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Up-regulation of microRNA-136 induces apoptosis and radiosensitivity of esophageal squamous cell carcinoma cells by inhibiting the expression of MUC1

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ABSTRACT

Objective: This present study is performed to figure out the role of microRNA-136 (miR-136) in radiosensitivity of esophageal squamous cell carcinoma (ESCC) cells through the regulation of MUC1.

Methods: Seventy-four ESCC patients were divided into radiotherapy sensitive group and radiotherapy resistance group. Colony formation assay and flow cytometry were used to test the radiosensitivity of radiotherapy resistant strain and parent strain. The expression of miR-136 between radiotherapy resistant strain and parent strain was detected by RT-qPCR, and the expression of miR-136 in Eca109 and TE-1 cells as well as Eca109-R and TE-1-R cells was detected after different doses of X-ray irradiation. Eca109 and TE-1 cells as well as Eca109-R and TE-1-R cells with overexpression of miR-136 or co-overexpression of miR-136 and MUC1 were constructed. Cell proliferation, colony formation and apoptosis was detected by CCK-8 assay, colony formation assay, and flow cytometry, respectively.

Results: The expression of miR-136 in ESCC tissues was lower and MUC1 mRNA and protein expression was higher than that in adjacent normal tissues. The expression of miR-136 was negatively correlated with the expression of MUC1 mRNA in ESCC. Low expression of miR-136 and high expression of MUC1 were associated with tumor size, lymph node metastasis and distant metastasis. The expression of miR-136 increased while the expression of MUC1 decreased in the radiotherapy sensitive group of ESCC patients relative to the radiotherapy resistant group. The colony formation ability of radiation resistant cell line was stronger than that of parent cell line, and the apoptosis rate showed an opposite trend. Up-regulation of miR-136 reduced the survival rate, suppressed colony formation ability and induced apoptosis of ESCC cells under irradiation, which was reversed by upregulated MUC1.

Conclusion: This study demonstrates that up-regulation of miR-136 induces apoptosis and radiosensitivity of ESCC cells by inhibiting the expression of MUC1.

1. Introduction

Esophageal cancer (EC) represents the eighth most commonly occurring cancer, and accounts for the sixth most frequent cause of death stemming from cancer worldwide (Okuda et al., 2017). EC can be divided into two main subtypes: esophageal adenocarcinoma (EAC) and esophageal squamous cell carcinoma (ESCC), with the former exhibiting rising incidences in recent years, while the latter is very common in eastern Africa, Central Asia and China (Kim and Shah, 2017). ESCC shows regional lymph node metastasis (LNM) or extensive local invasion at the time of initial diagnosis, thus, it is one of the significant aggressive diseases with poor outcome (Ye et al., 2011). ESCC is generally limited to treatment approaches of surgical resection, chemotherapy and radiotherapy, however, the results are often largely unsatisfactory (Nakajima and Kato, 2013), which is reflected by the poor 5-year survival rate observed among patients with ESCC (Cao

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et al., 2014). Recent studies have indicated that ESCC progression is involved with the mutation of some oncogenes and anti-oncogenes, which are featured with the synergetic role of multiple genes (Zhu et al., 2010; Shigeoka et al., 2015). Therefore, more effort is still necessary to explore both the genetic and molecular changes underlying in ESCC development.

MicroRNA (miRNAs) have been suggested to be implicated in some biological processes, including cell cycle, proliferation, polarity, proliferation and migration (Cheung et al., 2012; Noguchi et al., 2012; Haapa-Paananen et al., 2013). Meanwhile, studies also have revealed that miRNAs may play a role in determination of chemotherapeutic behavior to improve antineoplastic drug sensitivity and elimination of chemotherapy-resistant cancer cells (Sun et al., 2012; Feng et al., 2012; Bao et al., 2012). One such miRNAs, miR-136, is poorly expressed in a number of human cancers, such as non-small cell lung cancer and human glioma (Shen et al., 2014; Yang et al., 2012). Evidence has shown that up-expressed miR-136 suppressed proliferation, migration, invasion, and induced apoptosis in melanoma cells (Wang et al., 2017). However, the expression and its biological function of miR-136 in ESCC remain to be established. Mucins are defined as a group of high molecular glycoproteins, which are commonly expressed in the pancreatic, gastrointestinal, as well as respiratory tracts (Higashi et al., 2015). MUC1 is reported to expressed in majority of epithelial tissues, and its upregulation has been found in the pancreas and breast (Chhieng et al., 2003). Patients with overexpressed MUC1 often exhibits advanced stage or LNM, implying relationship between MUC1 expression and cell invasion or metastasis of ESCC (Sagara et al., 1999). Based on aforementioned results, we conducted this present study to figure out the role of miR-136 in radiosensitivity of ESCC cells through regulation of MUC1.

2. Materials and methods

2.1. Ethics statement

This study was approved by the ethics committee of the First People's Hospital of Jingmen City. All the participants provided the informed consent.

2.2. Source and grouping of tissue specimens

Endoscopic biopsy specimens confirmed by pathology in patients with EC were collected in the Digestive Department of the First People's Hospital of Jingmen City between March 2014 and March 2017. There were 74 cases of ESCC tissues and 74 cases of adjacent normal tissues. Among 74 patients with ESCC, there were 50 males and 24 females, aged 50 to 74 years; there were 41 smokers and 33 non-smokers; 12 had family history and 62 had no family history; 18 cases of upper esophageal carcinoma, 33 cases of middle esophageal carcinoma and 23 cases of lower esophageal carcinoma; there were 37 cases with tumor size $\leq$4.0 cm, 23 cases with 4.1–6.0 cm, and 14 cases $>$ 6.0 cm. Tissue specimens were included if they met the following criteria: (1) pathological diagnosis of ESCC, (2) no treatment for ESCC before this time of treatment, (3) Karnofsky (KPS) score $\geq$ 80 points before treatment, (4) receiving and completing standard radical radiotherapy in accordance with the criteria of the First People's Hospital of Jingmen City, (5) receiving follow-up examination on time one month after the end of radiotherapy. Seventy-four cases of ESCC were divided into radiotherapy-sensitive group and radiotherapy-resistant group according to the criteria of short-term curative effect evaluation. We defined the efficacy of complete remission (CR) or partial remission (PR) as radiotherapy-sensitive patients (n = 20), and progressive disease (PD) or stable disease (SD) as radiotherapy-resistant patients (n = 54). The short-term efficacy was evaluated by imaging methods (esophageal barium angiography, CT scan or endoscopy) according to the criteria for evaluating the efficacy of solid tumors (RECIST, 2000): CR: The target lesion is lost. PR: Compared to a baseline state, the longest diameter of target lesions decrease by at least 30%. PD: Compared to the minimum target lesions recorded after treatment, the longest diameter of target lesion increased by 20%, or appearance of one or more new lesions. SD: Between PD and PR.

2.3. Cell culture

Human ESCC cell line Eca109 and TE-1 were purchased from the cell bank of the Chinese Academy of Sciences (Shanghai, China). Eca109 cells and TE-1 cells were adherent cells. They grow in RPMI-1640 medium containing 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin, and incubated in a cell incubator with 5% CO_2 at 37 °C. Every other day, the liquid was changed, and the cells in logarithmic growth period (1×10^7 cells were harvested at 70% confluence) were used in the experiment.

2.4. Construction of radiation resistant strain

The ESCC cells (Eca109, TE-1) were irradiated with linear accelerator X-ray (X-ray tube voltage: 150 KV, tube current: 20 mA, air specific energy release rate of probe position: 7.180 Gy/m, air specific energy release rate of exposed object: 4.81 Gy/m, focus and specimen items: 350 mm, first dose: 1 Gy) when they were in logarithmic growth period and in good condition. After each X-ray irradiation, the fresh medium was replaced and then put back into the incubator. When the cells grew to 90% confluence, subculture was performed. When the cells re-entered the logarithmic growth phase, they were continued to irradiate 1 Gy, and the dose was gradually increased: 1 Gy, three times; 2 Gy, three times; 4 Gy, seven times. After all irradiation, there obtained radiation resistant strain named as Eca109-R and TE-1-R.

2.5. Cell transfection

The well-grown ESCC parent cells (Eca109 and TE-1) and radiotherapy resistant strains (Eca109-R and TE-1-R) were inoculated into a 6-well plate, in which Eca109 and TE-1 cells as well as Eca109-R and TE-1-R cells were transfected with miR-136 mimics, mimics negative control (NC), miR-136 mimics + pcDNA3.1 and miR-136 mimics + pc-MUC1. The cells were transfected with the instructions of Lipofectamine 2000 (Invitrogen Inc., California, USA). On the first day the cells after transfection were divided into 6-well plates, and the number of cells per well was 1×10^6 cells/well. Each well was added with 1.5 mL medium without antibiotics, and the cell density was 50–70%. miR-136 mimics (sequence: 5′-ACUCCAUUUGUUUUGAUGAUGGA-3′), mimics NC (sequence: 5′-GUCCCACUUCGACCGUGCUUCCA-3′) and pc-MUC1 were purchased from Guangzhou RiboBio Co., Ltd. (Guangzhou, China).

2.6. Cell counting kit-8 (CCK-8) assay

In Eca109 and TE-1 cells as well as Eca109-R and TE-1-R cells, 3–8 $\times 10^5$ cells/well were inoculated into a 6-well plate, which were transfected after the cell adhered to the wall. After 24 h of transfection, the cells were detached by trypsin and resuspended with a small amount of culture medium, and then the cells were counted. The cells were diluted to 20 cells/μL in proportion, and about 2×10^3 cells/well were inoculated on a 96-well plate, that is, 100 μL cell suspension was added to each well. In order to avoid the influence of water volatilization on the cell growth and the experimental results, 100 μL phosphate buffer saline (PBS) was added to every well around the culture plate. The cells were exposed to 6 Gy after the cell adhered to the wall. Cell proliferation was detected at 0 h, 12 h, 24 h and 48 h after irradiation. Each well was incubated with 10 μL CCK-8 solution for 3 h. The optical density (OD) value of each well was detected by a microplate reader (450 nm as absorption wavelength and 620 nm as reference

wavelength). Cell survival rate = (OD value in each irradiation group - OD value in each blank group)/(OD value in the non-irradiation group - OD value in each blank group) × 100%. The experiment was repeated three times.

2.7. Colony formation assay

The cells in good growth condition were selected and detached by 0.25% trypsin, and then triturated into single cells and centrifuged. The number of cells was counted with a counting board. Eca109-R, TE-1-R and its parent cells or Eca109-R, TE-1-R and parental cells transfected with miR-136 mimics, mimics NC, miR-136 mimics + pcDNA3.1 and miR-136 mimics + pc-MUC1 were inoculated separately in a 6-well plate after cells were resuspended in culture medium containing 10% FBS. 0 Gy group: 500 cells/well, 2 Gy group: 1000 cells/well, 4 Gy group, 2000 cells/well, 6 Gy group, 3000 cells/well, 8 Gy group, 4000 cells/well. Three replicate wells were set in each group. After overnight inoculation, the cells were exposed to 0 Gy, 2 Gy, 4 Gy, 6 Gy, 8 Gy X-ray, and then cultured in a cell incubator for 10 days. When the cell colonies grew to be visible to the naked eye, about 50 cells were counted under the microscope, the experiment was terminated. Next, the cells were fixed with 4% polyformaldehyde (500 μL) for 15 min, stained with crystal purple dye solution (500 μL) for 15 min and observed. Plating efficiency (PE) = colony number/inoculation number × 100%, and survival fraction (SF) = colony rate in the experimental group/colony rate in the control group × 100%. Cell dose survival curve was made by formula $S = 1 - (1 - e^{-KD})^N$ by using GraphPad. Prism. 6 software. The experiment was repeated three times.

2.8. Annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI) double staining

According to the experimental design, the resistant strain and the parent strain were divided into two groups: irradiation group and non-irradiation group. The cells were inoculated into 6-well plates with 3×10^5 cells. The cells were exposed to 0 Gy or 6 Gy radiation for 24 h when cells reached 50–60% confluence. According to the requirements of the experiment, the irradiation group and the non-irradiation group was divided into blank control group, miR-136 mimics group, mimics NC group, miR-136 mimics + pcDNA3.1 group and miR-136 mimics + pc-MUC1 group, respectively. The cells were inoculated into 6-well plates with 3–8 × 10^5 cells and then exposed to 0 Gy or 6 Gy radiation for 24 h when cells reached 50–60% confluence. MilliQ water was used to prepare 10 × Annexin V Binding Buffer into 1 × Binding buffer (Invitrogen Corporation, California, USA). PI solution (5 μL, 1 mg/mL) was added into 45 μL 1 × Annexin V Binding Buffer. The cells were detached with ethylene diamine tetraacetic acid (EDTA)-free trypsin and then washed with pre-cooled PBS. The floating dead cells in the medium were also collected. The cells were suspended with 100 μL 1 × Binding Buffer and prepared into single cell suspension with density of 1×10^6 cells/mL. The above cell suspension was added with 5 μL Alexa FluorR 488 annexin V and 1 μL 100 μg/mL PI, and incubated at room temperature for 15 min. After incubation, the cells were added with 400 μL 1 × Annexin V Binding Buffer and placed on ice in dark. Flow cytometer (Beckman Coulter Inc., Fullerton, CA, USA) was used to detect cell apoptosis. The experiment was repeated three times.

2.9. RNA isolation and quantification

Trizol (Takara Co., Ltd., Dalian, China) method was used to extract total RNA in cells and tissues, which was used to determine the concentration and purity of RNA. As for the detection of miR-124, miRNA reverse transcription primers (synthesized by Guangzhou RiboBio Co., Ltd., Guangzhou, China) were reverse transcribed by reverse transcription kit (Fermentas, Burlington, ON, Canada). SYBRGREEN real-time PCR Master Mix was used to perform real-time polymerase chain reaction (PCR) reaction in ABI 7500 real-time PCR system. The sequence of miRNA primers was as follows: miR-136 (reverse transcription primers): 5′-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTG GATACGACTCCAT-3′. miR-136-F: 5′-GCGCACTCCATTTGTTTTGAT-3′. miR-136-R: 5′-GTGCAGGGTCCGAGGT-3′. U6-F: 5′-CTCGCTTCGCAGC ACA-3′. U6-R: 5′-AACGCTTCACGAATTTGCGT-3′. As for the detection of MUC1, cDNA was reverse transcribed by reverse transcription kit (Fermentas, Burlington, ON, Canada). SYBR GREEN real-time PCR Master Mix was used for real-time PCR reaction. MUC1-F: 5′-TGAGTG ATGTGC-3′. MUC1-R: 5′-CTGCCCGTAGTTCTTTCG-3′. Wtp53-F: 5′-TTCCTCTTCCTGCAGTACTC-3′; Wtp53-R: 5′-GCAAATTTCCTTCCAC TCGG-3′. GAPDH-F: 5′-AACGGATTTGGTCGTATTGGG-3′. GAPDH-R: 5′-TCGCTCCTGGAAGATGGTGAT-3′. U6 and glyceraldehyde phosphate dehydrogenase (GAPDH) were used as internal controls, $2^{-\triangle\triangle Ct}$ (Tuo et al., 2015) was used for expression of genes. The experiment was repeated three times.

2.10. Western blot analysis

The total protein of cells in each group was extracted. The protein concentration was determined according to the bicinchoninic acid (BCA) protein assay kit. The extracted protein added with 5 × sodium dodecyl sulfate (SDS) loading buffer, separated with SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were transferred onto polyvinylidene fluoride (PVDF) membranes and blocked with 5% bovine serum albumin (BSA) for 1 h. Next, the membranes were supplemented with primary antibody against MUC1 and GAPDH (1: 1000, Abcam, Cambridge, MA, USA) and incubated at 4 °C overnight, followed by washing three times with Tris-buffered saline with Tween 20 (TBST). The membranes were supplemented with horseradish peroxidase (HRP)-labeled corresponding secondary antibody and incubated for 1 h at 37 °C. The membranes were washed with TBST. Chemiluminescence reagents were employed to develop images. The gray values of target protein bands were analyzed by ImageJ software. The experiment was conducted in triplicate.

2.11. Dual luciferase reporter gene assay

The target binding sites of MUC1 to miR-136 were determined by online prediction software http://www.targetscan.org. The primers were designed and synthesized with the sequence of MUC1 3′UTR. The restriction endonuclease Hind III and *Spe* I restriction sites were introduced into upstream and downstream primers, respectively. The mutation sequence of the binding site was designed and the target sequence was synthesized by GenScript (Nanjing) Co., Ltd. (Nanjing, China). The amplified target fragment and the vector plasmid of pMIR-REPORT™ Luciferase were digested by restriction enzyme Hind III and Spe I, and the products were recycled. The products were ligated by T4 DNA ligase and transformed into DH5α competent *Escherichia coli*. The recombinant plasmid was identified by restriction enzyme digestion and sequencing. In the 12-well plate, 1×10^5 Eca109 and TE-1 cells were seeded into each well respectively. The cells were co-transfected with recombinant plasmid and miR-136 mimics for 48 h according to the corresponding group. The firefly luciferase activities of the treated cells were normalized to their renilla luciferase activities and are expressed as a percentage of activity of untreated cells.

2.12. Statistical analysis

SPSS21.0 software (IBM Corp, Armonk, NY, USA) was used for date analysis. The enumeration data were expressed in the form of percentage or rate, and chi-square test was used for the analysis. Kolmogorov-Smirnov test verified that the measurement data was in normal distribution, and the results were expressed in the form of mean ± standard deviation. The paired *t*-test was used for the comparison within groups, the independent sample *t*-test was used in the

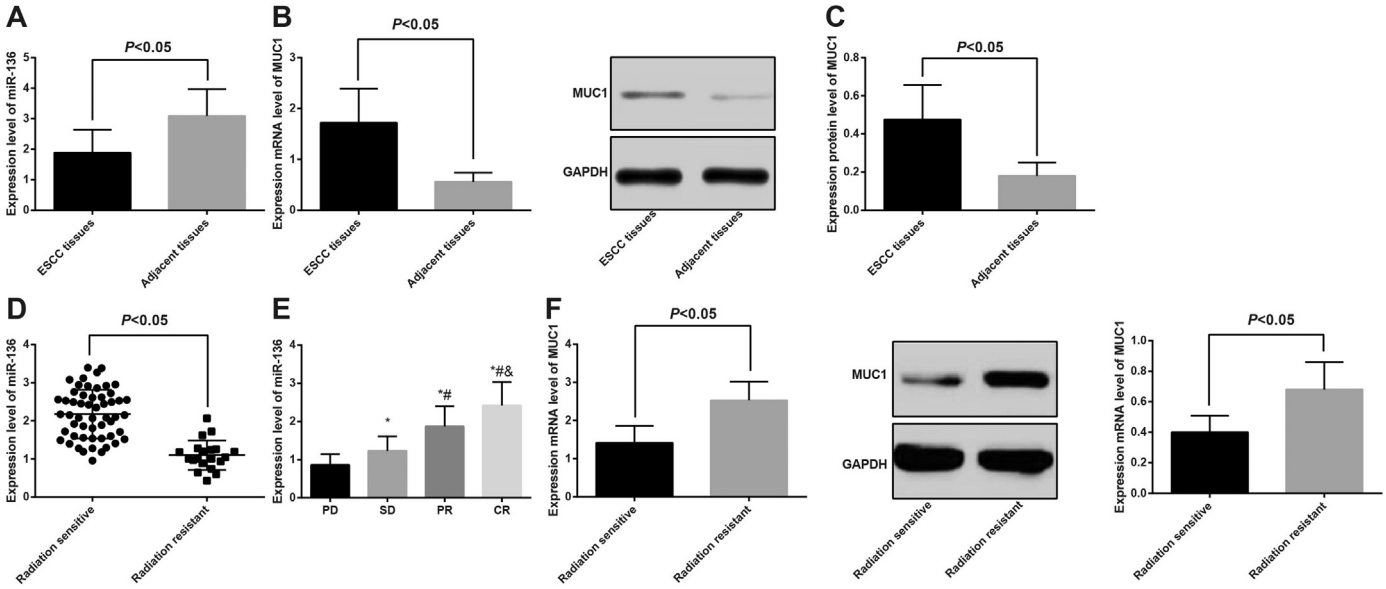

Fig. 1. Expression of miR-136 and MUC1 in tissue specimens and their correlations with clinicopathologic characteristics and short-term curative effect of radiotherapy. A. Expression of miR-136 in ESCC tissues and adjacent normal tissues. B. Expression of MUC1 in ESCC tissues and adjacent normal tissues. C. Correlation between the expression of miR-136 and MUC1 mRNA in ESCC tissues. D. miR-136 expression in radiation resistant and radiation sensitive groups. E. Expression of miR-136 in different radiotherapy effects groups. F. MUC1 expression in radiation resistant and radiation sensitive groups. $^{*}P < .05$ vs the PD group; $^{\#}P < .05$ vs the SD group; $^{\&}P < .05$ vs the PR group. The results were expressed as mean ± standard deviation. The paired *t*-test was used in Panel A and B; Pearson correlation analysis was used in Panel C; the independent sample *t*-test was used in Panel D and F; one-way ANOVA was used for analysis, and LSD-*t*-test fpr the pairwise comparison after ANOVA analysis in Panel E.

comparison between two groups, and one-way analysis of variance (ANOVA) was used in the comparison among multiple groups. The Fisher's least significant difference *t*-test (LSD-t) was employed for pairwise comparison after ANOVA analysis. Pearson was used for correlation analysis. When *P* value was < 0.05, statistical significance was supposed.

3. Results

3.1. Expression of miR-136 and MUC1 in tissue specimens and their correlations with clinicopathological features and short-term curative effect of radiotherapy

Initially, we examined the expression of miR-136 and MUC1 in 74 ESCC tissues and adjacent normal tissues. The results showed that the expression of miR-136 in ESCC tissues was lower and MUC1 mRNA and protein expression was higher than that in adjacent normal tissues (all $P < .05$; Fig. 1A–B). meanwhile, we analyzed the correlation between the expression of miR-136 and the expression of MUC1 mRNA in ESCC. The results suggested that the expression of miR-136 was negatively correlated with the expression of MUC1 mRNA in ESCC ($r = -0.809$, $P < .001$; Fig. 1C).

According to the average relative expression of miR-136 in ESCC, it was divided into low expression group and high expression group, and the relationship between the expression of miR-136 and MUC1 and clinicopathological features of ESCC showed that low expression of miR-136 and high expression of MUC1 were associated with tumor size, lymph node metastasis and distant metastasis (all $P < .05$). However, miR-136 low expression and high expression of MUC1 were not related to gender, age, smoking, family history, and tumor location (all $P > .05$) (Table 1).

In 74 cases of ESCC, there were 20 cases in the radiotherapy resistance group and 54 cases in the radiotherapy sensitive group. miR-136 expression in ESCC tissues from patients in both groups was detected by RT-qPCR. The results suggested that the expression of miR-136 in patients with ESCC in the radiotherapy sensitive group was

Table 1
Association of miR-136 expression and MUC1 mRNA expression with clinicopathologic characteristics of ESCC.

Characteristic	Case	miR-136 expression		*P*	MUC1 mRNA expression		*P*
		Low (n = 40)	High (n = 34)		Low (n = 30)	High (n = 44)	
Gender				0.804			0.454
Male	50	28	22		22	28	
Female	24	12	12		8	16	
Age (years)				0.343			0.627
≥60	46	27	19		20	26	
< 60	28	13	15		10	18	
Smoking				0.242			0.342
Yes	41	25	16		19	22	
No	33	15	18		11	22	
Family history				0.528			0.751
Yes	12	8	4		4	8	
No	62	32	30		26	36	
Tumor location				0.244			0.395
Upper	18	7	11		7	11	
Middle	33	18	15		16	17	
Lower	23	15	8		7	16	
Tumor size (cm)				0.038			0.011
≤4.0	37	15	22		21	16	
4.1–6.0	23	14	9		7	16	
> 6.0	14	11	3		2	12	
Lymph node metastasis				0.008			0.014
Yes	28	21	7		6	22	
No	46	19	27		24	22	
Distant metastasis				0.022			0.010
Yes	16	13	3		2	14	
No	58	27	31		28	30	

Note: The enumeration data were expressed in the form of percentage or rate, and chi-square test was used for the analysis.

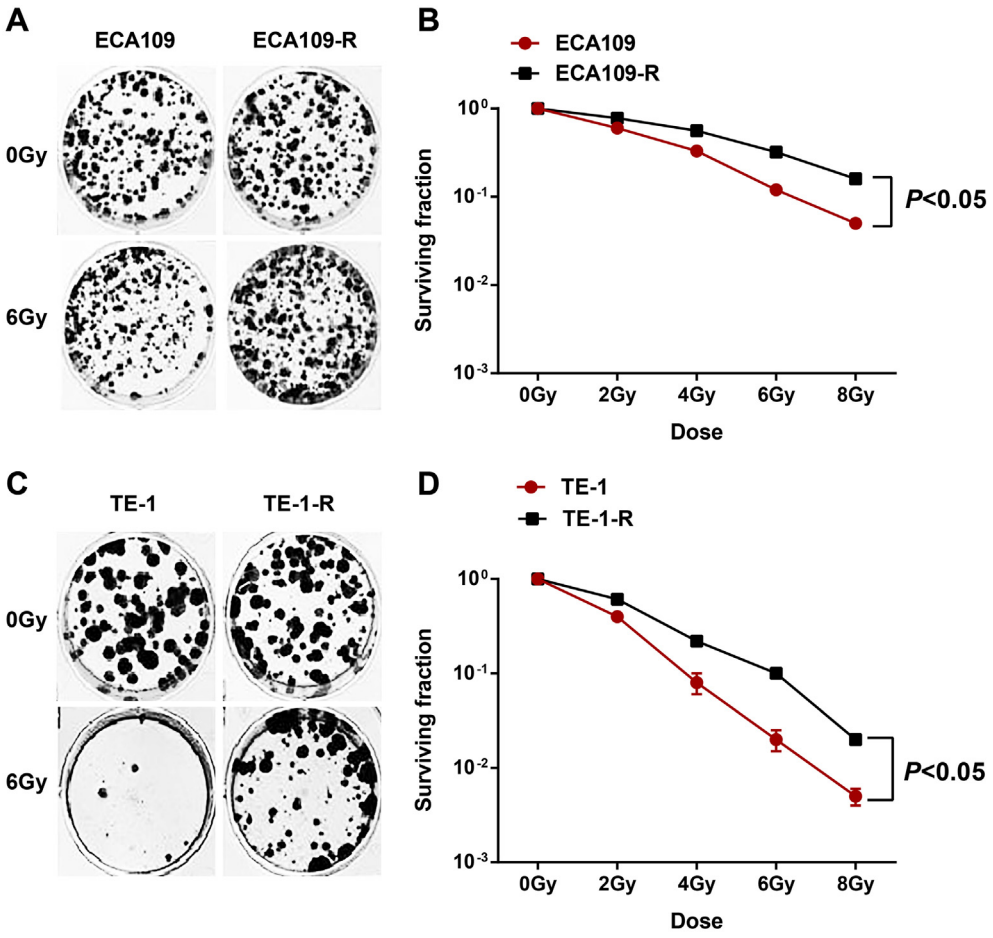

Fig. 2. Radiosensitivity of radiotherapy resistant strain and parent strain. A. Cell colony formation of Eca109 cells and Eca109-R cells. B. Cell survival fraction of Eca109 cells and Eca109-R cells. C. Cell colony formation of TE-1 and TE-1-R cells. D. Cell survival fraction of TE-1 and TE-1-R cells. The results were expressed as mean ± standard deviation. The independent sample *t*-test was used for the comparison between two groups.

significantly higher than that in the radiotherapy resistance group ($P < .05$; Fig. 1D). According to the evaluation of the radiotherapy response, we included the short-term curative effect of the tumor specimen in patients with ESCC. Seventy-four patients with ESCC received radiotherapy, including 7 patients with PD, 13 patients with SD, 24 patients with PR, and 30 patients with CR. The higher relative expression of miR-136 reflected the better short-term effect of ESCC after radical radiotherapy. There was significant difference in the expression of miR-136 in different radiotherapy effect groups ($P < .05$; Fig. 1E). Furthermore, the mRNA and protein expression of MUC1 in ESCC tissues was detected by RT-qPCR and western blot analysis. The results showed that the expression of MUC1 mRNA and protein in the radiotherapy sensitive group was significantly lower than that in the radiotherapy resistance group ($P < .05$; Fig. 1F).

3.2. Radiosensitivity of radiotherapy resistant strains and parent strains

The colony formation ability of cells under radiation is the key index to reflect the radiosensitivity or resistance of cells. Therefore, we tested the difference of colony formation ability between parent strains and constructed resistant strains by this experiment. As shown in Fig. 2A–B, under 2 Gy, 4 Gy, 6 Gy, 8 Gy X-ray irradiation, the SF of Eca109-R cells was higher than that of Eca109 cells, suggesting that Eca109-R cells are more resistant to radiation. In addition, there was significant difference of SF8 (SF under 8 Gy X-ray irradiation) between Eca109-R cells and Eca109 cells ($P < .05$). Likely, as shown in Fig. 2C–D, under 2 Gy, 4 Gy, 6 Gy, 8 Gy X-ray irradiation, the SF of TE-1-R cells was higher than that of TE-1 cells, suggesting that TE-1-R cells are more resistant to radiation. In addition, there was significant difference of SF8 between TE-1-R cells and TE-1 cells ($P < .05$). Furthermore, the radiosensitivity of TE-1 cells was higher than that of Eca109 cells.

3.3. Effect of radiation on apoptosis of the resistant strains and the parent strains of ESCC

Radiation-induced apoptosis is the main way for tumor cells to die after exposure to radiation. The results of flow cytometry (Fig. 3A) suggested that the apoptosis of Eca109 cells was similar to that of Eca109-R cells without irradiation ($P > .05$). When irradiated with 6 Gy X-ray, the apoptosis of both cell lines increased, but the apoptosis of Eca109-R cells was less than that of Eca109 cells ($P < .05$). As displayed in Fig. 3B, the apoptosis of TE-1-R cells was similar to that of TE-1 cells without irradiation ($P > .05$). When irradiated with 6 Gy X-ray, the apoptosis of both cell lines increased, but the apoptosis of TE-1-R cells was less than that of TE-1 cells ($P < .05$).

3.4. Expression of miR-136 and MUC1 between radiation resistant strains (Eca109-R and TE-1-R) and parent strains (Eca109 and TE-1) are related to the radiosensitivity of ESCC cells

In order to observe the expression of miR-136 between radiotherapy resistant and parent strains of ESCC cells, we extracted total RNA from the cells. The difference of miR-136 expression between radiation resistant strains (Eca109-R and TE-1-R) and parent strains (Eca109 and TE-1) was detected by RT-qPCR. As shown in Fig. 4A, with the expression of miR-136 in parent strain as 1, the expression of miR-136 in Eca109-R and TE-1-R cells was significantly deceased ($P < .05$). The results indicated that the expression of miR-136 in ESCC cells presented with high radiosensitivity, suggesting that the expression of miR-136 might be related to the radiosensitivity of ESCC cells.

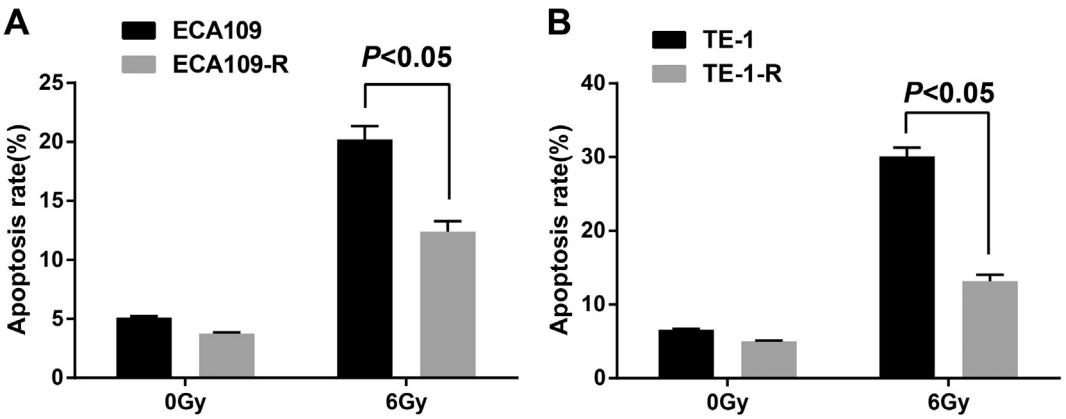

Fig. 3. Apoptosis characteristics of parent and resistant strains of ESCC cells under irradiation. A. Comparison of apoptosis between Eca109 and Eca109-R cells under irradiation. B. Comparison of apoptosis between TE-1 and TE-1-R cells under irradiation. The results were expressed as mean ± standard deviation. The independent sample *t*-test was used for the comparison between two groups.

Besides, the expression of MUC1 mRNA and protein between radiotherapy resistant and parent strains of ESCC cells were detected by RT-qPCR and western blot analysis. The obtained results suggested that increased expression of MUC1 mRNA and protein was found in ECA109-R and TE-1-R cells compared to the corresponding parental strains (ECA109 and TE-1) ($P < .05$; Fig. 4B).

3.5. Expression of miR-136 and MUC1 in ECA109 and TE-1 cells under different doses of X-ray irradiation

The results of RT-qPCR showed that the expression of miR-136 in Eca109 and TE-1 cells decreased significantly after 0, 2, 4, 6 and 8 Gy irradiation, and the increased irradiation dose reflected the decreased expression of miR-136 (Fig. 5A). After 0, 2, 4, 6 and 8 Gy irradiation, the expression levels of MUC1 mRNA and protein in ECA109 and TE-1 cells were significantly increased, and the higher expression of MUC1 mRNA and protein was increased with the irradiation dose (Fig. 5B).

3.6. Cells transfected with miR-136 mimics upregulates the expression of miR-136 and downregulates the expression of MUC1 in Eca109 and TE-1 cells

In order to observe the effect of miR-136 on radioresistance of ESCC, Eca109 and TE-1 cells as well as Eca109-R and TE-1-R cells were transfected with synthetic miR-136 mimics and corresponding control to up-regulate the expression of miR-136. After 6 h of transfection, the transfection rate of Eca109 and TE-1 cells as well as Eca109-R and TE-1-R cells transfected with miR-136 (Fig. 6A–B) showed that the cells emitting green fluorescence were the cells which were successfully transfected. The transfection efficiency of radiotherapy resistant and parent strains of ESCC cells was > 90%. After 48 h of transfection, the expression of miR-136 in transfected cells was detected by RT-qPCR, and the results of which suggested that after transfection with miR-136 mimics, the expression of miR-136 in Eca109 and TE-1 cells as well as Eca109-R and TE-1-R cells was significantly up-regulated (all $P < .05$;

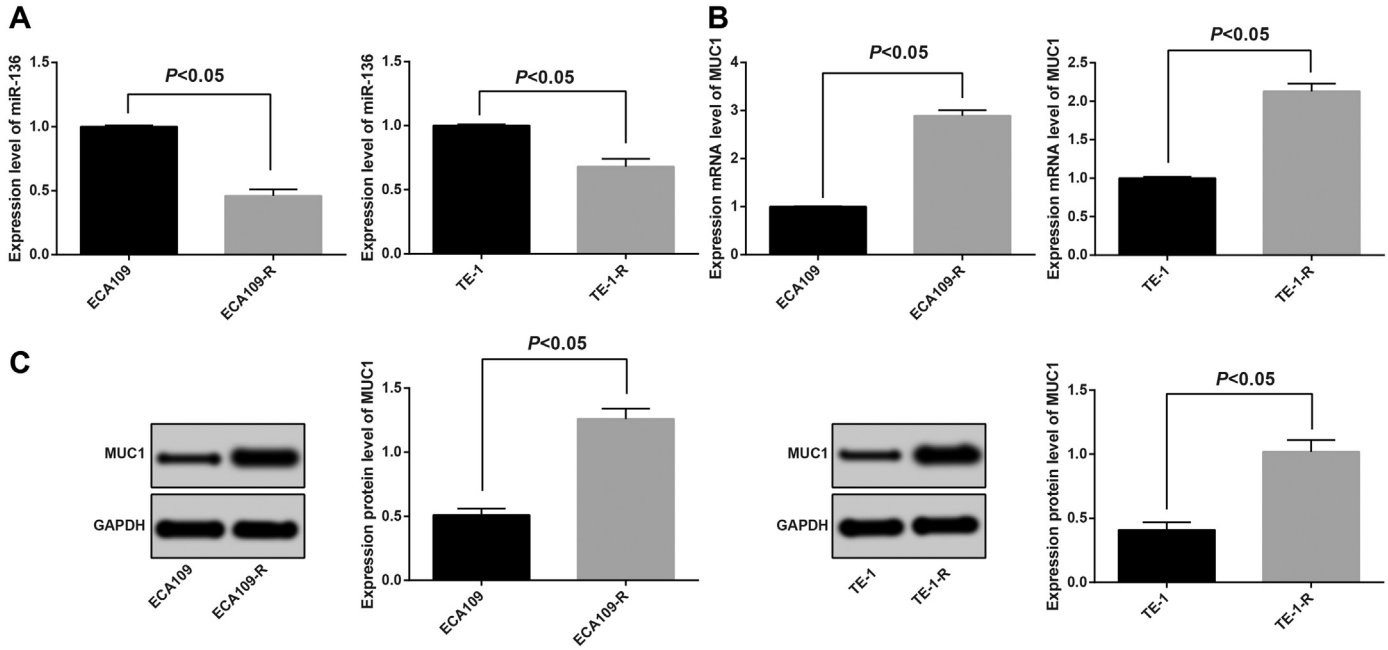

Fig. 4. Expression of miR-136 and MUC1 in parental and resistant cell lines of ESCC. A. Comparison of miR-136 expression in Eca109 and TE-1 cells as well as Eca109-R and TE-1-R cells. B. Comparison of MUC1 mRNA expression in Eca109 and TE-1 cells as well as Eca109-R and TE-1-R cells. C. Comparison of MUC1 protein expression in Eca109 and TE-1 cells as well as Eca109-R and TE-1-R cells. The results were expressed as mean ± standard deviation. The independent sample *t*-test was used for the comparison between two groups.

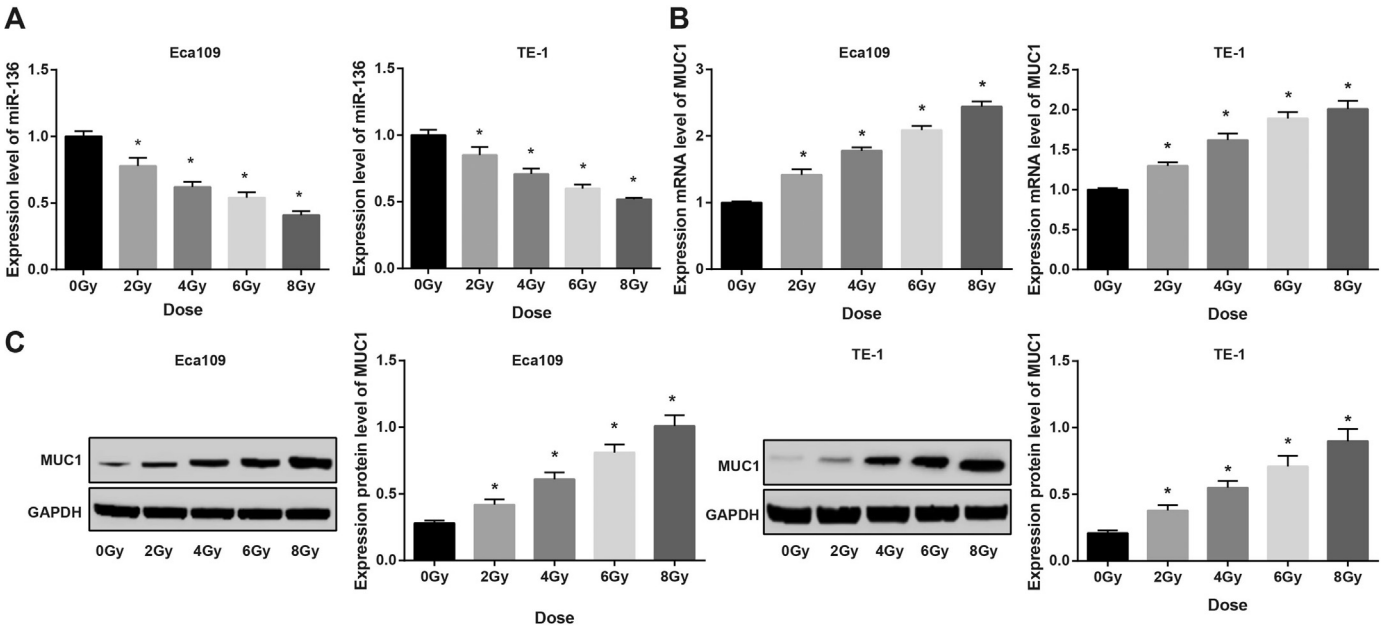

Fig. 5. Expression of miR-136 and MUC1 in Eca109 and TE-1 cells after different doses of X-ray irradiation. A. Expression of miR-136 in Eca109 and TE-1 cells after different doses of X-ray irradiation. B. Expression of MUC1 mRNA in Eca109 and TE-1 cells after different doses of X-ray irradiation. C. Expression of MUC1 protein in Eca109 and TE-1 cells after different doses of X-ray irradiation. $^{*}P < .05$ vs 0 Gy. The results were expressed as mean ± standard deviation. The paired *t*-test was used for the comparison within groups.

Fig. 6A–B).

Additionally, we used RT-qPCR and Western blot analysis to detect the expression of MUC1 mRNA and protein in cells after miR-136 up-regulation. The corresponding findings suggested that in Eca109 and TE-1 cells as well as Eca109-R and TE-1-R cells, the expression of MUC1 mRNA and protein declined in cells transfected with miR-136 mimics (all $P < .05$; Fig. 6C, E), indicating miR-136 can inhibit the expression of MUC1 in cells. In contrast to the miR-136 mimics + pcDNA3.1 group, the expression of MUC1 mRNA and protein increased in the miR-136 mimics + pc-MUC1 group (all $P < .05$; Fig. 6D, F).

3.7. Up-regulation of miR-136 reduces the survival rate of ESCC cells under irradiation, which is reversed by up-regulating the expression of MUC1

In order to observe the effect of miR-136 on cell proliferation of ESCC cells under radiation, we transfected miR-136 mimics into Eca109 and TE-1 cells as well as Eca109-R and TE-1-R cells, respectively to measure the OD value at 0 h, 12 h, 24 h and 48 h by CCK-8 assay. The results showed that in overexpression of miR-136 could decrease the survival rate of Eca109 and TE-1 cells after radiotherapy as well as Eca109-R and TE-1-R cells, as shown in Fig. 7A and C. In order to further elucidate the effect of expression of miR-136 on cell proliferation of ESCC cells under radiation, we transfected the miR-136 mimics + pc-MUC1 to detect the OD value of the cells after radiotherapy. As shown in Fig. 7B and D, the survival rate of in Eca109 and TE-1 cells after radiotherapy as well as Eca109-R and TE-1-R cells was increased with overexpressed miR-136 and overexpressed MUC1 ($P < .05$). It is suggested that up-regulation of miR-136 can decrease the survival rate of ESCC cells under radiation, which is reversed by the increased expression of MUC1.

3.8. Up-regulation of miR-136 reduces the colony formation rate of ESCC cells under irradiation, which is reversed by up-regulating the expression of MUC1

Subsequently, colony information assay was used to detect whether or not miR-136 can enhance the sensitivity of ESCC cells to radiation. Fig. 8A and C show that in Eca109 and TE-1 cells as well as Eca109-R and TE-1-R cells, under 2 Gy, 4 Gy, 6 Gy, and 8 Gy X-ray irradiation, the SF of cells with over-expressed miR-136 was decreased, and there was significant difference of SF8 between the mimics NC and the miR-136 mimics groups (all $P < .05$), indicating that miR-136 can significantly enhance the sensitivity of Eca109 and TE-1 cells to radiation as well as Eca109-R and TE-1-R cells. In order to further explore the effect of expression of miR-136 on the colony formation of ESCC cells under radiation by targeting MUC1, we transfected miR-136 mimics + pc-MUC1 in Eca109 and TE-1 cells as well as Eca109-R and TE-1-R cells under the irradiation of 2 Gy, 4 Gy, 6 Gy, and 8 Gy X-ray, the results of which demonstrated that the SF of cells in the miR-136 mimics + pc-MUC1 group were increased compared to the miR-136 mimics + pcDNA3 group ($P < .05$; Fig. 8B and D). The results suggest that the up-regulation of miR-136 can decrease the colony-forming ability of ESCC cells under radiation, which is reversed by the down-regulation of MUC1.

3.9. Up-regulation of miR-136 induces apoptosis of ESCC cells under irradiation, which is reversed by up-regulating the expression of MUC1

Annexin V/PI double staining was employed to determine the role of overexpressed miR-136 on cell apoptosis of ESCC cells under irradiation. The results suggested that there was no significant difference for the cell apoptosis rate in Eca109 and TE-1 cells as well as Eca109-R and TE-1-R cells without irritation between the mimics NC and the miR-136 mimics groups (all $P > .05$). After 6 Gy irradiation, the apoptosis rate in the miR-136 mimics group was higher than that in the mimics NC group ($P < .05$; Fig. 9A and C). This suggests that up-regulation of miR-136 inhibits apoptosis induced by radiation in ESCC cells. In order to further investigate the effect of miR-136 on the apoptosis of ESCC cells under radiation through regulation of MUC1, we transfected miR-136 mimics + pc-MUC1 in Eca109 and TE-1 cells as well as Eca109-R and TE-1-R cells. The results indicated that there was no significant difference in apoptosis rate of cells between the miR-136 mimics + pcDNA3.1 group and the miR-136 mimics + pc-MUC1 group without radiation ($P > .05$). After 6 Gy irradiation, the apoptosis rate of cells in

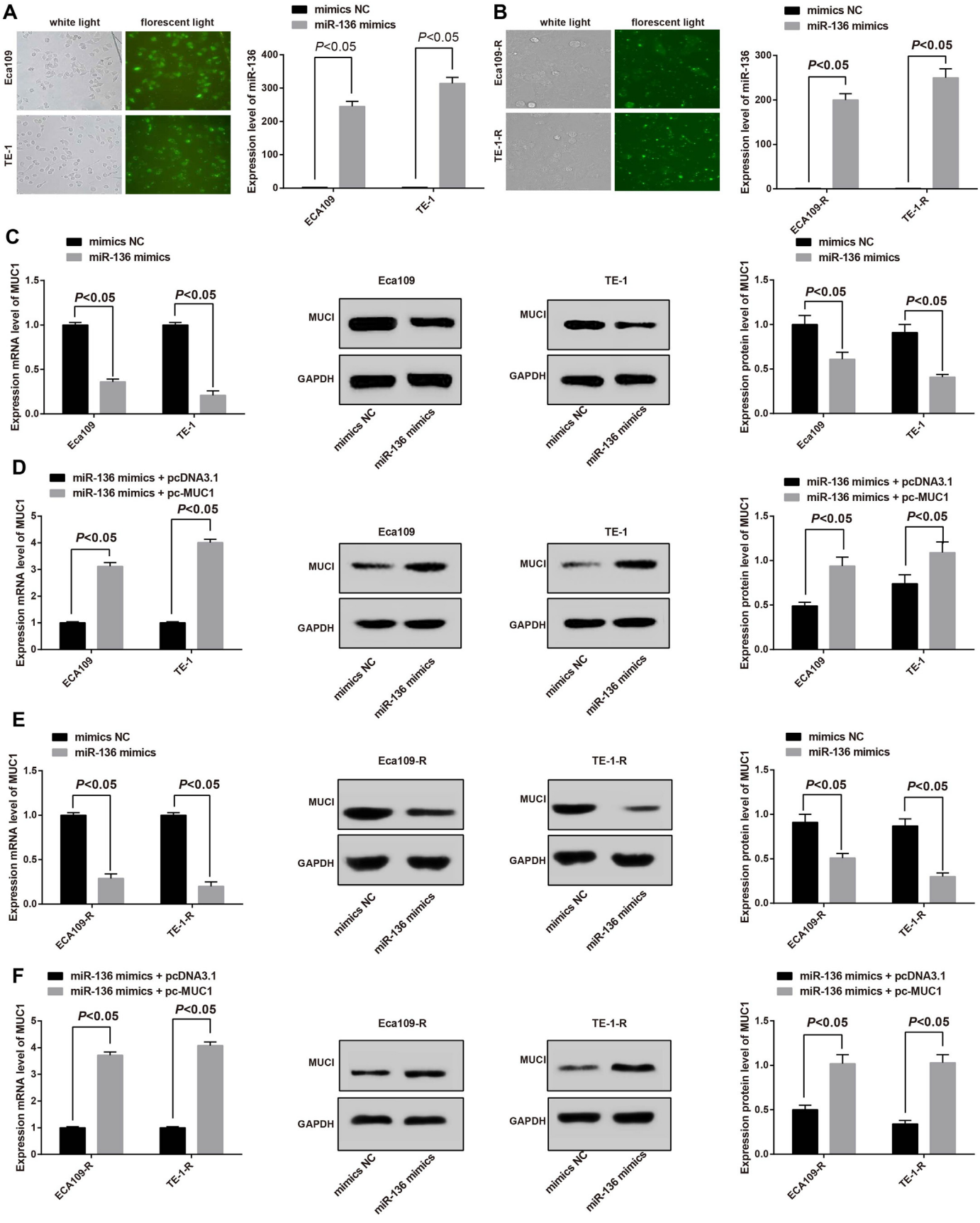

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Fig. 6. Cells transfected with miR-136 mimics upregulates the expression of miR-136 and downregulates the expression of MUC1 in Eca109 and TE-1 cells as well as Eca109-R and TE-1-R cells. A. The efficiency of miR-136 transfection in Eca109 and TE-1 cells. B. The efficiency of miR-136 transfection in Eca109-R and TE-1-R cells. C. Overexpression of miR-136 on the expression of MUC1 in Eca109 and TE-1 cells. D. Overexpressed miR-136 and overexpressed MUC1 on the expression of MUC1 in Eca109 and TE-1 cells. E. Overexpression of miR-136 on the expression of MUC1 in Eca109-R and TE-1-R cells. F. Overexpressed miR-136 and overexpressed MUC1 on the expression of MUC1 in Eca109-R and TE-1-R cells. The results were expressed as mean ± standard deviation. The independent sample *t*-test was used for the comparison between two groups.

the miR-136 mimics + pc-MUC1 group was significantly lower than that in the miR-136 mimics + pcDNA3.1 group ($P < .05$; Fig. 9C and D). These results suggest that up-regulation of miR-136 enhances apoptosis of ESCC cells under radiation, which is reversed by up-regulation of MUC1.

3.10. Effect of overexpression of miR-136 on the expression of p53 in ESCC cells

The expression of wild-type p53 gene in Eca109 and TE-1 cells was detected by RT-qPCR. The results showed that the expression of wild-type p53 gene in TE-1 cells was higher than that in Eca109 cells (Fig. 10A). After transfection of miR-136 mimics in Eca109 and TE-1 cells, the expression of wild-type p53 was increased ($P < .05$; Fig. 10B). After overexpression of miR-136 and overexpression of MUC1 in Eca109 and TE-1 cells, the expression of wild-type p53 was decreased in the miR-136 mimics + pc-MUC1 group compared with the miR-136 mimics + pcDNA3.1 group ($P < .05$; Fig. 10C).

3.11. MUC1 is determined as a target gene of miR-136

The binding site of MUC1 and miR-136 was determined by online prediction software Target Scan. The sequence of MUC1 3′-UTR region and miR-136 is shown in Fig. 11A. Additionally, luciferase activity detection suggested thatECA109 and TE-1 cells were co-transfected with recombinant plasmids miR-136 mimics and WT-miR-136/MUC1 or MUT-miR-136/MUC1, respectively. MiR-136 mimics had no effect on luciferase activity of MUT-miR-136/MUC1 but reduced luciferase activity WT-miR-136/MUC1 in Eca109 and TE-1 cells ($P < .05$; Fig. 11B–C).

4. Discussion

ESCC, a widely common tumor around the world, accounts for an estimated 90% of the most malignant esophageal tumors (Wang et al., 2016). Despite the continuous improvement of ESCC treatment, the prognosis of patients suffering from the disease remains poor, partly due to the associated high postoperative recurrence rate, with recurrence observed among 50% of patients who undergo excision (Liu et al., 2011). Therefore, identification of novel miRNAs that are involved in ESCC progression could contribute to the prognostic biomarker and therapeutic strategy for ESCC. Hence, the current study was carried out to elucidate the role of miR-136 in radiosensitivity of ESCC cells through regulation of MUC1.

Initially, the results of this study suggest that the expression of miR-136 in ESCC tissues was lower and MUC1 mRNA and protein expression was higher than that in adjacent normal tissues, and the expression of miR-136 was negatively correlated with the expression of MUC1 mRNA in ESCC. Accumulating evidence indicated that miR-136 acted as a suppressor in the progression of various human cancers, which might provide a therapeutic regimen in the prediction and treatment of human cancers (Zhao et al., 2015). Haapa-Paananen et al. supported that miR-136 decreased in human glioma cells and then induced cell apoptosis of glioma cells through the inhibition of AEG-1 and Bcl-2 (Haapa-Paananen et al., 2013; Yang et al., 2012). Another study has revealed that miR-136 had a tumor-suppressive effect on breast cancer cells through binding to PTEN, a tumor inhibitor (Lee et al., 2010). The gain- and loss-of-function experiments in a study demonstrated that miR-136 expression is able to reverse cisplatin resistance and promote the response to cisplatin treatment (Chen et al., 2014). Mucins are considered as high-molecular-weight glycoproteins which are regarded as markers of adverse prognosis and also, as attractive therapeutic

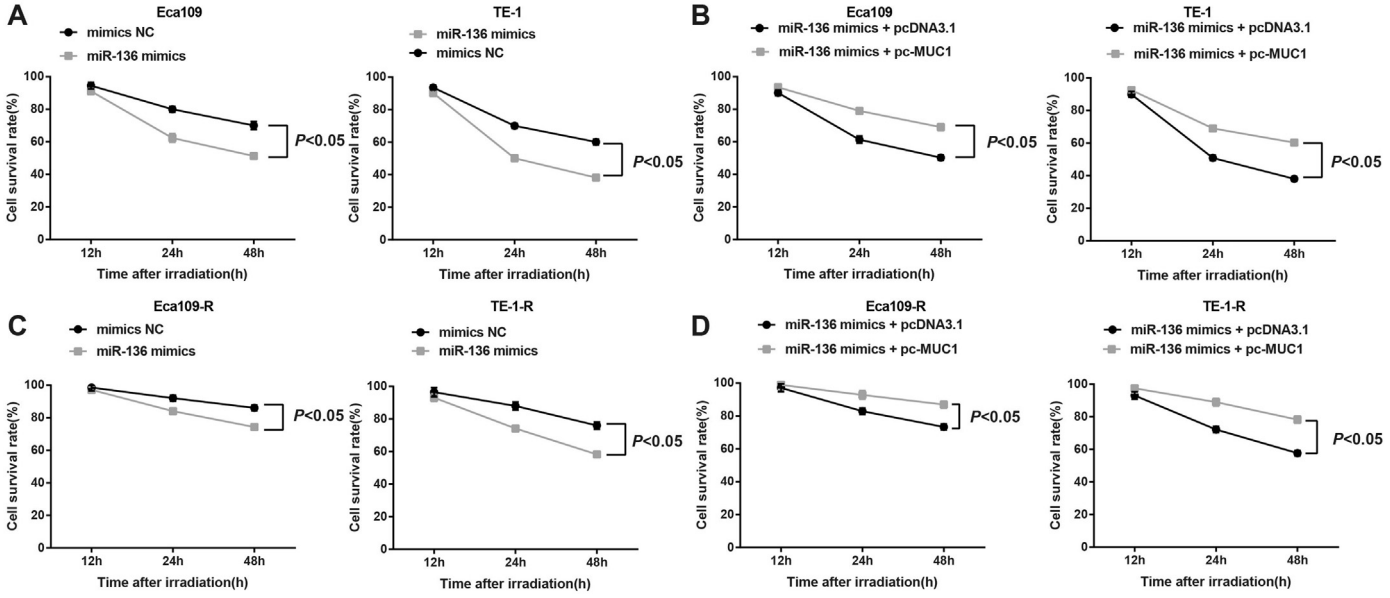

Fig. 7. Up-regulation of miR-136 reduces the survival rate of ESCC cells under irradiation, which is reversed by up-regulating the expression of MUC1. A. Effect of overexpression of miR-136 on the survival rate of Eca109 and TE-1 cells after radiotherapy. B. Effect of overexpression of miR-136 and MUC1 on the survival rate of Eca109 and TE-1 cells after radiotherapy. C. Effect of overexpression of miR-136 on the survival rate of Eca109-R and TE-1-R cells after radiotherapy. D. Effect of overexpression of miR-136 and MUC1 on the survival rate of Eca109-R and TE-1-R cells after radiotherapy. The results were expressed as mean ± standard deviation. The independent sample *t*-test was used for the comparison between two groups.

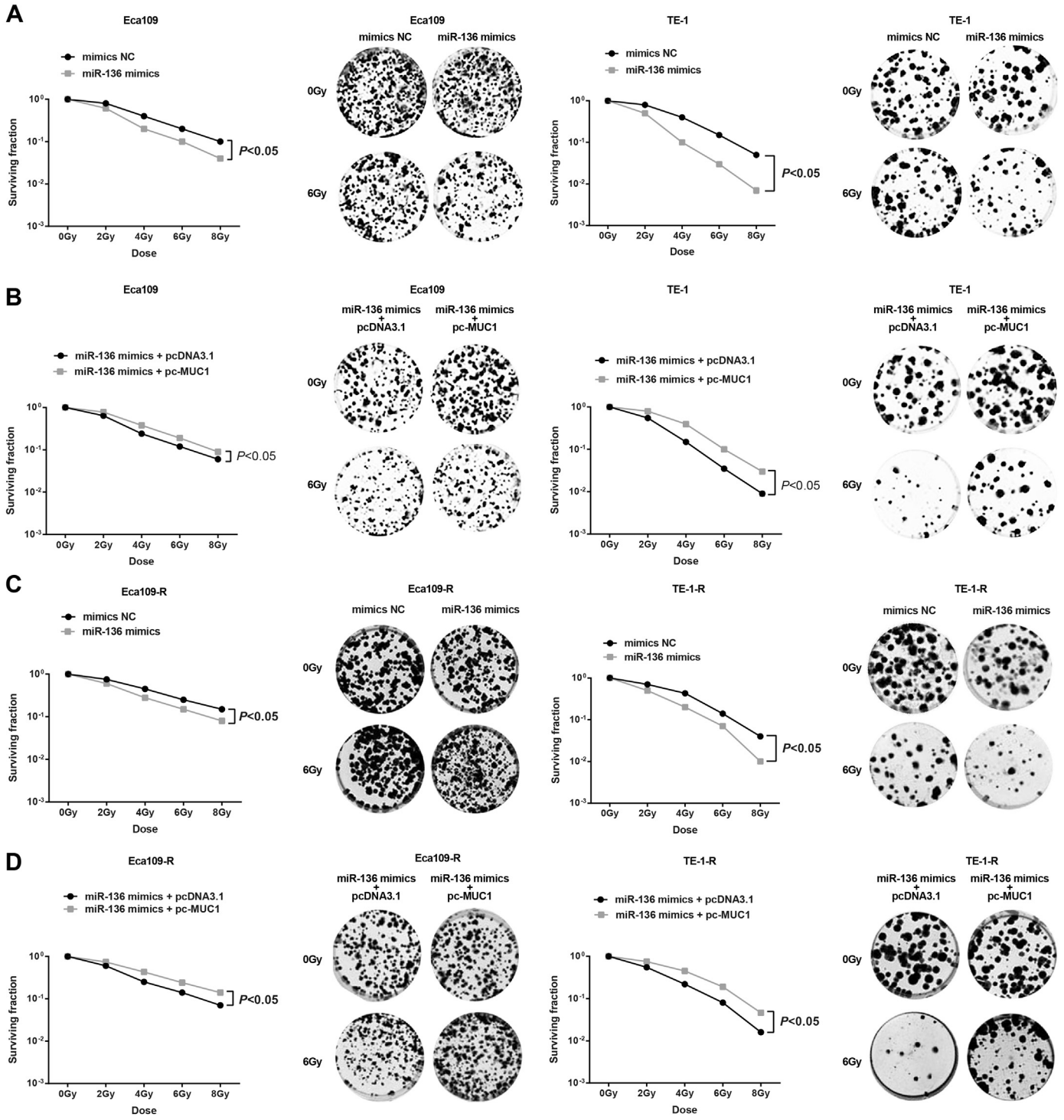

Fig. 8. Up-regulation of miR-136 reduces the colony formation rate of ESCC cells under irradiation, which is reversed by up-regulating the expression of MUC1. A. Effect of overexpression of miR-136 on colony formation ability of Eca109 and TE-1 cells after radiotherapy. B. Effect of overexpression of miR-136 and MUC1 on colony formation ability of Eca109 and TE-1 cells after radiotherapy. C. Effect of overexpression of miR-136 on colony formation ability of Eca109-R and TE-1-R cells after radiotherapy. D. Effect of overexpression of miR-136 and MUCA on colony formation ability of Eca109-R and TE-1-R cells after radiotherapy. The results were expressed as mean ± standard deviation. The independent sample *t*-test was used for the comparison between two groups.

targets (Kufe, 2009). Overexpression of MUC1 was suggested to be related to LNM and poor prognosis in ESCC patients, implying that MUC1 may be used as a new biomarker for predicting LNM and prognosis in ESCC (Song et al., 2003).

Additionally, MUC1 was a target gene of miR-136 based on bioinformatics analysis and luciferase activity assay. miRNAs bind to the 3′-untranslated region (3′UTR) of their target mRNAs to decreased their stability and the target mRNAs expression at the post-transcriptional level (Bartel, 2009), which play a role in different biological processes, such as cell growth, proliferation, differentiation as well as death (Kong et al., 2014; Yang et al., 2015; Zhang et al., 2015). Similar to our results, the bioinformatics databases in a study predicted that as an upstream miRNA, miR-136 bound to the MIEN1 3′-UTR directly, and the results of which was also verified by luciferase activity assay (Ren et al.,

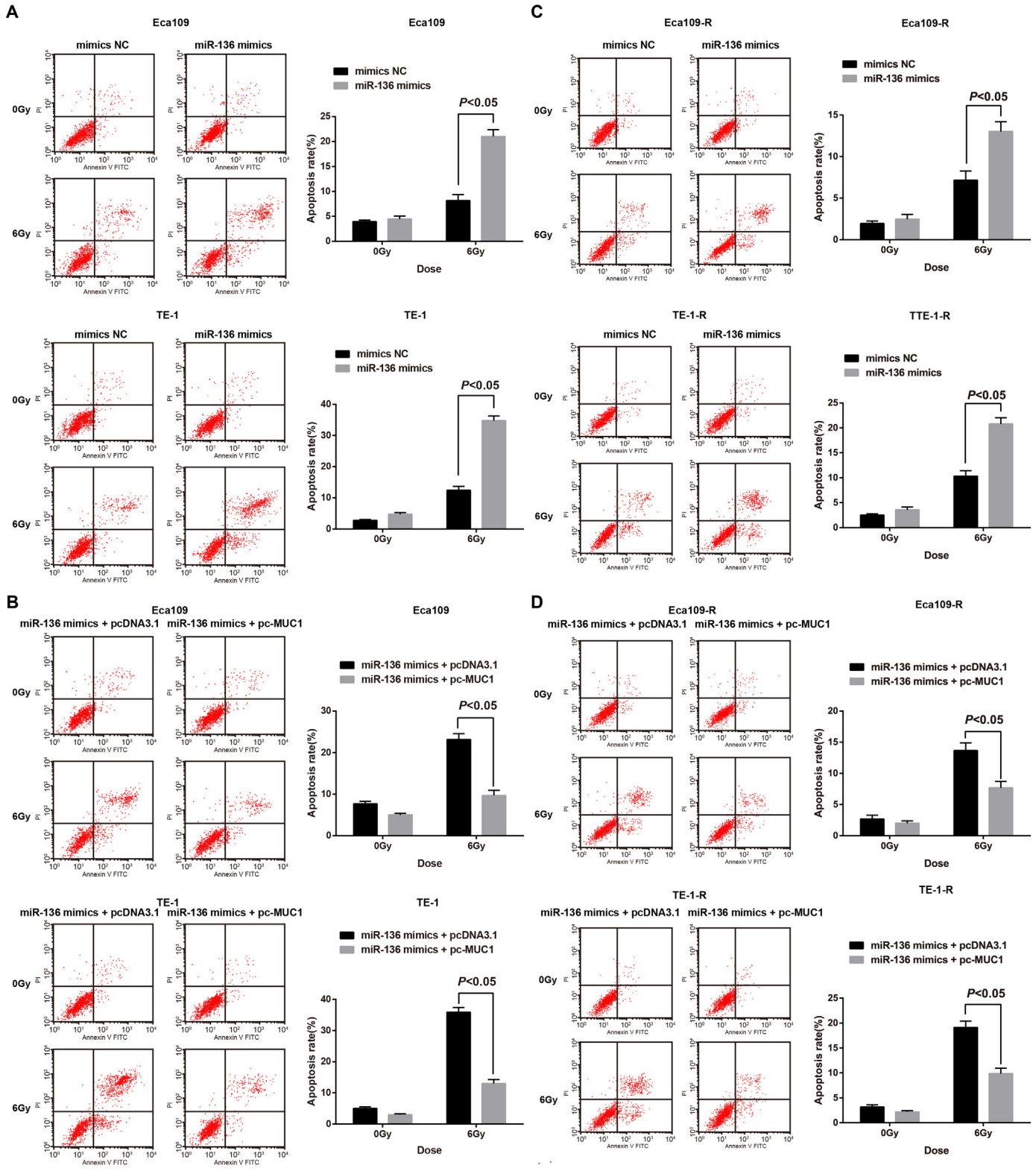

Fig. 9. Up-regulation of miR-136 induces apoptosis of ESCC cells under irradiation, which is reversed by up-regulating the expression of MUC1. A. Effect of overexpression of miR-136 on cell apoptosis of Eca109 and TE-1 cells after radiotherapy. B. Effect of overexpression of miR-136 and MUCA on cell apoptosis of Eca109 and TE-1 cells after radiotherapy. C. Effect of overexpression of miR-136 on cell apoptosis of Eca109-R and TE-1-R cells after radiotherapy. D. Effect of overexpression of miR-136 and MUCA on cell apoptosis of Eca109-R and TE-1-R cells after radiotherapy. The results were expressed as mean ± standard deviation. The independent sample *t*-test was used for the comparison between two groups.

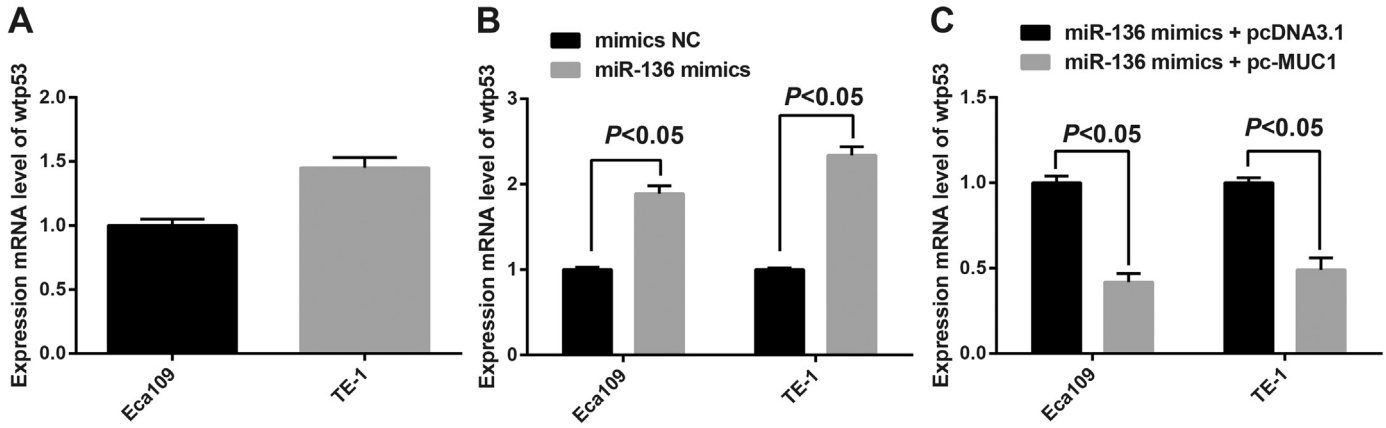

Fig. 10. Effect of overexpression of miR-136 on the expression of p53 in ESCC cells. A. Expression of wild type p53 gene in Eca109 and TE-1 cells. B. Effect of overexpression of miR-136 on wild type p53 gene in Eca109 and TE-1 cells. C. Effect of overexpression of miR-136 and MUCA on wild type p53 gene in Eca109 and TE-1 cells. The results were expressed as mean ± standard deviation. The independent sample *t*-test was used for the comparison between two groups.

2018). Besides, another study also confirmed that miR-1291 bound to the MUC1 3′-UTR to decrease stability and/or suppress translation participated in viability, invasion and apoptosis of ESCC cells (Luo et al., 2015).

Furthermore, out study also suggested that up-regulation of miR-136 reduced the survival rate, suppressed colony formation ability and induced apoptosis of ESCC cells under irradiation, which was reversed by up-regulating the expression of MUC1. Actually, miRNAs are able to change the cellular response to an exact drug or a class of drugs through some mechanisms, such as interference with DNA repair and drug targets (Giovannetti et al., 2012). miR-136 is reported to be down-regulated in cisplatin-resistant ovarian cancer, which is also participated in platinum-resistance by impacting DNA repair and cell apoptosis (Zhao et al., 2015). A recent study also elucidated that overexpression of miR-136 suppressed migration and angiogenesis of cells, indicating that miR-136 could be a potential strategy to reverse chemoresistance in ovarian cancer regardless of its relationship with Notch3 (Jeong et al., 2017). Besides, it has been demonstrated that the MUC1 expression in tumors may act as an anti-adhesion molecule which suppresses cell-cell aggregation, enhancing dissemination of cells from tumor nests (Ye et al., 2011).

In the process of radiotherapy, the normal function of p53 gene plays a key role in apoptosis and radiosensitivity of tumor cells. Wild type p53 gene blocks tumor cell cycle by activating or inhibiting a series of genes. It can inhibit the repair of radiation injury of tumor cells, promote the apoptosis of tumor cells, and enhance the sensitivity of tumor cells to radiotherapy. Lowe et al. (Lowe et al., 1993) studied the sensitizing effect of p53 gene as early as 20 years ago, and they irradiated mouse thymocytes expressing three different states of p53 gene. It was found that the cells with p53 mutation survived after 2000 cGy irradiation, while the cells expressing wild type p53 gene lost their vitality at the dose of 2000 cGy, and the radiation reaction of the cells in

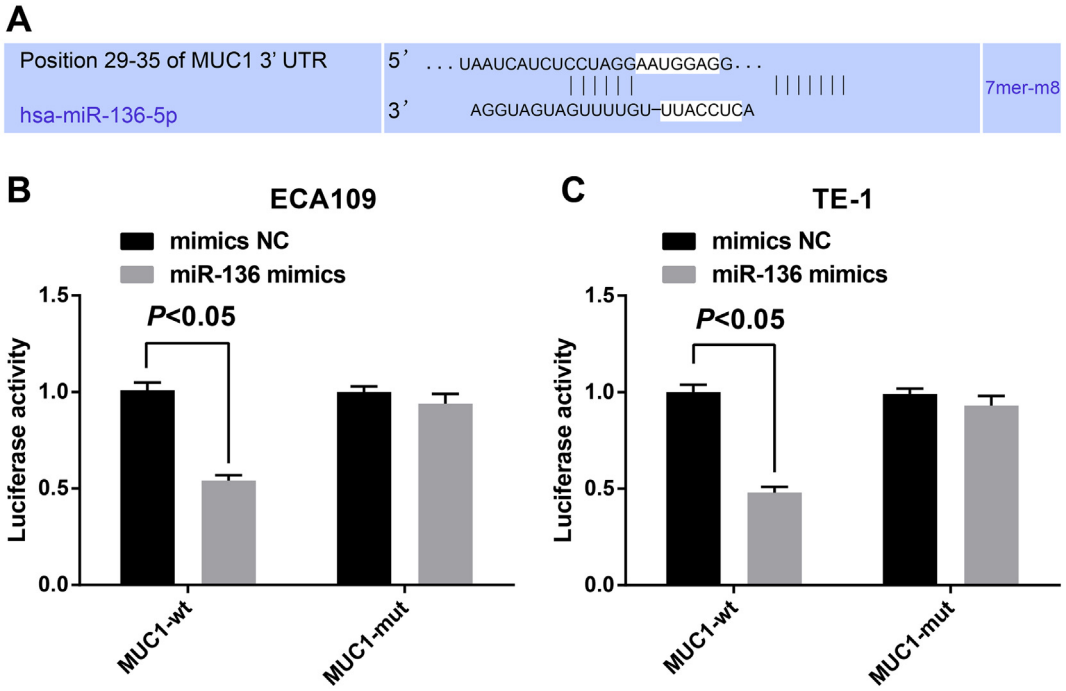

Fig. 11. miR-136 inhibits the expression of MUC1. A. Binding sites between miR-136 and MUC1 predicted by online software. B. Luciferase activity assay verified the targeting relationship between miR-136 and MUC1 in Eca109 cells. C. Luciferase activity assay verified the targeting relationship between miR-136 and MUC1 in TE-1 cells. The results were expressed as mean ± standard deviation. The independent sample *t*-test was used for the comparison between two groups.

the heterozygous state was between the two. Sakaguchi et al. (Sakaguchi et al., 1998) found that wild type p53 gene was prone to apoptosis under radiation, while mutant p53 gene mediated cell resistance to radiation induced apoptosis. Shimaitsu et al. (Shiomitsu et al., 2008) studied the relationship between the human osteosarcoma cells transfected with wild-type p53 gene and the radiosensitivity. The results showed that the carrying of normal function p53 can enhance the radiosensitivity, and will greatly increase the treatment gain ratio at the dose of l-4 Gy, which provides a reference value for clinical research. Based on this, the normal function of p53 gene plays a key role in apoptosis and radiosensitivity of tumor cells. In the present study, we found that the expression of wild-type p53 in Eca109 and TE-1 cells was increased after overexpression of miR-136. At the same time, overexpression of miR-136 promoted apoptosis and increased radiosensitivity of ESCC cells. Therefore, it is hypothesized that up-regulation of miR-136 promotes apoptosis and increases the radiosensitivity of ESCC cells, which may be related to up-regulation of wild-type p53. As for the more specific regulatory mechanisms, we have not conducted a more in-depth discussion due to time and economic constraints, but we will conduct research in the future study work.

In conclusion, this present study highlights that up-regulation of miR-136 induces apoptosis and radiosensitivity of ESCC cells through the inhibition of MUC1. Therefore, the identification of miR-136/MUC1 axis in apoptosis and radiosensitivity of ESCC may aid in facilitating the existing understanding of mechanisms of ESCC, with the potential of serving as a prognostic marker for of ESCC treatments in future.

Declaration of Competing Interest

None.

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References

Bao, L., et al., 2012. Increased expression of P-glycoprotein and doxorubicin chemoresistance of metastatic breast cancer is regulated by miR-298. Am. J. Pathol. 180 (6), 2490–2503.
Bartel, D.P., 2009. MicroRNAs: target recognition and regulatory functions. Cell 136 (2), 215–233.
Cao, H.H., et al., 2014. A molecular prognostic model predicts esophageal squamous cell carcinoma prognosis. PLoS One 9 (8), e106007.
Chen, W., et al., 2014. MiR-136 targets E2F1 to reverse cisplatin chemosensitivity in glioma cells. J. Neuro-Oncol. 120 (1), 43–53.
Cheung, T.H., et al., 2012. Maintenance of muscle stem-cell quiescence by microRNA-489. Nature 482 (7386), 524–528.
Chhieng, D.C., et al., 2003. MUC1 and MUC2 expression in pancreatic ductal carcinoma obtained by fine-needle aspiration. Cancer 99 (6), 365–371.
Feng, B., Wang, R., Chen, L.B., 2012. MiR-100 resensitizes docetaxel-resistant human lung adenocarcinoma cells (SPC-A1) to docetaxel by targeting Plk1. Cancer Lett. 317 (2), 184–191.
Giovannetti, E., et al., 2012. Molecular mechanisms underlying the role of microRNAs (miRNAs) in anticancer drug resistance and implications for clinical practice. Crit. Rev. Oncol. Hematol. 81 (2), 103–122.
Haapa-Paananen, S., et al., 2013. Functional profiling of precursor MicroRNAs identifies MicroRNAs essential for glioma proliferation. PLoS One 8 (4), e60930.
Higashi, M., et al., 2015. Mucin expression in endoscopic ultrasound-guided fine-needle aspiration specimens is a useful prognostic factor in pancreatic ductal adenocarcinoma. Pancreas 44 (5), 728–734.
Jeong, J.Y., et al., 2017. MicroRNA-136 inhibits cancer stem cell activity and enhances the anti-tumor effect of paclitaxel against chemoresistant ovarian cancer cells by targeting Notch3. Cancer Lett. 386, 168–178.
Kim, J.A., Shah, P.M., 2017. Screening and prevention strategies and endoscopic management of early esophageal cancer. Chin. Clin. Oncol. 6 (5), 50.
Kong, W., et al., 2014. Upregulation of miRNA-155 promotes tumour angiogenesis by targeting VHL and is associated with poor prognosis and triple-negative breast cancer. Oncogene 33 (6), 679–689.
Kufe, D.W., 2009. Mucins in cancer: function, prognosis and therapy. Nat. Rev. Cancer 9 (12), 874–885.
Lee, D.Y., et al., 2010. Expression of versican 3′-untranslated region modulates endogenous microRNA functions. PLoS One 5 (10), e13599.
Liu, J.B., et al., 2011. Plasma DNA methylation of Wnt antagonists predicts recurrence of esophageal squamous cell carcinoma. World J. Gastroenterol. 17 (44), 4917–4921.
Lowe, S.W., Schmitt, E.M., Smith, S.W., et al., 1993. p53 is required for radiation-induced apoptosis in mouse thymocytes. Nature 362 (6423), 847.
Luo, H., et al., 2015. miR-1291 targets mucin 1 inhibiting cell proliferation and invasion to promote cell apoptosis in esophageal squamous cell carcinoma. Oncol. Rep. 34 (5), 2665–2673.
Nakajima, M., Kato, H., 2013. Treatment options for esophageal squamous cell carcinoma. Expert. Opin. Pharmacother. 14 (10), 1345–1354.
Noguchi, S., et al., 2012. Anti-oncogenic microRNA-203 induces senescence by targeting E2F3 protein in human melanoma cells. J. Biol. Chem. 287 (15), 11769–11777.
Okuda, M., et al., 2017. Subcloning and characterization of highly metastatic cells derived from human esophageal squamous cell carcinoma KYSE150 cells by in vivo selection. Oncotarget 8 (21), 34670–34677.
Ren, H., et al., 2018. miR-136 targets MIEN1 and involves the metastasis of colon cancer by suppressing epithelial-to-mesenchymal transition. OncoTargets Ther. 11, 67–74.
Sagara, M., et al., 1999. Expression of mucin 1 (MUC1) in esophageal squamous-cell carcinoma: its relationship with prognosis. Int. J. Cancer 84 (3), 251–257.
Sakaguchi, T., et al., 1998. Prognostic value of cyclin E and p53 expression in gastric carcinoma. Cancer 82 (7), 1238–1243.
Shen, S., et al., 2014. Upregulation of miR-136 in human non-small cell lung cancer cells promotes Erk1/2 activation by targeting PPP2R2A. Tumour Biol. 35 (1), 631–640.
Shigeoka, M., et al., 2015. Cyr61 promotes CD204 expression and the migration of macrophages via MEK/ERK pathway in esophageal squamous cell carcinoma. Cancer Med. 4 (3), 437–446.
Shiomitsu, K., et al., 2008. Radiosensitivity of canine osteosarcoma cells transfected with wild-type p53 in vitro. Vet. Comp. Oncol. 6 (3), 193–200.
Song, Z.B., et al., 2003. Expression of MUC1 in esophageal squamous-cell carcinoma and its relationship with prognosis of patients from Linzhou city, a high incidence area of northern China. World J. Gastroenterol. 9 (3), 404–407.
Sun, L., et al., 2012. MiR-200b and miR-15b regulate chemotherapy-induced epithelial-mesenchymal transition in human tongue cancer cells by targeting BMI1. Oncogene 31 (4), 432–445.
Tuo, Y.L., Li, X.M., Luo, J., 2015. Long noncoding RNA UCA1 modulates breast cancer cell growth and apoptosis through decreasing tumor suppressive miR-143. Eur. Rev. Med. Pharmacol. Sci. 19 (18), 3403–3411.
Wang, X., et al., 2016. lncRNA UCA1 inhibits esophageal squamous-cell carcinoma growth by regulating the Wnt signaling pathway. J. Toxic. Environ. Health A 79 (9–10), 407–418.
Wang, J.J., et al., 2017. Effects of microRNA-136 on melanoma cell proliferation, apoptosis, and epithelial-mesenchymal transition by targetting PMEL through the Wnt signaling pathway. Biosci. Rep. 37 (5).
Yang, Y., et al., 2012. MiR-136 promotes apoptosis of glioma cells by targeting AEG-1 and Bcl-2. FEBS Lett. 586 (20), 3608–3612.
Yang, Y.K., et al., 2015. MicroRNA-494 promotes cervical cancer proliferation through the regulation of PTEN. Oncol. Rep. 33 (5), 2393–2401.
Ye, Q., et al., 2011. MUC1 induces metastasis in esophageal squamous cell carcinoma by upregulating matrix metalloproteinase 13. Lab. Investig. 91 (5), 778–787.
Zhang, Y., et al., 2015. Inhibition of microRNA-17/20a suppresses cell proliferation in gastric cancer by modulating UBE2C expression. Oncol. Rep. 33 (5), 2529–2536.
Zhao, H., et al., 2015. Expression of miR-136 is associated with the primary cisplatin resistance of human epithelial ovarian cancer. Oncol. Rep. 33 (2), 591–598.
Zhu, X., et al., 2010. Identification of galectin-7 as a potential biomarker for esophageal squamous cell carcinoma by proteomic analysis. BMC Cancer 10, 290.