Article

Discovery Medicine 2023; 35(176): 418–428
https://doi.org/10.24976/Discov.Med.202335176.42

Curcumin Increases Radiosensitivity of Radioresistant Nasopharyngeal Cancer

Guoyu Wang[1,†], Jie Zhu[2,†], Zengxian Wang[1], Zuliang Xu[1], Yiming Shi[1], Lingjie Luo[3,*]

[1]Department of Radiology, Taizhou Central Hospital, Taizhou University Hospital, 318000 Taizhou, Zhejiang, China
[2]Department of Clinical Laboratory, Taizhou Central Hospital, Taizhou University Hospital, 318000 Taizhou, Zhejiang, China
[3]Department of Ultrasound, Maternity and Child Health Care of Jiaojiang, 318000 Taizhou, Zhejiang, China
*Correspondence: luolingjiemrs@163.com (Lingjie Luo)
[†]These authors contributed equally.
Published: 2 June 2023

Objectives: To study the effects of curcumin on the proliferation, invasion, apoptosis, and radiosensitivity of the radioresistant nasopharyngeal carcinoma (NPC) C6661-IR strain as well as the potential radiosensitization mechanism.

Methods: NPC cells were continuously irradiated with different intensities of radiation to induce radiation-resistant cell lines. A plate clone formation assay was used to evaluate the effect of curcumin on the radiosensitivity of NPC cells. 3-(4,5-Dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide thiazolyl blue (MTT) assay was conducted to detect changes in cell viability. Flow cytometry was employed to analyze apoptosis percentage as well as Transwell® assay and immunofluorescence assay to observe cell invasion. Western blotting was applied to detect the expression levels of Bax, Bcl-2, and pro/cleaved-caspase 3. MiR-205-5p mimics and si-TP53INP1 were synthesized and transfected into C6661-IR cells, and the cells were then incubated with 10 μm/L curcumin. Real-time quantitative reverse transcription PCR (RT-qPCR) was used to measure miR-205-5p levels and western blotting was conducted to detect the expression of TP53INP1.

Results: The optimal radiation dose of X-ray was 6 Gy, and this dose was used in all subsequent experiments. Curcumin treatment significantly inhibited the proliferation and invasion of C6661-IR cells, promoted apoptosis and enhanced radiosensitivity. Compared to the 0 Gy+Cur group and the 6 Gy+Cur group, the miR-205-5p levels were higher in the C6661-IR cells of the 0 Gy and 6 Gy groups. Moreover, miR-204-5p was found to directly target TP53INP1. Curcumin downregulated miR-205-5p levels and upregulated TP53INP1 expression ($p < 0.05$). Thus, modulation of miR-205-5p or TP53INP1 expression attenuates the biological effects of curcumin on C6661-IR cells.

Conclusions: Curcumin inhibited the proliferation and invasion of C6661-IR, promoted apoptosis, and enhanced its radiosensitivity to X-rays by mediating miR-205-5p/TP53INP1 expression.

Keywords: curcumin; radioresistant NPC strain; radiosensitization; miR-205-5p; TP53INP1

Introduction

Nasopharyngeal carcinoma (NPC) is the most common malignancy among head and neck tumors, originating on the upper and lateral walls of the nasopharynx [1]. The treatment methods for NPC include radiotherapy, induction chemotherapy, minimally invasive surgery, endoscopic resection, targeted therapy, immunotherapy, or combination therapy [2]. Among these therapies, radiotherapy is still the current preferred treatment method. However, this therapy has certain disadvantages. Radioresistant NPC cells (10–20%) will lead to the recurrence and poor prognosis of NPC; For instance, patients with NPC who undergo radiotherapy alone have a 5-year survival rate of below 50% [3]. Therefore, it is necessary to find drugs that can enhance the radiosensitivity of radioresistant NPC cells, which can compensate for the deficiency of radiotherapy.

Because of the advantages of low toxicity, high efficiency, multiple targets, and fewer side effects, recent studies have focused on the application of traditional Chinese medicine in radiosensitization. Traditional Chinese medicine radiosensitizers exert their effects through multiple mechanisms such as inducing apoptosis [4], causing cell cycle arrest [2], and inhibiting DNA damage repair [5]. Curcumin, as a polyphenolic substance extracted from the rhizomes of *Zingiberaceae*, has anticancer, antioxidant, anti-inflammatory, hypolipidemic, and radiosensitizing properties [6]. For example, curcumin reduced chemotherapy resistance of hepatoma cells by regulating introduction hydroxycarboxylic acid receptor 1 (HCAR1) [7]; it functions as a radiosensitizer by inhibiting the expression of Nuclear factor-κ B (NF-κB) in laryngeal squamous carcinoma and renal cancer [8,9]; and enhances the radiosensitivity of human urethral scar fibroblasts by downregulating Smad4 [10]. Although a few studies have reported the

radiosensitization effect of curcumin on NPC cells, the specific mechanism and other biological changes remain to be elucidated.

MicroRNAs (miRNAs) have a significant regulatory role in tumor development, and some miRNAs can modulate the chemosensitivity of tumor cells [11]. Therefore, studying how to improve the radiosensitivity of tumor cells via miRNA pathways has become a new idea with great development prospects. For example, miR-339-5p can predict treatment response and side effects in esophageal cancer, and it can enhance radiosensitivity in tumor cells via Cdc25A regulation, as supported by subsequent research [12]. MiR-365 can be used as a radiation sensitizer in non-small cell lung cancer, and overexpression of miR-365 can effectively enhance the radiosensitivity of tumor cells [13]. MiR-205-5p is a functional miRNA located on chromosome lq32.2LOC642587, and it is abnormally expressed in a variety of tumors. For instance, miR-205-5p is downregulated in breast cancer [14], prostate cancer [15], colorectal cancer [16], and other tumors to inhibit cancer progression. But it plays the role of an oncogene in endometrial cancer [17], head and neck squamous cell carcinoma [18], and ovarian cancer [19].

MiR-205-5p has been suggested as a diagnostic molecular marker for NPC based on previous research [20], its expression and significance in radioresistant NPC strains have not yet been reported.

Our present study investigated the effects of curcumin on the proliferation, apoptosis, invasion, and radiosensitivity of the radioresistant NPC cell line C6661-IR and determined the relevant mechanisms. This study's findings will aid in assessing curcumin's efficacy in enhancing radiosensitivity while also identifying new molecular markers for treating and diagnosing NPC.

Materials and Methods

Cell Culture

The human NPC C6661 and HEK293T cell lines, both verified by STR, were purchased from ATCC (Manassas, VA, USA). The cells were cultured in RPMI-1640 medium with 10% FBS and 1% double antibiotics for C6661 and C6661-IR, and DMEM medium with 5% FBS and 1% double antibiotics for HEK293T. All cells were treated with radiation before interfering RNA and incubated at 5% CO_2 and 37 °C using a Thermo Fisher Scientific incubator.

Construction of the C6661-IR Cell Line

Using C6661 cells in the logarithmic growth phase, cell suspensions were created with a concentration of 2×10^5 cells/mL. Then, 100 μL of the cell suspension was inoculated into each well of a 6-well plate. The cells were then supplemented with 3 mL of RPMI-1640 medium and allowed to culture for 24 h. When the adherent cells reached the logarithmic growth phase, the radioresistant NPC subclone (C6661-IR) was constructed following a previously reported method [2]. C6661 cells were irradiated with 11 Gy (sublethal dose) X-rays to screen for surviving cells. This process lasted for 5 rounds. Finally, the radioresistant subclone C6661-IR with stable cell morphology and proliferation status was obtained.

Irradiation

C6661 and C6661-IR cell lines were cultured in the logarithmic growth phase and suspended at a concentration of 5×10^4 cells/mL. The suspensions were each inoculated on 6-well plates and cultivated until the adherent cells reached logarithmic growth. After reaching this point, the NPC cells underwent 6MV X-ray irradiation at doses ranging from 0, 2, 4, 6, 8, and 10 Gy. The 6-well plates were returned to the incubator for continued culture immediately following irradiation, and fresh medium was added after 24 hours.

Curcumin Treatment

Curcumin solution was prepared by dissolving 20 mg curcumin standard (C110685, Aladdin, Shanghai, China) in DMSO. The mixture was then ultrasonicated for 30 min to fully dissolve it, and finally, an 80 mmol/L liquor was obtained. Then, the 96-well plates (or 6-well plates) were rinsed with PBS, and the medium was replaced with RPMI-1640 medium containing 10 μm/L curcumin (for the dosage of curcumin, following the previous related research [21]). The plates were placed again in the incubator and further incubated for 24 h.

Plate Clone Formation Assay

Six-well plates were rinsed with PBS, and 4% paraformaldehyde was added to each well for fixation for 12 min; the liquid was then aspirated. Next, a 2 mL crystal violet solution (Cat# C0121, Beyotime, Shanghai, China) was added for 20 min, and the excess dye solution was then washed off with running water. The number of clones in each group was counted. Among them, colonies with more than 50 cells were considered viable clones.

MTT Assay

For each group, C6661 and C6661-IR cells were gathered and 100 μL of 2×10^4 cells/mL cell suspension was added to a 96-well plate. The plate was placed in a cell culture incubator for 24 hours, after which 0.5 mg/mL MTT solution was added for 4 hours. Following this, a 100 μL DMSO solution was added, and the mixture was shaken on a shaker for 10 minutes until the crystals dissolved. OD490 values were measured for three wells using an automatic microplate reader to determine cell viability.

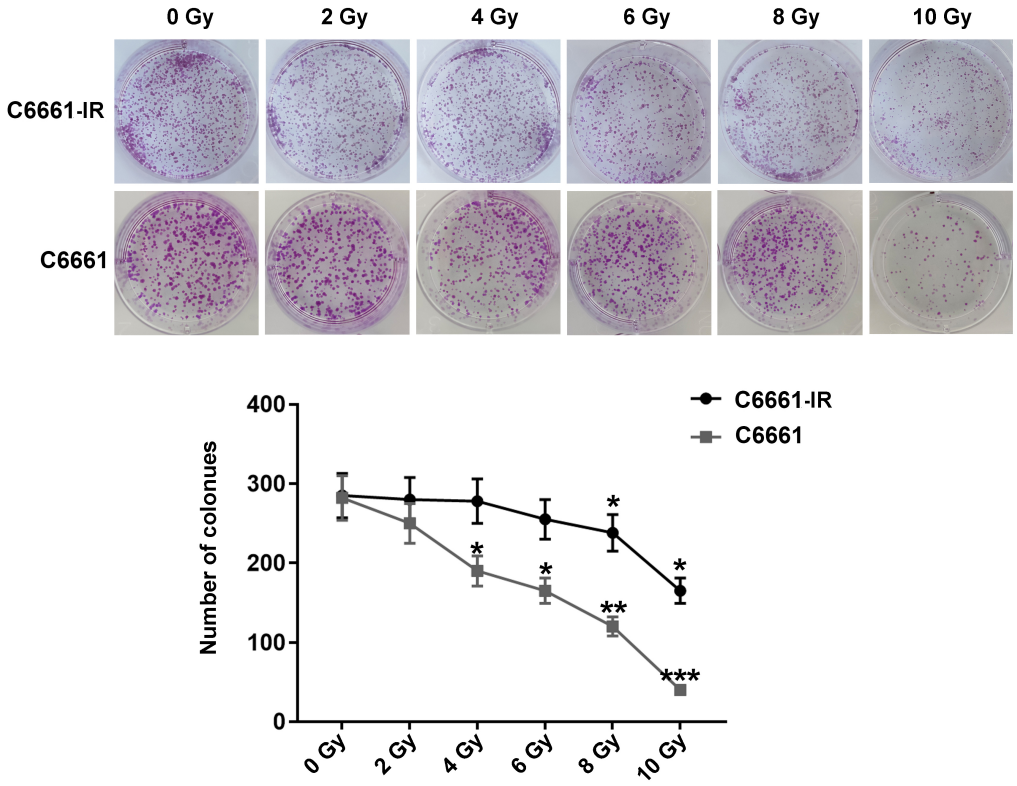

Fig. 1. Effects of different X-ray doses on the clone number of C6661 and C6661-IR cells. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ vs. 0Gy.

Flow Cytometry

We collected NPC cells and washed them twice in PBS, then centrifuged at 518 ×g for four minutes before resuspending. 200 mL of binding buffer was added, followed by 5 mL each of Annexin V-FITC and PI solution (Yeasen, Co., Ltd., Shanghai, China). The suspension was mixed thoroughly, and flow cytometry was used to detect the percentage of apoptosis.

Western Blotting

To quantify total protein extracted from of RIPA lysis buffer containing 1% PMSF was added and then centrifuged at 13,400 ×g for 12 minutes. Protein concentration was quantified using a BCA kit (Solarbio, Beijing, China). Proteins were separated via SDS-PAGE and transferred to PVDF membranes, which were then blocked using 5% skimmed milk powder in TBST for 1 hour. The membranes were incubated overnight at 4 °C with primary antibodies (anti-TP53INP1, ab202026, 1:500; cleaved caspase-3, ab32042, 1:500; anti-GAPDH, ab8245, 1:500; anti-Bax, ab32503, 1:500; anti-Bcl-2, ab32124, 1:500). After being washed with TBST, the membrane was incubated with secondary antibodies labeled with HRP (ab205718, 1:1000, Abcam, Cambridge, UK) for 1 hour, followed by imaging using an ECL kit (Yeasen, Co., Ltd., Shanghai, China). ImageJ software (version 2.0.0, ImageJ Software Inc., Bethesda, MD, USA) was used to analyze the resulting images and calculate the gray value of each band.

Transwell® Assay

To prepare for the Transwell® assay (BD Biosciences, San Jose, CA, USA), the chamber was adjusted to room temperature inside a cell incubator and then filled with RPMI-1640 medium. Following this, Matrigel was added and incubated for 2 hours before removing the medium. NPC cells in the logarithmic growth phase were then added to the upper chamber at a concentration of 4×10^5 cells/mL, with 200 μL of cell suspension added to the upper chamber and 600 μL of medium added to the lower chamber. After being incubated for 20 hours, the medium was removed, and the chamber was washed with PBS. The cells were then fixed with 4% paraformaldehyde for 20 minutes and washed three times with PBS before being stained with 1% crystal violet for 0.5 hours. Finally, the cells were gently washed with running water, dried, observed, and counted using an inverted microscope.

Immunofluorescence (IF) Assay

After washing twice with PBS, NPC cells were fixed for 30 minutes using 4% paraformaldehyde. The cells were then treated with 0.2% Triton X-100 for 10 minutes, washed thrice with PBS for 10 minutes each, and blocked with 5%

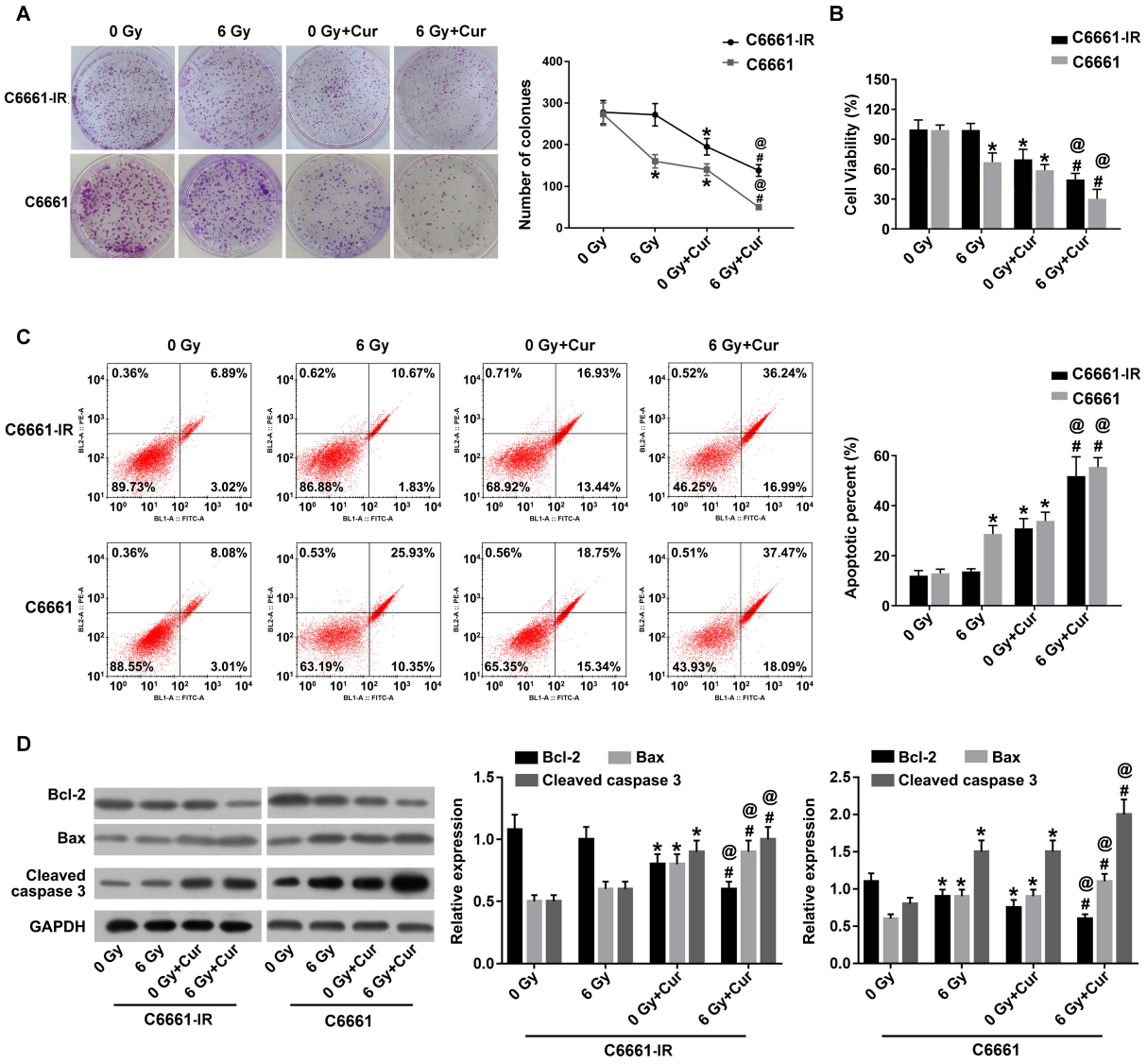

Fig. 2. Curcumin inhibits the proliferation of NPC cells and promotes their apoptosis. (A) Colony formation ability of C6661 and C6661-IR cells after irradiation with 6 Gy X-rays and/or treatment with curcumin. (B) Cell viability. (C) Apoptosis percentage. (D) Expression of Bax, Bcl-2, and pro/cleaved-caspase 3. *$p < 0.05$ vs. 0 Gy; #$p < 0.05$ vs. 6 Gy; @$p < 0.05$ vs. 0 Gy + Cur.

bovine serum albumin (BSA) for 30 minutes. Primary antibodies (E-cadherin, 1:50; N-cadherin, 1:30) from Beyotime (Shanghai, China) were added to the slides and incubated overnight at 4 °C. Residual primary antibodies were washed off with PBST, and a CY-3-labeled fluorescent secondary antibody (1:100, Beyotime, Shanghai, China) was added and incubated in the dark for 2 hours. Residual secondary antibody was washed off with PBST, and DAPI was added for 5 minutes. The cover glasses were sealed with an antifluorescence quenching sealing agent, and the slides were observed and scanned using a fluorescence microscope (BX52 System Microscope, Olympus, Hamburg, Germany).

RNA Interference and Cell Transfection

C6661-IR cells at 50–55% density were collected and mixed with 50 nM vector-liposome 2000 solution (Invitrogen, Camarillo, CA, USA); the cells were then incubated in a 37 °C incubator for 48 h. The relevant vectors were synthesized by Shanghai Sangon (Shanghai, China), including miR-NC (5′-UCGCUUGGUGCAGGUCGGGAA-3′), miR-205-5p mimics (5′-GACAAGGACGACUUGACUCGGU-3′), si-con (5′-GAACGATTATCCATTCAAA-3′), and si-TP53INP1 (5′-UUGUGAUGGUUUUGCUGAGCCA-3′).

RT-qPCR

Total RNA was extracted from NPC cells using TRIzol (Invitrogen, Camarillo, CA, USA), and cDNA was syn-

thesized using a reverse transcription kit (Yeasen, Co., Ltd., Shanghai, China). The specific miR-205-5p and U6 primer sequences were synthesized by Shanghai as follows: miR-205-5p forward: 5′-CTTGTCCTTCATTCCACCGGA-3′; miR-205-5p reverse: 5′-TGCCGCCTGAACTTCACTCC-3′; U6 forward: 5′-TGCGGGTGCTCGCTTCGGCAGC-3′; U6 reverse: 5′-CCAGTGCAGGGTCCGAGGT-3′. RT-qPCR was performed using the corresponding reaction system following the instructions of the SYBR Green Master Mix Kit. Each sample was repeated three times, and the relative levels of miR-205-5p were calculated by the $2^{-\Delta\Delta ct}$ method.

TargetScan Prediction

The miR-205-5p was entered as the search term to predict its potential target genes in the TargetScan database (http://www.targetscan.org/).

Dual-Luciferase Reporter Assay

HEK293T cells were inoculated in a 24-well plate at a density of 5×10^4 cells/well and cultured until reaching 70–80% confluency prior to transfection. TP53INP1-WT and TP53INP1-MUT were co-transfected with miR-NC or miR-205-5p mimics into the HEK293T cells for 48 hours. The Dual-Luciferase Reporter Assay System was used to measure both firefly and renilla luciferase activities, and the ratio between the activity of firefly and renilla luciferase served as an indicator of their relative activity. The specific sequence of TP53INP1-WT was 5′-UUGUGAUGGGUUUUGCUGAGCCA-3′ and the specific sequence of TP53INP1-WUT was 5′-UUGUGAUGGGUUUUGUGCGCAAC-3′.

Statistical Analysis

The SPSS 20.0 software (IBM Corp., Chicago, IL, USA) was used for statistical analysis of all data in the study, and results were expressed as mean ± SD. A *t*-test was employed for inter-group comparisons, with statistical significance defined as $p < 0.05$.

Results

Identification of C6661-IR Cells and Selection of the Radiological Dose

As described in the Methods section, we repeatedly irradiated the C6661 with X-rays at the sublethal dose of 11 Gy to obtain its radioresistant subclone C6661-IR. Next, we treated C6661 and C6661-IR with gradient doses (0, 2, 4, 6, 8, and 10 Gy) of X-ray irradiation to confirm the optimal irradiation dose. As shown in Fig. 1, the clone number of C6661 and C6661-IR cells decreased after X-ray irradiation, and the trend was dose-dependent. The clone number of C6661 cells decreased more significantly ($p < 0.05$ and $p < 0.01$) than that for C6661-IR cells. Compared with the 0 Gy group, the clone number of C6661-IR in the 8 Gy group was remarkably reduced ($p < 0.05$). Considering the toxicity of irradiation treatment to cells, we chose 6 Gy as the optimal irradiation dose and used it for all subsequent experiments (a moderate X-ray dose of 6 Gy was applied, according to previous studies [22,23]).

Curcumin Inhibits Viability and Induces Apoptosis of C6661-IR Cells

As shown in Fig. 2, no significant differences were observed in cell activity, clone number, and apoptosis percentage of C6661-IR cells between the 0 Gy and 6 Gy groups ($p > 0.05$). However, the clone number and cell viability of C6661 in the 6 Gy group were significantly lower than those in the 0 Gy group, accompanied by a significant increase in the apoptosis percentage ($p < 0.05$). Then, we incubated C6661 and C6661-IR cells with 10 μm/L curcumin for 24 h. The results indicated that the clone number, cell viability, and Bcl-2 expression of C6661 and C6661-IR were remarkably downregulated, while the apoptosis percentage, Bax and cleaved caspase-3 expression were significantly upregulated ($p < 0.05$). This finding demonstrated that curcumin effectively inhibited proliferation, enhanced radiosensitivity, and promoted apoptosis of NPC cells.

Curcumin Inhibits the Invasion of C6661-IR Cells

It is reported that the invasive ability of C6661-IR cells is significantly stronger than that of C6661 cells (parental strain) [2]. To verify the effect of curcumin on the invasion of NPC cells, the Transwell® assay and IF were performed to detect the invasion of C6661 and C6661-IR and changes in invasion-related proteins' expression. Curcumin was found to lower the invasiveness and impede growth of transmembrane NPC cells. It was also observed to decrease N-cadherin expression while increasing E-cadherin expression ($p < 0.05$) (Fig. 3).

Curcumin Downregulates miR-205-5p and Upregulates TP53INP1 in C6661-IR Cells

In Fig. 4A,B, it was observed that the expression of miR-205-5p in C6661 cells was downregulated significantly upon irradiation with 6 Gy, whereas TP53INP1 expression was increased significantly ($p < 0.05$). However, no significant change was observed in the expression levels of TP53INP1 and miR-205-5p in C6661-IR ($p > 0.05$). Additionally, the administration of curcumin led to a significant decrease in miR-205-5P levels and an increase in TP53INP1 expression levels in both NPC cell lines ($p < 0.05$). TargetScan predicted that TP53INP1 is a potential target gene for miR-205-5p. By co-transfection with miR-205-5p mimics, the activity of TP53INP1-WT was greatly reduced, while the activity of TP53INP1-MUT remained unchanged ($p < 0.05$) (Fig. 4C,D), thus indicating that miR-205-5p directly targeted TP53INP1. Both miR-205-5p and TP53INP1 were involved in curcumin-mediated biological effects on NPC cells.

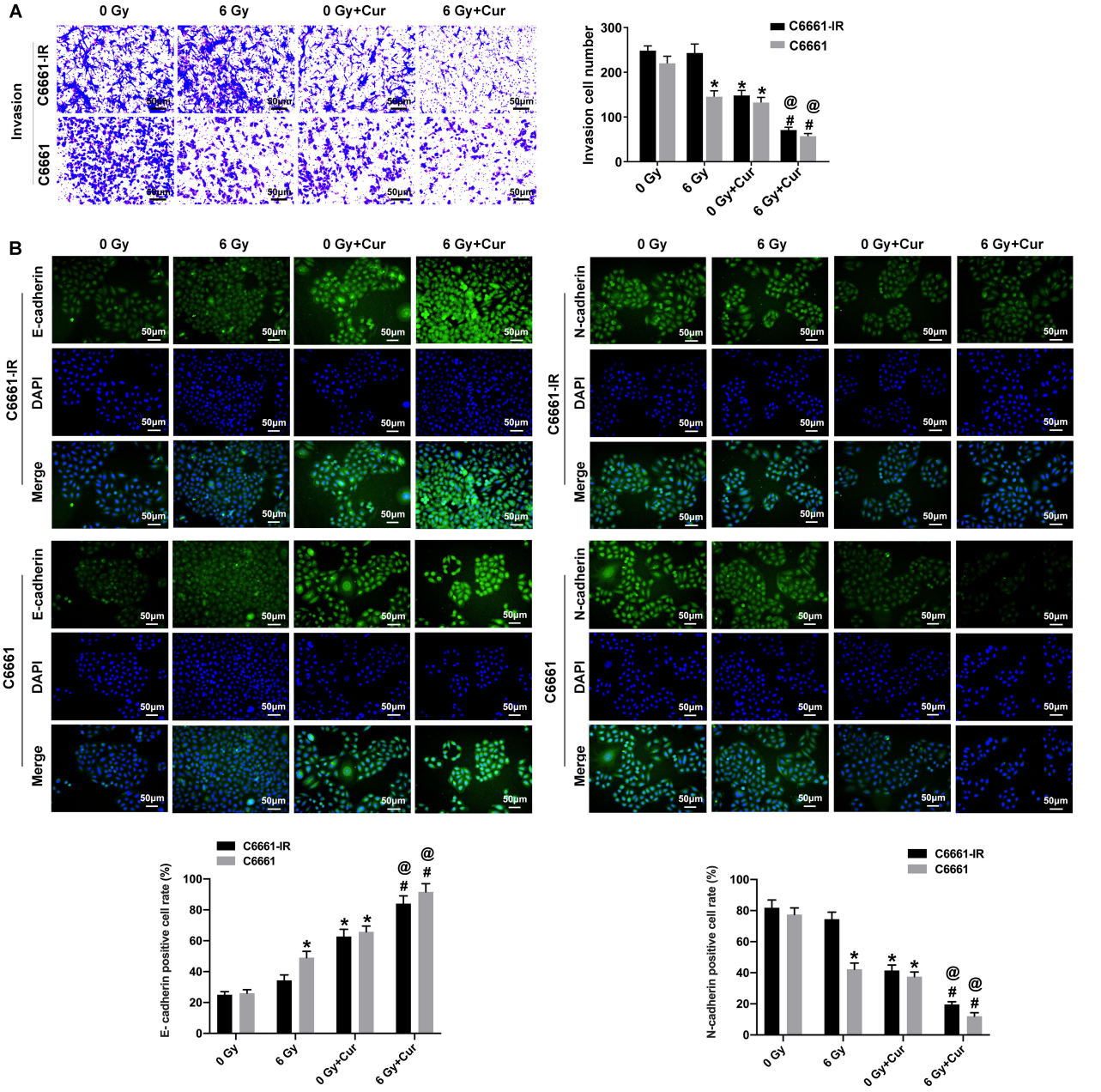

Fig. 3. Curcumin inhibits the invasion of NPC cells. (A) Invasion ability of C666 and C6661-IR cells after irradiation with 6 Gy X-rays and/or treatment with curcumin (50 μm). (B) Expression of E-cadherin and N-cadherin (50 μm). *$p < 0.05$ vs. 0 Gy; #$p < 0.05$ vs. 6 Gy; @$p < 0.05$ vs. 0 Gy + Cur.

Overexpression of miR-205-5p or Knockdown of TP53INP1 Attenuates the Effect of Curcumin on the Proliferation and Apoptosis of C6661-IR Cells

To further explore the role of miR-205-5p/TP53INP1 in curcumin-mediated proliferation and apoptosis, miR-205-5p mimics was used to upregulate the miR-205-5p levels in C6661-IR ($p < 0.05$) (Fig. 5A) and si-TP53INP1 was used to inhibit TP53INP1 expression ($p < 0.05$) (Fig. 5B). As demonstrated in Fig. 5C–E, upregulation of miR-205-5p or knockdown of TP53INP1 significantly weakened the effects of curcumin on cell proliferation and apoptosis. Compared with the 6 Gy+miR-NC+si-con group, the cell viability of C6661-IR was significantly decreased, the clone number and Bcl-2 expression were reduced, while the apoptosis percentage, Bax and cleaved-caspase 3 expression levels were increased after the administration of curcumin ($p < 0.05$) (Fig. 5F). Compared with the 6 Gy+Cur+miR-NC+si-con group, the transfection of miR-205-5p mimics or si-TP53INP1 increased C6661-IR's cell viability and clone number, but caused a decrease in apoptosis percentage ($p < 0.05$).

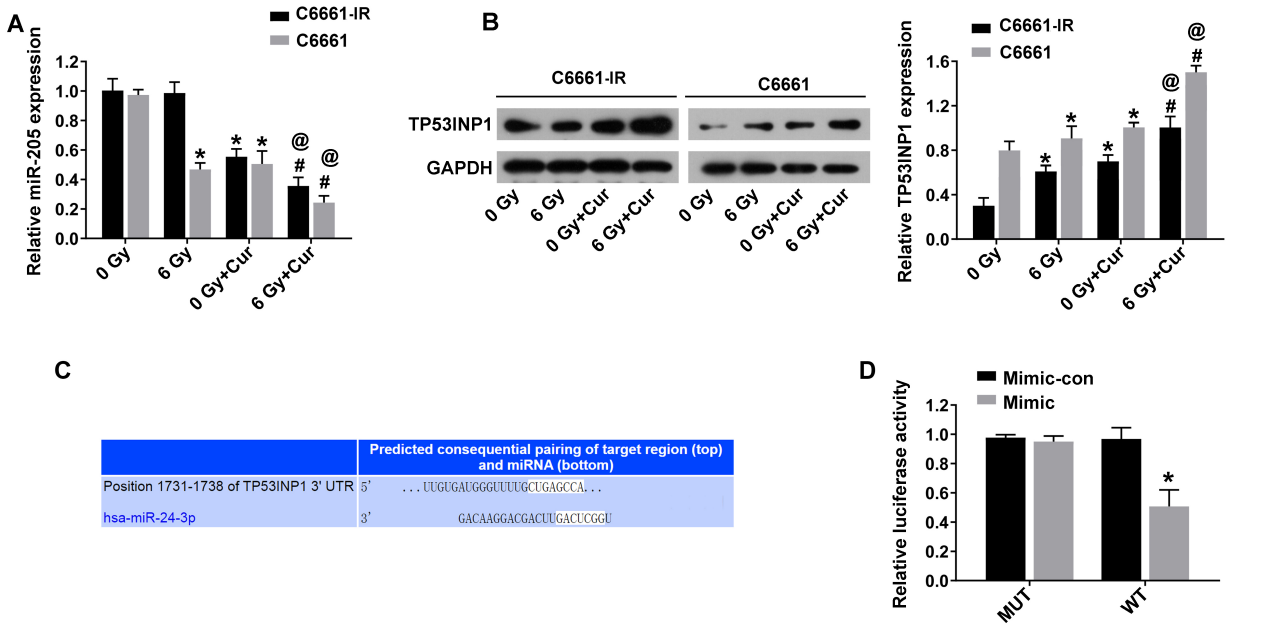

Fig. 4. Curcumin downregulates miR-205-5p and TP53INP1 in C6661-IR cells. (A) miR-205-5p levels in NPC cells of each group. (B) TP53INP1 expression in the NPC cells of each group. (C) TargetScan prediction. (D) Dual-luciferase reporter assay. *$p < 0.05$ vs. 0 Gy; #$p < 0.05$ vs. 6 Gy; @$p < 0.05$ vs. 0 Gy + Cur.

Overexpression of miR-205-5p or Knockdown of TP53INP1 Attenuates the Effect of Curcumin on the Invasion of C6661-IR Cells

Compared to the 6 Gy+miR-NC+si-con group, the administration of curcumin led to a significant reduction ($p < 0.05$) in the number of C6661-IR transmembrane cells (Fig. 6A). A decrease in N-cadherin expression was also observed, while an increase in E-cadherin expression was seen ($p < 0.05$) (Fig. 6B). Upregulation of miR-205-5p or knockdown of TP53INP1 significantly increased transmembrane cells, resulting in N-cadherin expression increasing and E-cadherin expression decreasing ($p < 0.05$). These results confirmed that the overexpression of miR-205-5p or knockdown of TP53INP1 antagonized the inhibitory effect of curcumin on invasion.

Discussion

The incidence of NPC is less than $1/10^5$ individuals worldwide, but it is as high as $50/10^5$ individuals in the south of China [24]. Because of the lack of targeted drugs with low toxicity and high efficiency, radiotherapy is still the main treatment for NPC, which also makes it difficult to cure some NPC patients because of radioresistance, which leads to distant metastasis of cancer cells. Therefore, it is necessary to find adjuvant radiosensitizers with minimal side effects and high efficiency to improve the patient's quality of life and reduce the sequelae of chemotherapy. Natural products such as curcumin [25], matrine [26], capsaicin [27] and berberine [28] can alleviate the chemotherapy resistance of NPC and can be used as radiosensitizers.

Curcumin is one of the effective components of turmeric, and it is also an important purified monomer. It shows broad-spectrum antitumor, anti-inflammatory, and antioxidant properties and fewer adverse reactions [29]. The sensitization mechanism of curcumin may be related to the following factors: (1) affecting cell cycle distribution and regulating the expression of cell cycle-related factors (CKI, CDK, and Cyclin); (2) inhibiting the expression of the MDR1 gene; and (3) blocking the growth-related pathways such as the NF-κB pathway [30] and the PI3K/AKT pathway [31] and promoting the apoptosis of tumor cells. At present, only a few articles have reported that curcumin can be used as a radiosensitizer for NPC cells. A recent study showed that curcumin has therapeutic and radiosensitizing properties in NPC cells [32,33]. One way in which curcumin enhances the sensitivity of nasopharyngeal carcinoma cells to radiation is through the mediation of MiR-593 and the MDR1 pathway [34]. In this study, the effects of curcumin on the biological functions of C6661-IR were investigated, and the underlying mechanism of radiosensitization was revealed. We successfully constructed a radioresistant strain C6661-IR with stable cell morphology and proliferation by repeated irradiation with a sublethal dose (11 Gy) of X-rays. C6661 and C6661-IR were induced by 6 Gy irradiation and treated with 10 μm/L curcumin. The proliferation and invasion of C6661-IR were significantly inhibited, which were reflected through the decrease in cell viability, clone number, transmembrane cell number and expression of N-cadherin and an increase in E-cadherin expression. In addition, we investigated the ef-

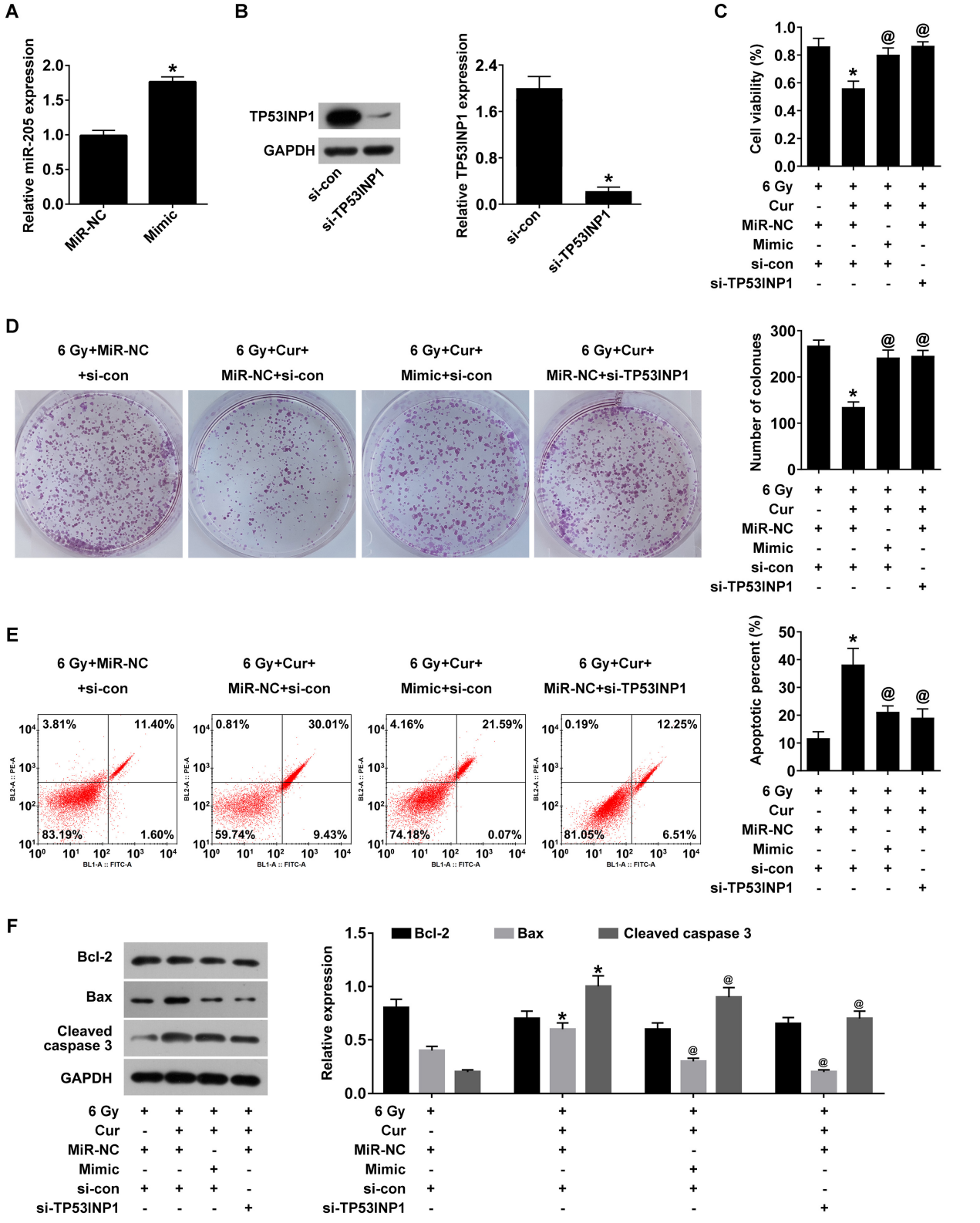

Fig. 5. Overexpression of miR-205-5p or knockdown of TP53INP1 attenuates the effect of curcumin on the proliferation and apoptosis of C6661-IR cells. (A) miR-205-5p levels in C6661-IR after transfection of miR-205-5p mimics. (B) TP53INP1 expression in C6661-IR after transfection of si-TP53INP1. (C) Cell viability of C6661-IR after treatment with curcumin and transfection of miR-205-5p mimics or si-TP53INP1. (D) Colony forming ability of C6661-IR. (E) Apoptosis percentage of C6661-IR. (F) Bax, Bcl-2, and pro/cleaved-caspase 3 expression levels. *$p < 0.05$ vs. 6 Gy + miR-NC + si-con; @$p < 0.05$ vs. 6 Gy + Cur + miR-NC + si-con.

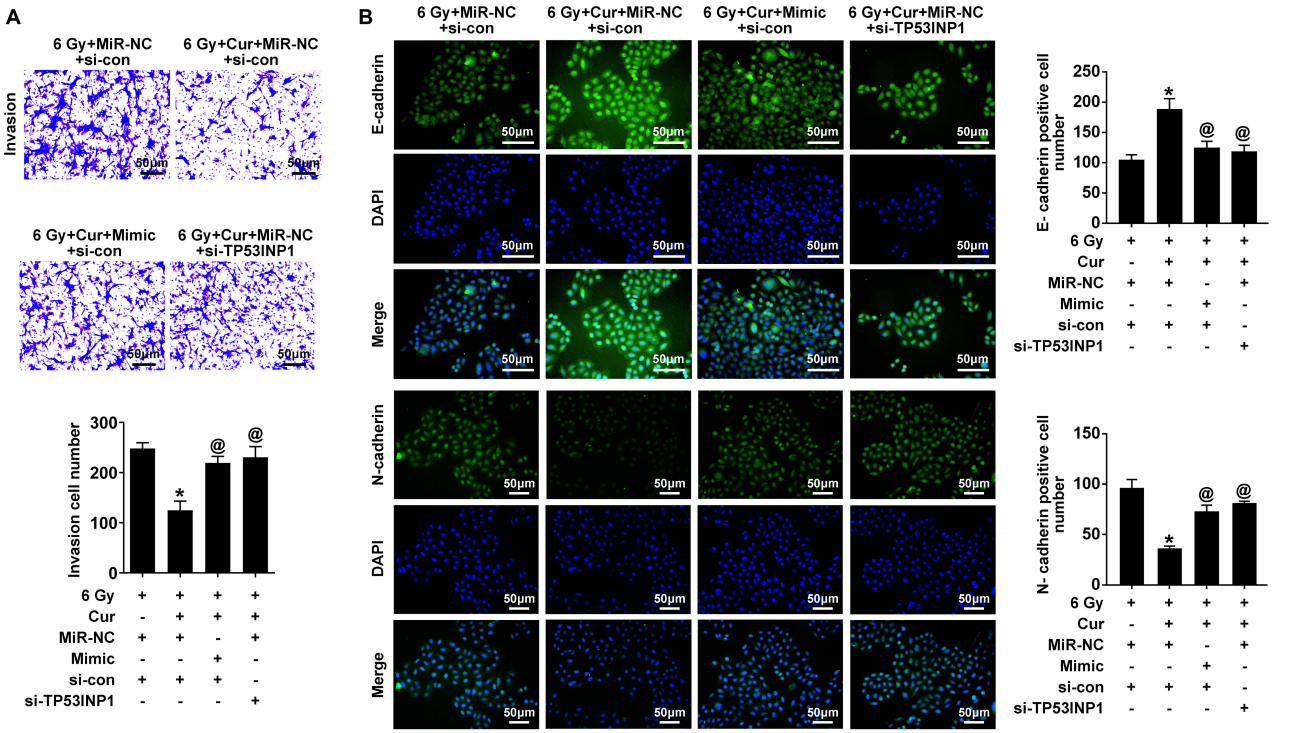

Fig. 6. Overexpression of miR-205-5p or knockdown of TP53INP1 attenuates the effect of curcumin on the invasion of C6661-IR cells. (A) Invasion ability of the C6661-IR cells after treatment with curcumin and transfection of miR-205-5p mimics or si-TP53INP1 (50 μm). (B) Expression of E-cadherin and N-cadherin (50 μm). *$p < 0.05$ vs. 6 Gy + miR-NC + si-con; @$p < 0.05$ vs. 6 Gy + Cur + miR-NC + si-con.

fect of curcumin on the apoptosis of C6661-IR cells. The results showed that the apoptosis percentage of C6661-IR and the expression of Bax and cleaved-caspase 3 increased significantly, while the expression of Bcl-2 decreased, that is, curcumin promoted the apoptosis of C6661-IR.

In recent years, several studies have discovered a large number of molecular markers and signaling pathways related to NPC. The development and application of these markers can facilitate the evaluation of the radiosensitivity of NPC cells and the prognosis of patients with NPC. For instance, miR-29c [35], miR-22 [36], miR-142-3p [37], and PD-L1 [38] can predict the prognosis and tumor-free survival rate of NPC patients; miR-23a, DAKP1, GRP78, and 14-3-3 sigma can assess the sensitivity of radiotherapy [39]. Kiwi fruit extract has been found to regulate miR-205-5p, resulting in the modulation of proliferation, metastasis, and radiosensitivity of nasopharyngeal carcinoma cells [40]. Our study found that miR-205-5p and TP53INP1 were involved in curcumin-mediated effects on the biological functions of C6661-IR, and the former directly targeted the latter. TP53INP1 is located on the human chromosome 8q22 and plays a vital role in the apoptotic network [41]. It can be activated by p53, p73, and E2F, leading to apoptosis and cell cycle arrest [42]. MiR-30a and miR-205 downregulation is observed in hypoxia related prostate cancer. These miRNA molecules modulate radiosensitivity in prostate cancer cells through TP53INP1-mediated inhibition of autophagy [43]. In C6661-IR cells, the overexpression of miR-205-5p or the knockdown of TP53INP1 antagonized the radiosensitization effect of curcumin by enhancing the proliferation and invasion ability of C6661-IR and inhibiting its apoptosis.

This study has some limitations. First, although WGCNA analysis revealed that *H. pylori* were not related to Cyan modules, this factor was not included in the qRT-PCR validation of human gastric mucosa because of the small sample size. We will incorporate *H. pylori* into the study in subsequent experimental experiments. In addition, the mechanism of GATA6-AS1 regulating CDX2 and intestinal markers was not sufficiently explored.

Conclusions

In conclusion, curcumin inhibited the proliferation and invasion of C6661-IR cells and induced their apoptosis by regulating miR-205-5p/TP53INP1, which demonstrated the effect of reversing NPC cells' radioresistance. These findings provide certain support for the diagnosis and treatment of NPC.

Availability of Data and Materials

The data used to support the findings of this study are available from the corresponding author upon request.

Author Contributions

GYW, JZ and LJL designed the research study. ZXW and ZLX performed the research. YMS provided help and advice on the experiments. GYW, JZ and LJL analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

Funding

This study was funded by Taizhou Science and Technology Bureau Project (No.1901KY43).

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Wei LN, Luo M, Wang XP, Liang T, Huang CJ, Chen H. PADI4, negatively regulated by miR-335-5p, participates in regulating the proliferation, migration, invasion and radiosensitivity of nasopharyngeal carcinoma cells. Journal of Biological Regulators and Homeostatic Agents. 2021; 35: 117–129.

[2] Li J, Jiang R, Liu WS, Liu Q, Xu M, Feng QS, *et al*. A large cohort study reveals the association of elevated peripheral blood lymphocyte-to-monocyte ratio with favorable prognosis in nasopharyngeal carcinoma. PLoS ONE. 2013; 8: e83069.

[3] Tham IWK, Hee SW, Yap SP, Tuan JKL, Wee J. Retropharyngeal nodal metastasis related to higher rate of distant metastasis in patients with N0 and N1 nasopharyngeal cancer. Head & Neck. 2009; 31: 468–474.

[4] Hasegawa M, Imai R, Nojima K, Sakurai H, Suzuki Y, Kawashima M, *et al*. Radiation-induced apoptosis in vivo: therapeutic significance of apoptosis in radiation therapy. Nihon Igaku Hoshasen Gakkai Zasshi. Nippon Acta Radiologica. 2002; 62: 535–539. (In Japanese)

[5] Cook JA, Choudhuri R, Degraff W, Gamson J, Mitchell JB. Halofuginone enhances the radiation sensitivity of human tumor cell lines. Cancer Letters. 2010; 289: 119–126.

[6] Gupta SC, Patchva S, Koh W, Aggarwal BB. Discovery of curcumin, a component of golden spice, and its miraculous biological activities. Clinical and Experimental Pharmacology & Physiology. 2012; 39: 283–299.

[7] Soni VK, Shukla D, Kumar A, Vishvakarma NK. Curcumin circumvent lactate-induced chemoresistance in hepatic cancer cells through modulation of hydroxycarboxylic acid receptor-1. The International Journal of Biochemistry & Cell Biology. 2020; 123: 105752.

[8] Deng XZ, Geng SS, Luo M, Chai JJ, Xu Y, Chen CL, *et al*. Curcumin potentiates laryngeal squamous carcinoma radiosensitivity via NF-κB inhibition by suppressing IKKγ expression. Journal of Receptor and Signal Transduction Research. 2020; 40: 541–549.

[9] Li G, Wang Z, Chong T, Yang J, Li H, Chen H. Curcumin enhances the radiosensitivity of renal cancer cells by suppressing NF-κB signaling pathway. Biomedicine & Pharmacotherapy. 2017; 94: 974–981.

[10] Gong Z, Yang J, Dong J, Li H, Wang B, Du K, *et al*. LncRNA *HIF1A-AS1* Regulates the Cellular Function of HUVECs by Globally Regulating mRNA and miRNA Expression. Frontiers in Bioscience-Landmark. 2022; 27: 330.

[11] Khan SH. Genome-Editing Technologies: Concept, Pros, and Cons of Various Genome-Editing Techniques and Bioethical Concerns for Clinical Application. Molecular Therapy. Nucleic Acids. 2019; 16: 326–334.

[12] Luo A, Zhou X, Shi X, Zhao Y, Men Y, Chang X, *et al*. Exosome-derived miR-339-5p mediates radiosensitivity by targeting Cdc25A in locally advanced esophageal squamous cell carcinoma. Oncogene. 2019; 38: 4990–5006.

[13] Li H, Jiang M, Cui M, Feng G, Dong J, Li Y, *et al*. MiR-365 enhances the radiosensitivity of non-small cell lung cancer cells through targeting CDC25A. Biochemical and Biophysical Research Communications. 2019; 512: 392–398.

[14] Ma C, Shi X, Guo W, Feng F, Wang G. miR-205-5p downregulation decreases gemcitabine sensitivity of breast cancer cells via ERp29 upregulation. Experimental and Therapeutic Medicine. 2019; 18: 3525–3533.

[15] Li L, Li S. miR-205-5p inhibits cell migration and invasion in prostatic carcinoma by targeting ZEB1. Oncology Letters. 2018; 16: 1715–1721.

[16] Chen S, Wang Y, Su Y, Zhang L, Zhang M, Li X, *et al*. miR 205 5p/PTK7 axis is involved in the proliferation, migration and invasion of colorectal cancer cells. Molecular Medicine Reports. 2018; 17: 6253–6260.

[17] Lu Z, Xu Y, Yao Y, Jiang S. miR-205-5p contributes to paclitaxel resistance and progression of endometrial cancer by downregulating FOXO1. Oncology Research. 2019. (online ahead of print)

[18] Zhou Z, Liu C, Liu K, Lv M, Li B, Lan Z, *et al*. Expression and Possible Molecular Mechanisms of microRNA-205-5p in Patients With Head and Neck Squamous Cell Carcinoma. Technology in Cancer Research & Treatment. 2020; 19: 1533033820980110.

[19] Tao P, Yang B, Zhang H, Sun L, Wang Y, Zheng W. The overexpression of lncRNA MEG3 inhibits cell viability and invasion and promotes apoptosis in ovarian cancer by sponging miR-205-5p. International Journal of Clinical and Experimental Pathology. 2020; 13: 869–879.

[20] Yang W, Tan S, Yang L, Chen X, Yang R, Oyang L, *et al*. Exosomal miR-205-5p enhances angiogenesis and nasopharyngeal carcinoma metastasis by targeting *desmocollin-2*. Molecular Therapy Oncolytics. 2022; 24: 612–623.

[21] Zhan JW, Jiao DM, Wang Y, Song J, Wu JH, Wu LJ, *et al*. Integrated microRNA and gene expression profiling reveals the crucial miRNAs in curcumin anti-lung cancer cell invasion. Thoracic Cancer. 2017; 8: 461–470.

[22] Liu Q, He X, Liu Y, Du B, Wang X, Zhang W, *et al*. NADPH oxidase-mediated generation of reactive oxygen species: A new mechanism for X-ray-induced HeLa cell death. Biochemical and Biophysical Research Communications. 2008; 377: 775–779.

[23] Lu J, Suzuki T, Lu S, Suzuki N. Involvement of glyceraldehyde-3-phosphate dehydrogenase in the X-Ray resistance of HeLa cells. Bioscience, Biotechnology, and Biochemistry. 2008; 72: 2432–2435.

[24] Tang Z, Chen W, Xu Y, Lin X, Liu X, Li Y, *et al*. miR-4721, Induced by EBV-miR-BART22, Targets *GSK3β* to Enhance the Tumorigenic Capacity of NPC through the *WNT/β-catenin* Pathway. Molecular Therapy. Nucleic Acids. 2020; 22: 557–571.

[25] Liu L, Yang J, Ji W, Wang C. Curcumin Inhibits Proliferation of Epstein-Barr Virus-Associated Human Nasopharyngeal Carcinoma Cells by Inhibiting EBV Nuclear Antigen 1 Expression. BioMed Research International. 2019; 2019: 8592921.

[26] Zhang J, Tang AZ. Reversal effect of matrine on drug resistance of human nasopharyngeal carcinoma cell line. Journal of Chinese Medicinal Materials. 2012; 35: 1989–1994. (In Chinese)

[27] Lin YT, Wang HC, Hsu YC, Cho CL, Yang MY, Chien CY. Capsaicin Induces Autophagy and Apoptosis in Human Nasopharyngeal Carcinoma Cells by Downregulating the PI3K/AKT/mTOR Pathway. International Journal of Molecular Sciences. 2017; 18: 1343.

[28] Wang J, Kang M, Wen Q, Qin YT, Wei ZX, Xiao JJ, *et al*. Berberine sensitizes nasopharyngeal carcinoma cells to radiation through inhibition of Sp1 and EMT. Oncology Reports. 2017; 37: 2425–2432.

[29] Qiu B, Xu X, Yi P, Hao Y. Curcumin reinforces MSC-derived exosomes in attenuating osteoarthritis via modulating the miR-124/NF-κB and miR-143/ROCK1/TLR9 signalling pathways. Journal of Cellular and Molecular Medicine. 2020; 24: 10855–10865.

[30] Coker-Gurkan A, Ozakaltun B, Akdeniz BS, Ergen B, Obakan-Yerlikaya P, Akkoc T, *et al*. Proinflammatory cytokine profile is critical in autocrine GH-triggered curcumin resistance engulf by atiprimod cotreatment in MCF-7 and MDA-MB-231 breast cancer cells. Molecular Biology Reports. 2020; 47: 8797–8808.

[31] Ren BC, Zhang YF, Liu SS, Cheng XJ, Yang X, Cui XG, *et al*. Curcumin alleviates oxidative stress and inhibits apoptosis in diabetic cardiomyopathy via Sirt1-Foxo1 and PI3K-Akt signalling pathways. Journal of Cellular and Molecular Medicine. 2020; 24: 12355–12367.

[32] Momtazi-Borojeni AA, Ghasemi F, Hesari A, Majeed M, Caraglia M, Sahebkar A. Anti-Cancer and Radio-Sensitizing Effects of Curcumin in Nasopharyngeal Carcinoma. Current Pharmaceutical Design. 2018; 24: 2121–2128.

[33] Pan Y, Wang M, Bu X, Zuo Y, Wang S, Wang D, *et al*. Curcumin analogue T83 exhibits potent antitumor activity and induces radiosensitivity through inactivation of Jab1 in nasopharyngeal carcinoma. BMC Cancer. 2013; 13: 323.

[34] Fan H, Shao M, Huang S, Liu Y, Liu J, Wang Z, *et al*. MiR-593 mediates curcumin-induced radiosensitization of nasopharyngeal carcinoma cells via MDR1. Oncology Letters. 2016; 11: 3729–3734.

[35] Niu M, Gao D, Wen Q, Wei P, Pan S, Shuai C, *et al*. MiR-29c regulates the expression of miR-34c and miR-449a by targeting DNA methyltransferase 3a and 3b in nasopharyngeal carcinoma. BMC Cancer. 2016; 16: 218.

[36] Wang X, Zou M, Li J, Wang B, Zhang Q, Liu F, *et al*. LncRNA H19 targets miR-22 to modulate H_2 O_2 -induced deregulation in nucleus pulposus cell senescence, proliferation, and ECM synthesis through Wnt signaling. Journal of Cellular Biochemistry. 2018; 119: 4990–5002.

[37] Qi X, Li J, Zhou C, Lv C, Tian M. MiR-142-3p Suppresses SOCS6 Expression and Promotes Cell Proliferation in Nasopharyngeal Carcinoma. Cellular Physiology and Biochemistry. 2015; 36: 1743–1752.

[38] Johnson D, Ma BBY. Targeting the PD-1/ PD-L1 interaction in nasopharyngeal carcinoma. Oral Oncology. 2021; 113: 105127.

[39] Zhang P, Lu X, Shi Z, Li X, Zhang Y, Zhao S, *et al*. miR-205-5p regulates epithelial-mesenchymal transition by targeting PTEN via PI3K/AKT signaling pathway in cisplatin-resistant nasopharyngeal carcinoma cells. Gene. 2019; 710: 103–113.

[40] Chen Q, Pan M, Lu Y, Wei F, Chen C, Huang H. Proliferation, Metastasis, and Radiosensitivity of Nasopharyngeal Carcinoma Cells in the Expression and Effect of Kiwifruit Extract through the Regulation of miR-205-5p. Journal of Oncology. 2022; 2022: 6925772.

[41] Xiao WY, Zong Z, Qiu ML, Chen XY, Shen HX, Lao LF. Paclitaxel Induce Apoptosis of Giant Cells Tumor of Bone via TP53INP1 Signaling. Orthopaedic Surgery. 2019; 11: 126–134.

[42] Shahbazi J, Lock R, Liu T. Tumor Protein 53-Induced Nuclear Protein 1 Enhances p53 Function and Represses Tumorigenesis. Frontiers in Genetics. 2013; 4: 80.

[43] Xu CG, Yang MF, Fan JX, Wang W. MiR-30a and miR-205 are downregulated in hypoxia and modulate radiosensitivity of prostate cancer cells by inhibiting autophagy via TP53INP1. European Review for Medical and Pharmacological Sciences. 2016; 20: 1501–1508.