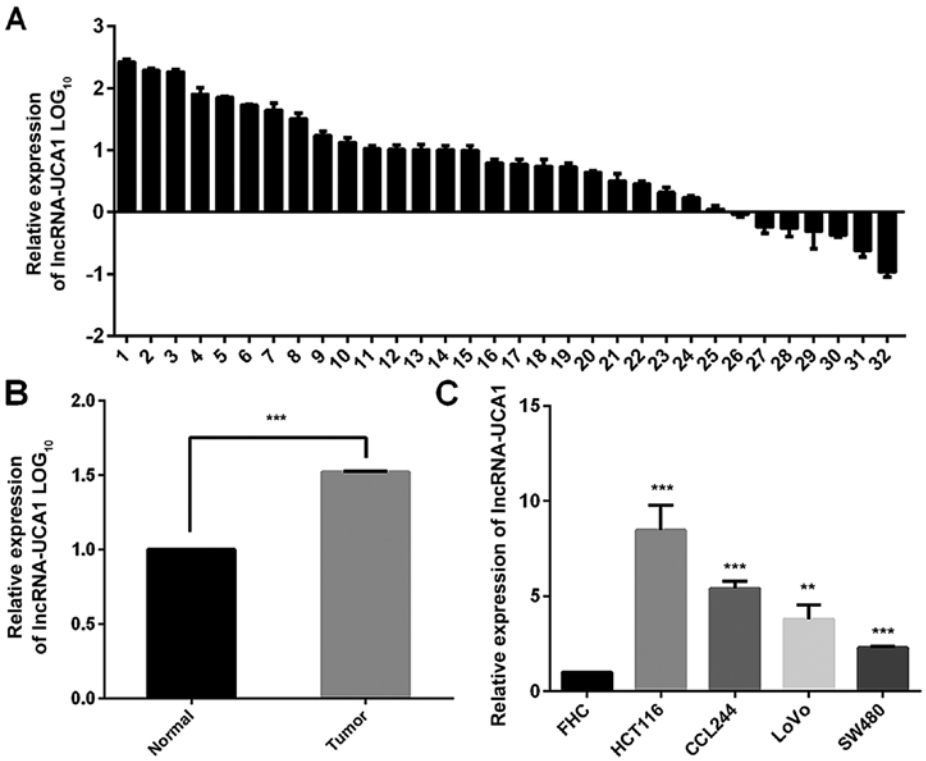

Figure 1. Expression of UCA1 in CRC tissues and cells. (A) Real-time RT-PCR was performed to detect the relative expression of UCA1 in 32 pairs of human CRC cancerous and adjacent non-cancerous tissues. GAPDH was used as an internal control. (B) The mean $2^{-\Delta\Delta Ct}$ of UCA1 in the 32 CRC tissues was compared with that in the matched non-cancerous colorectal tissues (***P<0.001). (C) Relative expression of UCA1 ($2^{-\Delta\Delta Ct}$) in 4 CRC cell lines (HCT116, CCL244, LoVo and SW480) and in one normal colonic mucosal cell line (FHC) (**P<0.01, ***P<0.001).

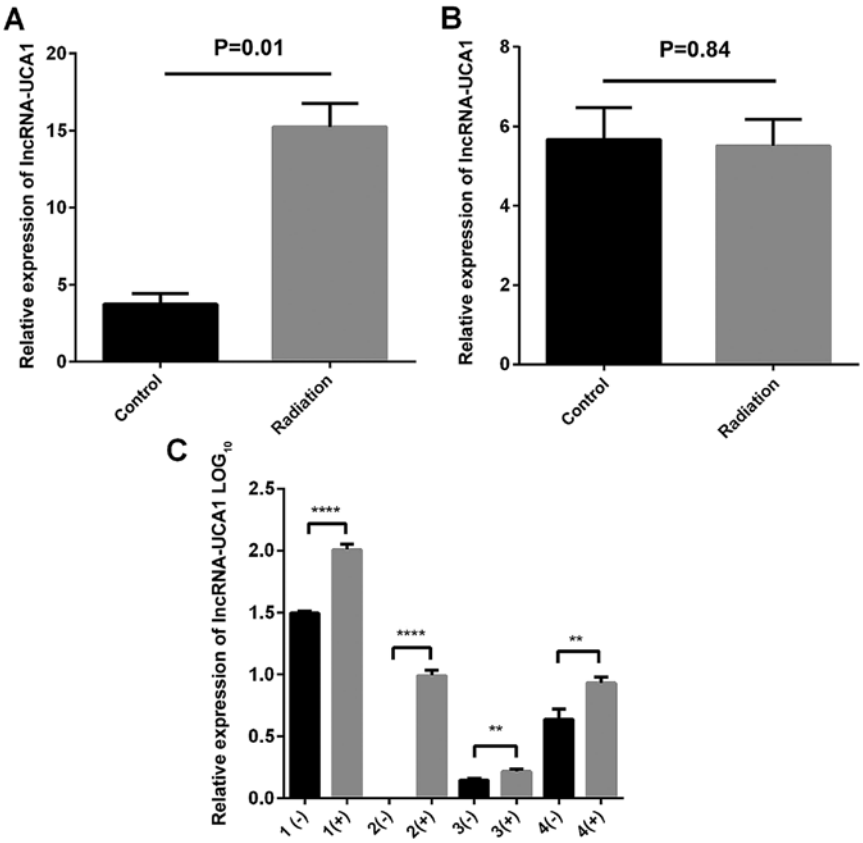

Figure 2. Expression of UCA1 in colorectal cancer cells and tissues before and after X-ray irradiation. (A) The expression of UCA1 in CCL244 cells before and after X-ray irradiation. CCL244 cells after irradiation exhibited higher expression of UCA1 when compared to cells that were not irradiated (P<0.01). (B) The expression of UCA1 in HCT116 cells before and after X-ray irradiation. There was no statistically significant difference in HCT116 cells (P=0.84). (C) The expression of UCA1 was upregulated in four clinical samples after chemoradiotherapy. (-), before chemoradiotherapy; (+), after chemoradiotherapy (**P<0.01, ****P<0.0001).

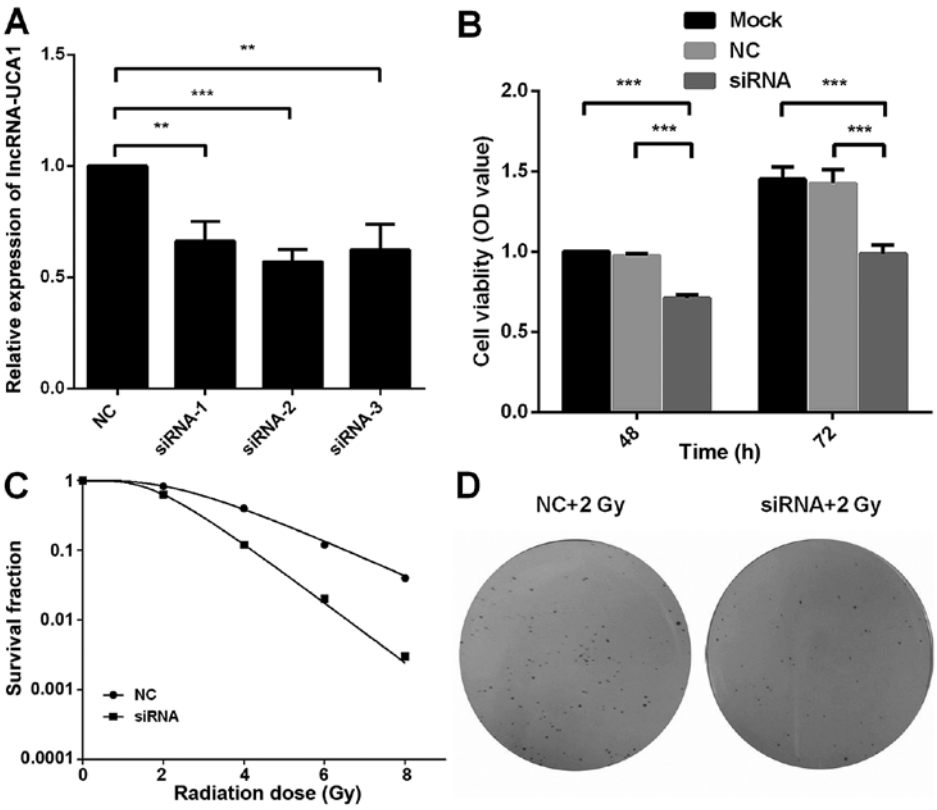

Figure 3. Downregulation of UCA1 influences the radiosensitivity of CCL244 cells. (A) Real-time RT-PCR was used to detect the silencing efficiency of three siRNA sequences (**P<0.01, ***P<0.001). (B) CCL244 cells were transfected with siRNA and NC. Then the cells were exposed to 8-Gy irradiation 24 h later. After 48 and 72 h, an MTT assay were used to detect cell viabilities (***P<0.001). (C) Tranfected with siRNA and NC cells were exposed to 2, 4, 6 and 8 Gy of X-ray irradiation. Ten days later, the survival curve was obtained by multi-target single hit model. (D) Representative colony images of UCA1-silenced CCL244 cells compared to the negative control exposed to 2 Gy of X-ray irradiation.

24 h. After 48 and 72 h, MTT and colony-formation assays were performed. The MTT assay demonstrated that the cell viability was reduced after UCA1 downregulation, compared to the mock-transfected cells (P<0.001) and negative control UCA1 (P<0.001) (Fig. 3B). CCL244 cells were transfected with siRNA or NC-siRNA 24 h prior to irradiation at 0, 2, 4, 6 and 8 Gy. The multi-target single hit model was used to obtain the dose-survival curves. A dose-dependent radiosensitization on UCA1-silenced cells was also observed with a sensitizing enhancement ratio (SER) of 1.41 (Fig. 3C). Representative colony images of UCA1-silenced CCL244 cells compared to the negative control exposed to 2 Gy of X-ray irradiation are shown in Fig. 3D. These results demonstrated that downregulation of UCA1 significantly increased the radiosensitivity of CCL244 cells.

Knockdown of UCA1 promotes radiation-induced apoptosis in CCL244 cells and regulates the expression of apoptosis-related proteins. Apoptosis was detected using Annexin V/PI staining with flow cytometry to investigate the role of UCA1 in radiation-induced apoptosis in CCL244 cells. Apoptosis was promoted in the CCL244 cells in the NC+IR group when compared to the NC group [NC (7.93±0.20%) vs. NC+IR (17.60±0.69%), P<0.001], as shown in Fig. 4A and B. The percentage of apoptosis in the irradiation plus the UCA1-knockdown group was significantly enhanced in contrast with the NC+IR group [siRNA+IR (33.97±1.03%) vs. NC+IR (17.60±0.69%), P<0.001]. Furthermore, the expression of apoptosis-related molecules such as Bcl-2 and caspase-3 was evaluated by western blot analysis. As shown in Fig. 4C, the expression of Bcl-2 was downregulated after transfection with siRNA compared to the negative control regardless of irradiation. In contrast with Bcl-2, the expression of caspase-3 was upregulated. All these results demonstrated that knockdown of UCA1 promoted radiation-induced apoptosis in CCL244 cells.

UCA1 silencing attenuates radiation-induced G2/M arrest. We then studied whether UCA1 had any effects on radiation-induced cell cycle changes. Transfected CCL244 cells were subjected to 6-Gy irradiation. Twenty-four hours later, the cells were harvested and detected by flow cytometry. As shown in Fig. 5, the results revealed that IR caused cell cycle arrest in the G2/M phase in the NC+IR group when compared to the NC group [NC (11.77±1.01%) vs. NC+IR (31.81±1.59%), P<0.001]. However, the percentage of cells in the G2/M phase was reduced when UCA1 was knocked down after irradiation compared to the NC+IR group [siRNA+IR (18.87±1.15%) vs. NC+IR (31.81±1.59%) P<0.01]. The percentage of radiation-induced G2/M arrest in the siRNA+IR group was reduced by 12.94% compared to the NC+IR group. These results indicated that knockdown of UCA1 can reduce the radiation-induced G2/M arrest.

Knockdown of UCA1 inhibits the migration and EMT of CCL244 cells after irradiation. Metastasis is an important factor of poor prognosis in CRC. A wound healing assay was

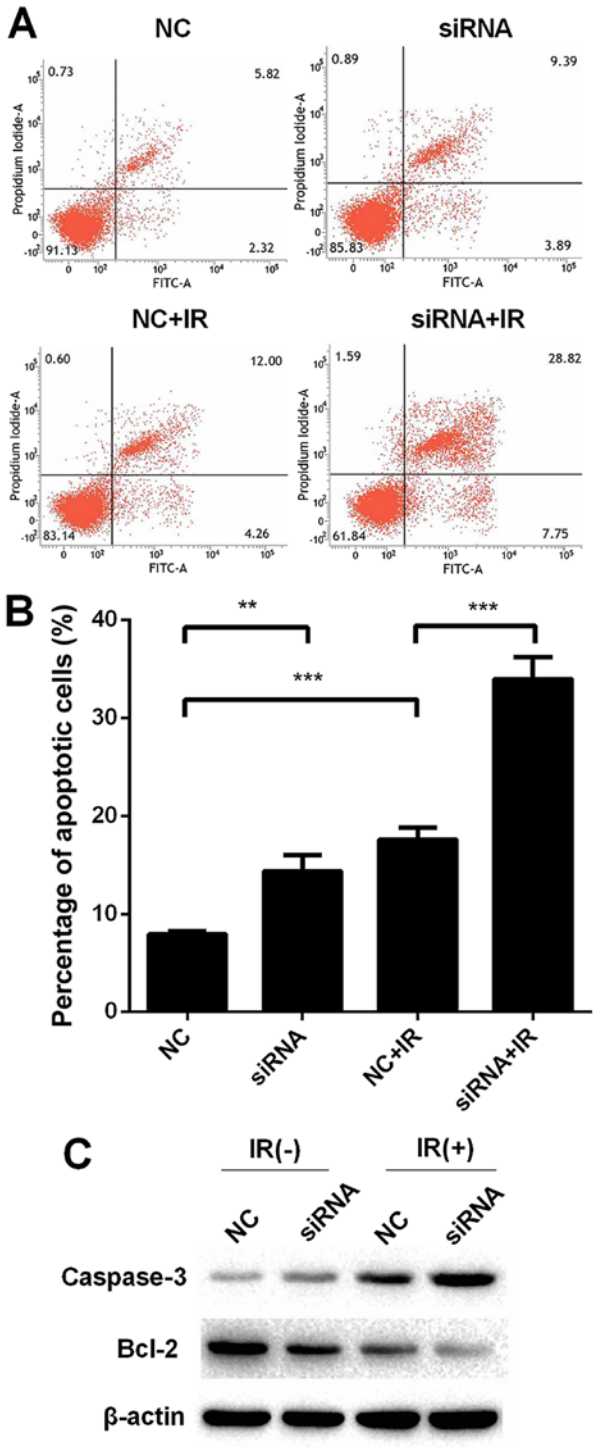

Figure 4. Downregulation of UCA1 promotes radiation-induced apoptosis of CCL-244 cells. (A) Annexin V/propidium iodide (PI) double-staining was performed to determine the apoptosis of CCL-244 cells transfected with siRNA or NC exposed to 8 Gy or sham-irradiation. (B) Percentage of apoptotic cells transfected with siRNA or NC exposed to 8 Gy or sham irradiation (**P<0.01, ***P<0.001). (C) Western blot analysis of the apoptotic marker proteins caspase-3 and Bcl-2 in CCL244 cells transfected with siRNA or NC exposed to 8 Gy or sham-irradiation. GAPDH was used as an internal control.

carried out to investigate the effect of UCA1 on the metastasis of CCL244 cells. Fig. 6A and B revealed that downregulation of UCA1 inhibited the migration of CCL244 cells compared to the negative control CCL244 cells (46.38 vs. 59.42%, P<0.05). After transfection and exposure to 6 Gy X-ray irradiation, knockdown of UCA1 (siRNA+IR) significantly inhibited the migration of CCL244 cells compared to cells with X-ray irradiation alone (NC+IR) (16.06 vs. 35.92%, P<0.05). As widely known, matrix metalloproteinases (MMPs) are of vital importance in tumor metastasis. ZEB1 promotes EMT by binding to DNA through E-boxes of target gene promoters such as E-cadherin (26). Vimentin is also a biomarker of EMT (27). Western blot analysis revealed that downregulation of UCA1 plus irradiation reduced the expression level of MMP2 and

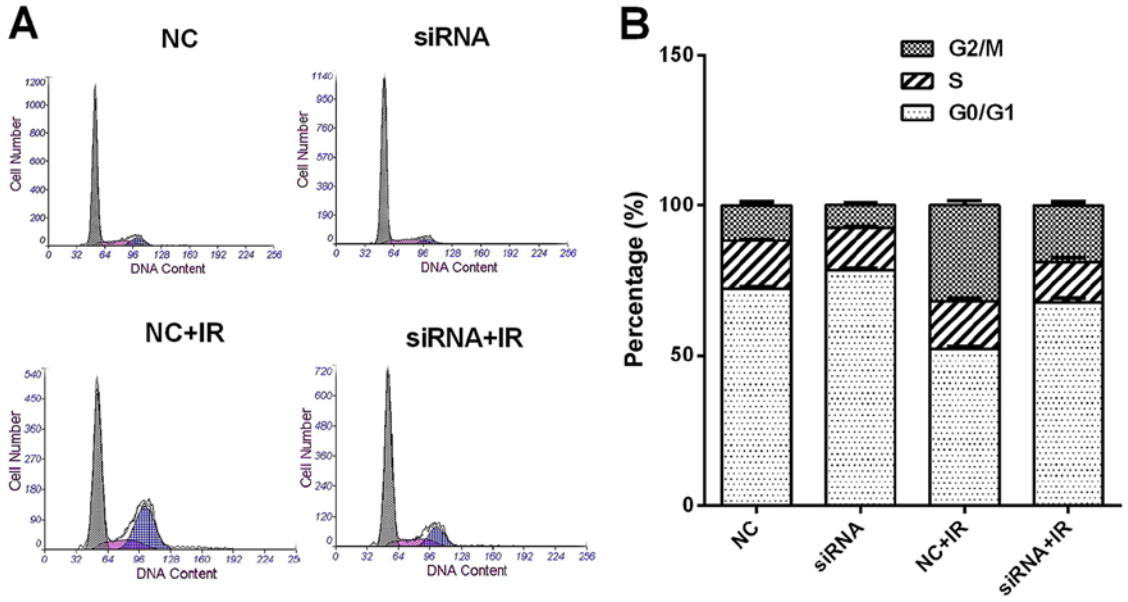

Figure 5. Downregulation of UCA1 attenuates radiation-induced G2/M arrest. (A) Representative images of the cell cycle distribution after UCA1 knockdown and NC cells with irradiation or not. (B) Quantification of the cell cycle distribution after UCA1 knockdown and NC cells with irradiation or not.

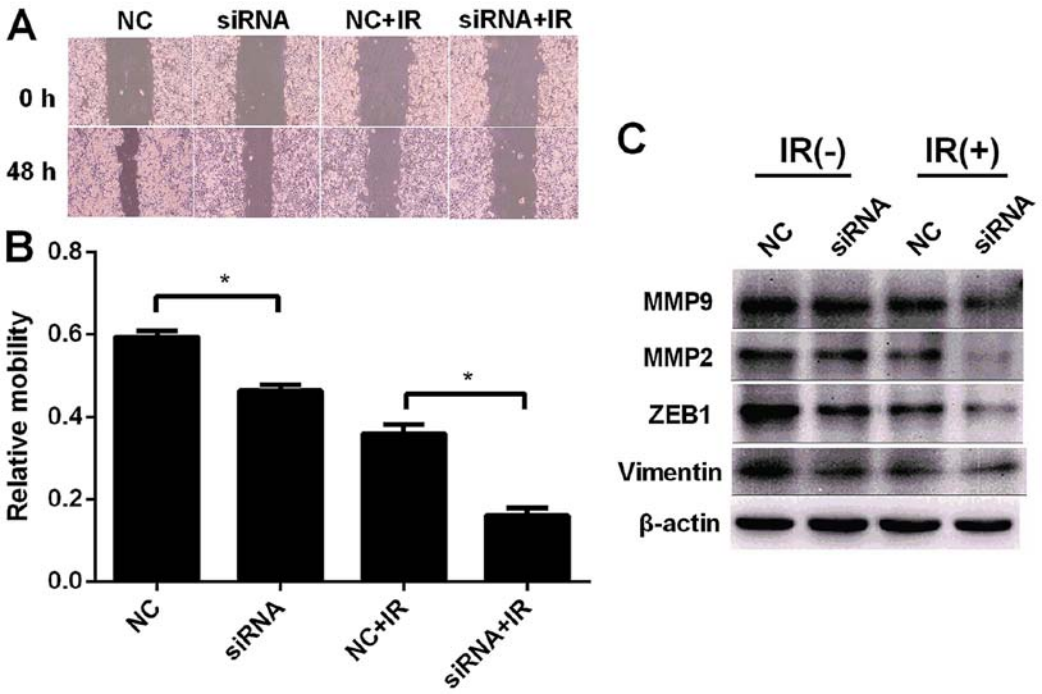

Figure 6. Effect of UCA1 silencing on cell migration and EMT. (A) Representative images of wound healing assays of CCL244 cells after UCA1 silencing plus 6 Gy X-ray irradiation. (B) The relative mobility of UCA1 knockdown and NC cells with irradiation or not (*P<0.05). (C) The expression level of migration- and EMT-associated proteins MMP2, MMP9, ZEB1 and vimentin.

MMP9. Similar results were obtained in the expression levels of ZEB1 and vimentin (Fig. 6C). These results demonstrated that knockdown of UCA1 inhibited the migration and EMT of CCL244 cells after irradiation.

Discussion

Radiotherapy is a primary treatment modality for early and locally advanced CRC cancer (28). However, radiation resistance has a greater impact on the treatment effect, which suggests a poor prognosis and high recurrence rate. Therefore, research has begun to focus on how to increase the radiosensitivity of tumor cells. Downregulation of c-myc and MG132 (carbobenzoxyl-leucinyl-leucinyl-leucinal-H) enhanced the radiosensitivity of A549 cells *in vivo* and *in vitro* (29). In addition, anti-apoptotic proteins, such as Bcl-2 and Bcl-xL, play an important role in the radioresistance of cancer cells. When acquired radioresistant breast cancer cell

line MDA-MB-231R was treated with ABT-737, Bcl-2 and Bcl-xL were downregulated, radiation-induced apoptosis was increased, restoring the radiosensitivity of the MDA-MB-231R cells (30). The PI3K/Akt/mTOR pathway is an important intracellular signaling pathway involved in the regulation of cell radioresistance (31,32). The PI3K/Akt/mTOR pathway inhibitors BEZ235 and PI103 increase the radiation sensitivity of prostate cancer cells via induction of apoptosis and reduction of autophagy (33). In our previous study, CCL244 was identified as a radioresistant cell line and HCT116 as a radiosensitive cell line as determined through clone formation assay (25). lncRNA UCA1 was identified as a vital lncRNA which was distinctly upregulated in CCL244 cells after irradiation. UCA1 has been established to be involved in many of the biological behaviors of tumor. However, there are no studies concerning UCA1 affecting the radiosensitivity of tumor cells.

In recent years, as long non-coding RNAs have become a hot topic, numerous lncRNAs have been demonstrated to be related with tumor cell radiosensitivity, although UCA1 was not among them. Downregulation of HOTAIR increased the radiosensitivity of cervical cancer when p21 was simultaneously upregulated (34). lncRNA-p21 enhanced the sensitivity of radiotherapy for CRC by targeting the β-catenin signaling pathway and it may provide a potential target for CRC radiotherapy (35). lncRNA-LIRR1 was able to modulate the radiosensitivity of BEAS-2B cells through the DDR signaling pathways in a p53-dependent manner (36). Downregulation of MALAT1 and upregulation of miR-145 increased the radio-sensitivity in HR-HPV$^+$ cervical cancer (37). In the present study, we identified that UCA1 was upregulated in CCL244 cells after irradiation which indicated that UCA1 may be involved in radioresistance of CRC cells. Since UCA1 was highly expressed in tissues and CCL244 cells, we used small interfering RNA to downregulate UCA1 and investigate the relevance between lncRNA-UCA1 and the radiosensitivity of CRC cells. An MTT assay indicated that silencing of UCA1 significantly decreased the proliferation rate compared to the negative control siRNA when cells were exposed to X-ray irradiation. Colony-formation assay revealed that UCA1-silencing was observed with a sensitizing enhancement ratio (SER) of 1.41 and the colony survival fraction was significantly reduced. All these results indicated that downregulation of UCA1 enhanced the radiosensitivity of CCL244 cells. In addition, UCA1 may be involved in the modulation of chemosensitivity of CRC cells.

Radiation-induced apoptosis was one of modes of radiation-induced cell death (38). lncRNAs were identified to be involved in the modulation of radiation-induced apoptosis. Downregulation of MALAT1 significantly enhanced radiation-induced apoptosis, as the expression of Bcl-2 was decreased and that of caspase-3 was increased (39). It was demonstrated that silencing of HOTAIR increased the radiation-induced apoptosis in CRC cells (40). In the present study, we examined the apoptosis of CCL244 cells after transfection and exposure to X-ray irradiation in order to confirm that UCA1 was involved in regulating the radiosensitivity of CCL244 cells. It was revealed that the downregulation of UCA1 resulted in a higher percentage of radiation-induced apoptosis than the negative control. Moreover, downregulation of UCA1 reduced the expression of Bcl-2 and improved the expression level of caspase-3. All of the aforementioned results demonstrated that downregulation of UCA1 enhanced the radiosensitivity via the promotion of radiation-induced apoptosis.

Cell cycle regulation may be the most important determinant of ionizing radiation sensitivity (41). It has been universally acknowledged that irradiation induces DNA double-strand breaks (DSBs) resulting in G2 arrest and then DNA DSB repair occurrs. The G2 arrest in tumor cells provides time for the repair processes to operate which are critical for ensuring cell survival after DNA damage (41). It was suggested that the combination of BI-69A11 and celecoxib impaired IR-induced DSB repair, attenuated the G2/M cell cycle and then enhanced the radiosensitivity of CRC cancer cells (42). In the present study, CCL244 cells with irradiation revealed prolonged G2/M phase arrest. However, downregulation of UCA1 attenuated the radiation-induced arrest of G2/M cell cycle. These findings indicated that silencing of UCA1 enhanced the radiosensitivity of CRC by impairing G2/M arrest.

In the last decade, EMT has been widely recognized to play a vital role in the promotion of carcinoma metastasis. lncRNAs have been reported to be involved in the process of EMT. Silencing of HOTAIR prevented the EMT process stimulated by TGF-β1 in CRC and breast cancer (43). EMT-associated proteins (ZEB1, ZEB2, Snail, E-cadherin, MMP2, MMP9 and vimentin) have been studied in the EMT process (27). In the present study, we found that downregulation of UCA1 plus irradiation reduced the expression levels of MMP2, MMP9, ZEB1 and vimentin. These results indicated that silencing of UCA1 significantly inhibited EMT in CCL244 cells.

In summary, the present study demonstrated that UCA1 was highly expressed in CRC cells and that downregulation of UCA1 enhanced the radiosensitivity and suppressed EMT of CCL244 cells. All these results demonstrated that lncRNA-UCA1 may be a novel therapeutic target for CRC in radiotherapy.

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Availability of data and materials

The datasets used during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

CX and ZY conceived and designed the study. WL and XY performed the experiments and wrote the paper. WL, XY, XX, JZ, YongW, KZ and SH were involved in the conception of the study. SZ, ML, YongyouW and JC provided technical support. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integ-rity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

All experimental protocols were approved by the Institutional Review Board of the Second Affiliated Hospital of Soochow University, Suzhou, Jiangsu 215004.

Consent for publication

Not applicable.

Competing interests

The authors state that they have no competing interests.

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