J Biosci (2019) 44:92
DOI: 10.1007/s12038-019-9918-y

Review

miR-183-5p enhances the radioresistance of colorectal cancer by directly targeting ATG5

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MS received 2 January 2019; accepted 14 June 2019; published online 5 August 2019

Radioresistance is a material obstacle for effective treatment of colorectal cancer (CRC). Thus, the discovery of a novel biomarker for determining the CRC radiosensitivity is necessary. Recent studies have confirmed that miR-183-3p regulates cell phenotypes and tumor growth in various cancers. However, the role and mechanism of this micro-ribonucleic acid in CRC radiosensitivity remains unclear. Here, the abundances of miR-183-5p and ATG5 mRNA were detected by a real-time quantitative reverse transcription polymerase chain reaction. Kaplan–Meier survival analysis was carried out to explore the correlation between miR-183-5p and patient prognosis. Cell viability was evaluated by the MTT assay. Survival fraction analysis through colony formation was performed to assess the cell radiation response. Bioinformatic, luciferase and western blot assays were employed to verify the targeted interaction between miR-183-5p and ATG5. The results showed that an elevated abundance of miR-183-5p and a reduced ATG5 level in CRC were associated with the poor prognosis. The knockdown of miR-183-5p enhanced the sensitivity of CRC cells to radiation, inflected by the decreased cell viability and survival fraction. Mechanically, ATG5 was targeted by miR-183-5p. The addition of ATG5 conferred the radiosensitivity of the CRC cells, which was revered by miR-183-5p restoration. Furthermore, miR-183-5p knockdown hindered the tumor growth by repressing ATG5 *in vivo* after radiation treatment. In summary, the output data indicated that miR-183-5p heightened the radiation response of the CRC cells by targeting ATG5, promising a novel therapeutic target for CRC patients with radioresistance.

Keywords. ATG5; colorectal cancer; miR-183-5p; radiosensitivity

Abbreviations: CRC, colorectal cancer; 3′-UTR, 3′-untranslated regions; FBS, fetal bovine serum; PVDF, polyvinylidene difluoride

1. Introduction

Colorectal cancer (CRC), one of the most common malignancies, is the third most frequent cause of cancer-related deaths among both men and women (Torre *et al.* 2015). As the statistics show, there are about 135,430 new cases and 50,260 deaths in the United States only in 2017, and people aged 50 or older with higher morbidity and mortality (Siegel *et al.* 2017). Radical surgery combined with adjuvant radiotherapy is considered as a mainstay for CRC treatment (Farniok and Levitt 2015). Unfortunately, the inherent and acquired radioresistance among a large number of CRC patients leads to treatment failure (Lin *et al.* 2015). Previous studies have highlighted that a series of genes, including EGFR (Toulany *et al.* 2005), P53 (Kurrey *et al.* 2009), Bcl-2 (An *et al.* 2007) and NF-κB (Li and Sethi 2010), are linked to the radiation response of various tumor cells. However, the clinical application of these markers for the response of radiotherapy remains controversial. Therefore, further clarification of the potential molecular mechanism involving the emergence of radioresistance and development of novel therapeutic strategies to improve the sensitivity of CRC to radiation are in urgent need.

MicroRNAs (miRNAs) are conservative small non-coding RNA molecules (19–24 nucleotides) that negatively mediate gene expression by base-pairing with the 3′-untranslated regions (3′-UTR) of target mRNA (Bartel 2009). Reliable evidence demonstrates that miRNAs are implicated in the

progression of human cancers via regulating multiple cell phenotypes including proliferation, apoptosis, differentiation, invasion and migration. Recent studies indicate that dysregulation of miRNAs is associated with the radiation response of several tumor cells (Volinia *et al.* 2006). For example, forced expression of miR-216a enhanced the sensitivity of pancreatic cancer to radiation by directly targeting Beclin-1, a major autophagy-related gene (Zhang *et al.* 2015a). MiR-9 and let-7g improved the efficacy of radiotherapy by suppressing NF-κB1 in lung cancer (Arora *et al.* 2011). Ectopic expression of miR-200c weakened radiation-induced autophagy and enhanced the radiosensitivity of breast cancer cells by repressing target gene UBQLN1 (Sun *et al.* 2015a).

MiR-183 has been identified as a major aberrantly expressed miRNA in some cancers including breast cancer (Cheng *et al.* 2016), prostate cancer (Ueno *et al.* 2013) and lung cancer (He *et al.* 2018). Dysregulation of miR-183-5p is implicated with the occurrence and progression of several cancers and triggers the malignant features by serving as an oncogene (Sarver *et al.* 2010). Additionally, the roles of miR-183-5p in the drug sensitivity of multiple cancers are also confirmed (Yan *et al.* 2013; Gao *et al.* 2019). In CRC, miR-183-5p is obviously down-regulated (Wang *et al.* 2016a; Falzone *et al.* 2018), hinting the possible involvement of miR-183-5p in CRC progression. However, its role and mechanism in the radiation response of CRC remains far from being elucidated.

Here, our study investigated the abundance of miR-183-5p in CRC tissues and cell lines, and analyzed the correlation between miR-183-5p expression and patient prognosis. Next, we further explored the role and mechanism of miR-183-5p in the radiosensitivity of CRC.

2. Materials and methods

2.1 *Patients and tissue specimens*

This study was performed with the approval of the Research Ethic Committee of Chongqing Three Gorges Central Hospital, and written informed consents were signed by patients and their relatives. Fresh tumor tissues and nearby normal tissues were obtained from 39 CRC patients by surgical resection at Chongqing Three Gorges Central Hospital during December 2010 to March 2012. All samples were quick-frozen in liquid nitrogen and stored in a freezer at − 80°C for RNA extraction.

Thirty-nine patients were divided into the low miR-183-5p expression group ($n = 13$) and high miR-183-5p expression group ($n = 26$) or low ATG5 expression group ($n = 21$) and high ATG5 expression group ($n = 18$) according to their mean expression levels in CRC tissues. All of them suffered from conventional radiotherapy were followed-up for Kaplan–Meier survival analysis. Those patients who died due to other diseases rather than CRC were excluded from the present study.

2.2 *Cell culture*

Human normal colonic epithelial cells NCM460 and human colon cancer cells Caco-2 were obtained from American type culture collection (ATCC, Manassas, VA, USA). Human colorectal cancer cell lines SW620, HT29, HCT116, SW480 and LoVo were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). All cells were grown at 37°C in a 5% CO_2 incubator with RPMI-1640 medium (Gibco, Grand Island, NY, USA) in the presence of 10% fetal bovine serum (Gibco) and 1% penicillin/streptomycin (HyClone, Logan, Utah, USA). The medium was replaced every 48 h until the confluence reached 80–90%.

2.3 *Plasmids and cell transfection*

MiR-183-5p inhibitor (anti-miR-183-5p) and matched control (anti-miR-NC), miR-183-5p mimics (miR-183-5p) and negative control (miR-NC) were obtained from GenePharma (Shanghai, China). An ATG5-overexpressed plasmid (pcDNA-ATG5) was established by inserting the full-length ATG5 sequences into the pcDNA3.1 vector (Invitrogen, Carlsbad, CA, USA), with the pcDNA3.1 empty vector (vector) as a negative control. HT29 and LoVo cells (1×10^6) were plated in six-well plates. Twenty-four hours later, cell transfection was performed using 40 nM oligonucleotides or 200 ng plasmids with Lipofectamine 2000 (Invitrogen) referring to the instructions provided by the manufacturer.

2.4 *RNA isolation and qRT-PCR*

Total RNA was extracted from tissues and cells using a Trizol reagent (Thermo Fisher, Wilmington, DE, USA) in accordance with the manufacturer's procedures, followed by the detection of RNA purity by using a microspectrophotometer (Thermo Fisher). For miR-183-5p expression, 1 μg RNA was reversely transcribed into First-Stand cDNA using the MicroRNA Reverse Transcription Synthesis Kit (Thermo Fisher), with U6 snRNA as an endogenous control. Then, the relative miR-183-5p expression was determined on Applied Biosystems 7500 Real-time PCR Systems (Thermo Fisher) using TaqMan Advanced miRNA Assay reagents (Thermo Fisher) and specific primers. Reserve transcription and qPCR primers for miR-183-5p and U6 were obtained from GenePharma. For ATG5 mRNA expression, cDNA was synthesized using a High Capacity Reverse Transcription System Kit (Takara, Dalian, China). Then, relative mRNA levels of ATG5 were measured using SYBR™ Select Master Mix (Thermo Fisher) and primers,

with GAPDH as a housekeeping gene. The PCR primers for mRNA were displayed as below: ATG5 (forward: 5′-ATGA CAGATGACAAAGATG-3′; reverse: 5′-CAAATAACCTT CTGAAAGTG-3′); GAPDH (forward: 5′-TATGATGATAT CAAGAGGGTAGT-3′; reverse: 5′-TGTATCCAAACTCAT TGTCATAC-3′). All experiments were repeated three times, and the results were calculated by the $2^{-\Delta\Delta Ct}$ method.

2.5 *Cell viability assay*

The cell viability was assessed by using the MTT kit (Solarbio, Beijing, China) according to the manufacturer's instructions. Briefly, transfected HT29 and LoVo cells (5×10^3) treated or untreated with X-ray radiation were seeded into 96-well plates. Afterwards, 10 μL MTT reagent were added into each well at different periods (24, 48, 72 and 96 h) after transfection. Following 4 h of culture, the supernatant from each well was removed and 180 μL formazan dissolving solution was added to terminate this reaction. The absorbance value at 490 nm was evaluated by using a microplate reader (Bio-Rad, Hercules, CA, USA).

2.6 *Colony formation assay*

HT29 and LoVo cells (1×10^5) were seeded into six-well plates, followed by the transfection of anti-miR-183-5p, pcDNA-ATG5, anti-miR-183-5p+pcDNA-ATG5 or matched controls. About 48 h after transfection, cells were irradiated with different doses of irradiation (2, 4, 6 and 8 Gy) using a RadSource 2000 X-ray irradiator (RadSource, Kansas City, Missouri, USA) at a dose rate of 2 Gy/min and incubated for another 14 d until the formation of colonies. After this, each well was fixed with methanol and stained with 1% crystal violet overnight at 4°C. After washing and drying, colonies with more than 50 cells were selected and counted.

2.7 *Western blot assay*

Total RNA in cells was isolated by RIPA lysis buffer (Solarbio) in line with the manufacturer's protocols, followed by the detection of protein concentrations using a BCA protein detection kit (Thermo Fisher). Equal amounts of protein were divided by sodium dodecyl sulfate polyacrylamide gel electrophoresis and then transferred onto polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). After being blocked with 5% non-fat milk in TBST overnight at 4°C, the membranes were incubated with rabbit anti-ATG5 (1:1000) or anti-β-actin (1:5000) (Abcam, Cambridge, MA, USA) for 2 h at 37°C and washed three times with TBST. Then, the membranes were further incubated with the HRP-conjugated secondary antibody for 1.5 h incubation at 37°C (Abcam). The protein bands were visualized using an enhanced chemiluminescence kit (Biorbyt, Shanghai, China) and the intensity of each band was quantified with Image Lab software (Bio-Rad).

2.8 *Luciferase assay*

Partial sequences of ATG5 3′-UTR with miR-183-5p wild-type binding sites were cloned into luciferase vector psi-CHECK-2 (Promega, Madison, WI, USA) to generate a wild-type ATG5 3′-UTR reporter (ATG5 3′-UTR-WT). Then, a TaKaRa MutanBEST kit (Takara, Dalian, China) was employed to mutate the sites of the ATG5 3′-UTR-WT reporter containing putative miR-183-5p binding sequences, and is named the mutant ATG5 3′-UTR reporter (ATG5 3′-UTR-MUT). For the luciferase reporter assay, the ATG5 3′-UTR-WT or ATG5 3′-UTR-MUT reporter was transfected into HT29 and LoVo cells along with miR-183-5p, anti-miR-183-5p or matched controls. About 48 h later, the cells were lysed and luciferase activities were evaluated by using a Dual-Luciferase Reporter Assay System (Promega).

2.9 *Tumor xenograft experiments*

All experiments *in vivo* were carried out in strict line with the Guidelines of Animal Care and Use approved by Animal Research committee of Chongqing Three Gorges Central Hospital. Four- to six-week-old female BALB/c nude mice were obtained from Charles River (Beijing, China) and fed under a designated condition for one-week. Anti-miR-NC or anti-miR-183-5p was transfected into HT-29 cells (2×10^6) along with pcDNA or pcDNA-ATG5. Afterwards, the transfected cells were subcutaneously inoculated into the back of BALB/c nude mice. When the tumor volume reached about 60 mm^3, mice were treated with a radiation dose of 4 Gy, with the untreated group as a blank control. The tumor volume was measured every 5 days in accordance with the following formula: volume = (length × $width^2$)/2. About 40 d after injection, mice were sacrificed and xenografts were resected for weight and miR-183-5p and ATG5 expression analyses.

2.10 *Statistical analysis*

All statistical analyses were conducted using SPSS 20.0 statistical software. Student's *t*-test and one-way ANOVA were performed to assess the statistical significance between two or more groups. The survival curve of patients was analyzed by the Kaplan–Meier method and log-rank test. The linear correlations between miR-183-5p and ATG5 levels in CRC tissues were investigated by spearman's correlation analysis. The results were shown as means ± standard

deviation. The P value less than 0.05 ($P<0.05$) was defined as statistically significant.

3. Results

3.1 *MiR-183-5p expression was up-regulated and ATG5 expression was decreased in CRC and associated with the poor prognosis*

Firstly, expression patterns of miR-183-5p in CRC tumor or adjacent tissues were determined by a real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR). The results displayed that miR-183-5p was significantly up-regulated in CRC tumor tissues (n = 39) compared to their counterparts (n = 39) (figure 1A). The Kaplan–Meier survival assay showed that high miR-183-5p expression caused a lower survival rate than that in the low miR-183-5p expression group (figure 1B). Moreover, the expression of miR-183-5p was also evidently elevated in six CRC cell lines (SW620, HT29, HCT116, SW480, Caco-2 and LoVo) compared to NCM460 cells (figure 1C). Meanwhile, the qRT-PCR assay revealed that the expression of ATG5 mRNA was significantly decreased in CRC tissues and cells compared with their corresponding controls (figure 1D and F). In addition, the low expression of ATG5 indicated poor outcomes of patients with CRC (figure 1E). These findings suggested that miR-183-3p and ATG5 might serve as independent prognosis factors for CRC.

3.2 *MiR-183-5p knockdown increased the radiosensitivity of CRC cells*

The cell viability and clonogenic survival fraction are considered as typical standards for determining the radiosensitivity (Banasiak *et al.* 2015). To explore the effects of miR-183-5p on radiosensitivity, CRC cells were transfected with anti-miR-183-5p or anti-miR-NC, and/or treated with 4 Gy radiation. The MTT assay showed that the knockdown of miR-183-5p enhanced the inhibitory effect of radiation on cell viability in HT29 and VoLo cells (figure 2A and B). Next, the CRC cells transfected with anti-miR-NC or anti-miR-183-5p were further treated with different radiation doses (2, 4, 6 and 8 Gy). The results of the colony formation assay revealed that miR-183-5p deficiency notably reduced the cell survival fraction in HT29 and LoVo cells following radiation treatment compared to the control group (figure 2C and D). These findings suggested that the depletion of miR-183-5p improved the sensitivity of CRC cells to irradiation.

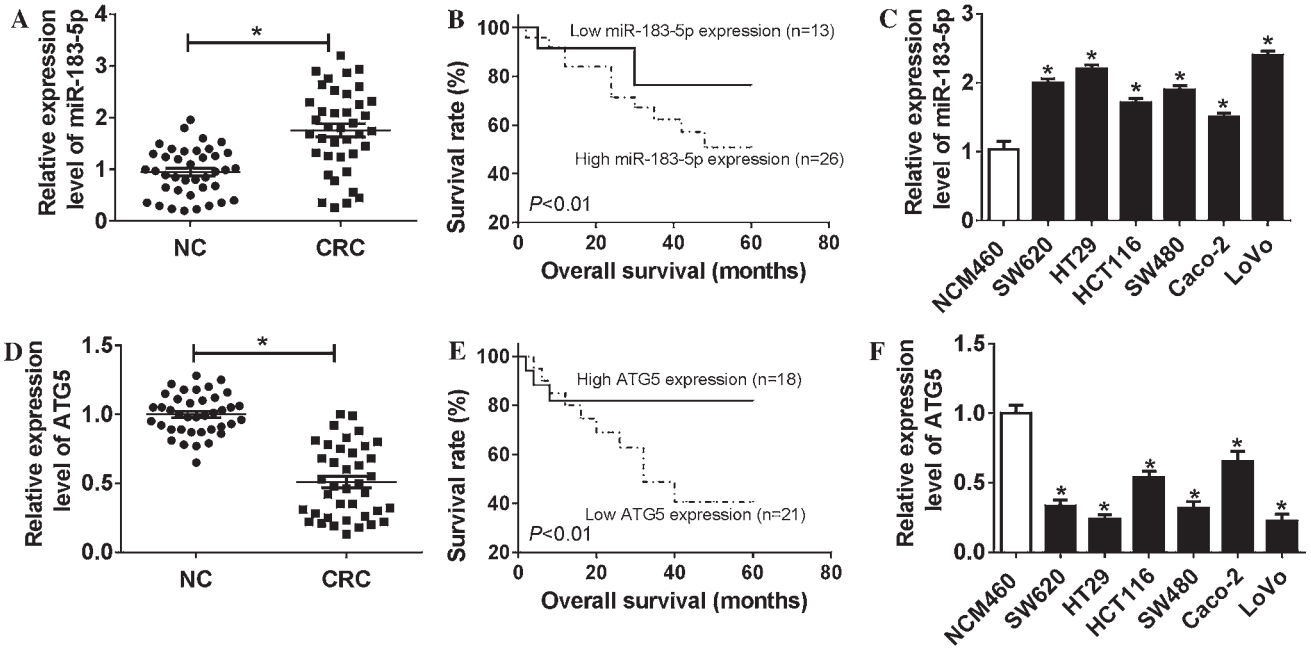

Figure 1. miR-183-5p was up-regulated in CRC tissues and cell lines and associated with poor prognosis. (**A**) miR-183-5p expression in CRC tissues (n = 39) and paired normal tissues (n = 39) was determined by the qRT-PCR. (**B**) Kaplan–Meier survival analysis was performed to evaluate the correlation between miR-183-5p expression and 5 year survival rates of 39 CRC patients. (**C**) The expressions of miR-183-5p in normal colonic epithelial cells NCM460 and six CRC cell lines (SW620. HT29, HCT116, SW480, Caco-2 and LoVo cells) were measured by the qRT-PCR. (**D**) The expression of ATG5 mRNA in CRC tissues (n = 39) was measured by the qRT-PCR. (**E**) The survival rate of patients was analyzed according to the expression level of ATG5 in CRC tissues. (**F**) The level of ATG5 mRNA was detected in CRC cells by the qRT-PCR. *$P<0.05$, compared to the NC or NCM460 group.

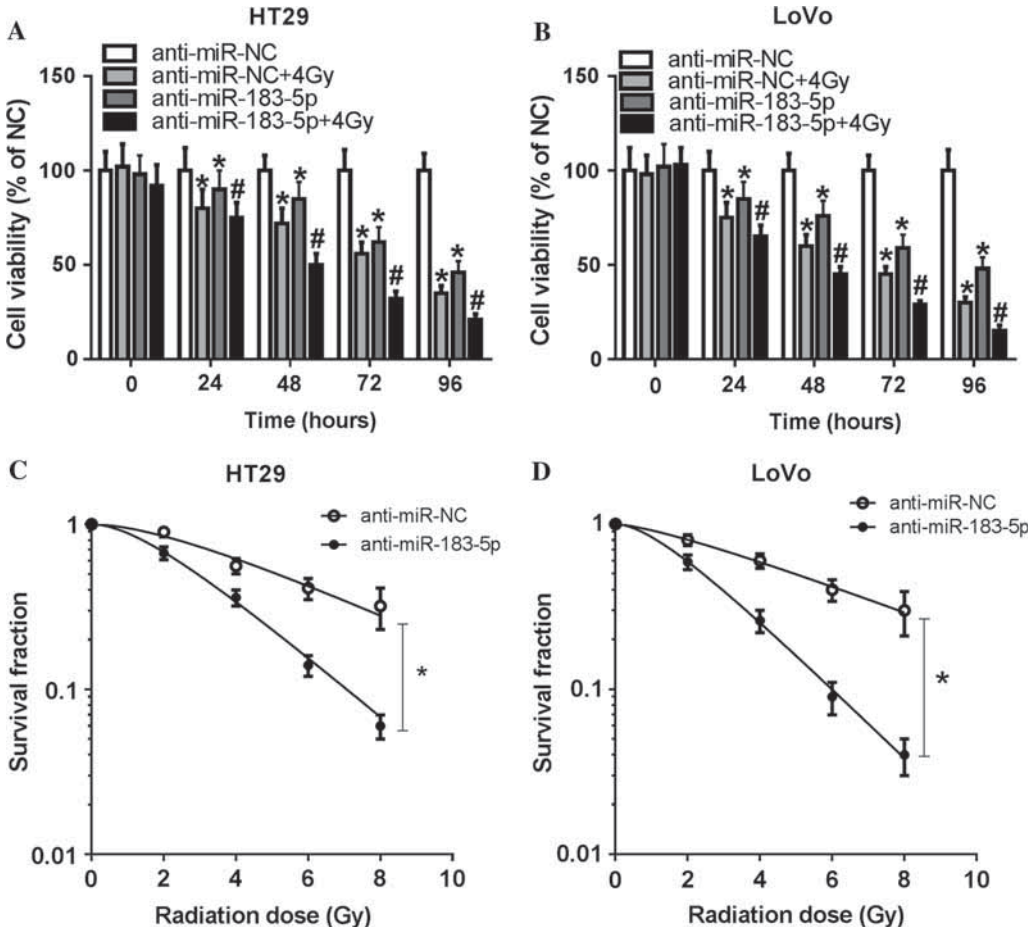

Figure 2. MiR-183-5p deficiency enhanced the sensitivity of CRC cells to radiation. (**A** and **B**) Anti-miR-NC or anti-miR-183-5p-transfected HT29 and LoVo cells were exposed to 4 Gy radiation, with no irradiation as a control, followed by the detection of cell viability by the MTT assay. (**C** and **D**) HT29 and LoVo cells were transfected with anti-miR-NC or anti-miR-183-5p, and exposed to different radiation doses. The survival fraction of cellular colony in HT29 and LoVo cells was calculated by the colony formation assay. *$P<0.05$, compared to anti-miR-NC; $^{\#}P<0.05$, compared to the anti-miR-183-5p group.

3.3 *ATG5 was an authentic target of miR-183-5p in CRC cells*

Given that miRNAs regulate a series of tumor processes, like cell growth, invasion, migration and tumor resistance by targeting cancer-related molecules (Iorio and Croce 2013; Pedroza-Torres *et al.* 2014), we investigated whether miR-183-5p is involved in the radiosensitivity of CRC cells by interacting with downstream target mRNA. The bioinformatics assay verified the existence of binding sites between miR-183-5p and the 3′-UTR of ATG5 using TargetScan online website (figure 3A). To further confirm the true interplay between miR-183-5p and ATG5, ATG5 3′-UTR-WT and ATG5 3′-UTR-MUT reporters were constructed, respectively. The results of the luciferase reporter assay showed that miR-183-5p overexpression notably suppressed the luciferase activity of the ATG5 3′-UTR-WT reporter in HT29 and LoVo cells relative to the miR-NC group, with no significant changes of ATG5 3′-UTR-MUT construct (figure 3B and C). The MiR-183-5p knockdown conferred the luciferase activity of the ATG5 3′-UTR-WT reporter, but had no impact on the luciferase activity of the ATG5 3′-UTR-MUT reporter (figure 3D and E). Next, the western blot assay showed that miR-183-5p enrichment suppressed, while miR-183-5p knockdown induced the expression of ATG5 protein in HT29 and LoVo cells (figure 3F). Moreover, a significant negative correlation was found between miR-183-5p and ATG5 expression in CRC tissues by spearman's correlation analysis (figure 3G). Taken together, these results indicated that ATG5 was negatively regulated by miR-183-5p by complementary binding.

3.4 *ATG5 improved radiosensitivity of CRC cells*

In order to evaluate the effect of ATG5 on radiosensitivity of CRC cells, HT29 and LoVo cells were transfected with pcDNA-ATG5 to overexpress ATG5 (figure 4A and B), and then MTT and colony forming assays were performed. As seen in figure 4C, the cell viability was obviously suppressed following radiation exposure, and ATG5 overexpression aggravated the

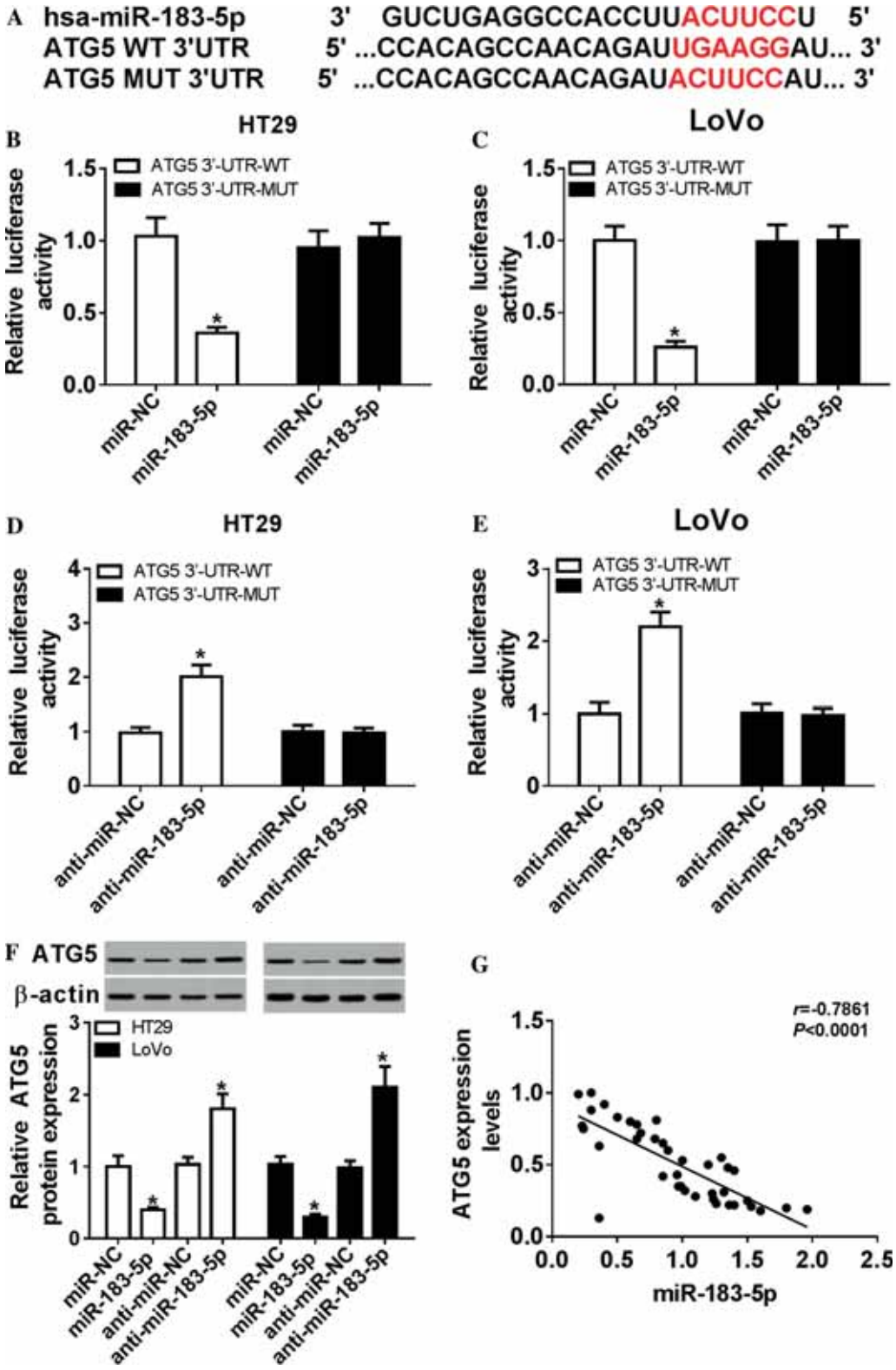

Figure 3. ATG5 was a direct target of miR-183-5p. (**A**) The putative miR-183-5p targeting ATG5 3′UTR sequences were predicted using TargetScan software. (**B**–**E**) Luciferase reporter containing wild-type or mutant miR-183-5p binding sites were transfected into HT29 and LoVo cells along with miR-183-5p, anti-miR-183-5p, or matched controls. About 48 h after transfection, the luciferase activity was determined by the dual-luciferase reporter assay. (**F**) The protein expressions of ATG5 in miR-183-5p-up-regulated or reduced HT29 and LoVo cells were detected by western blot assay. (**G**) Spearman's correlation assay was performed to assess the correlation between miR-183-5p and ATG5 expression in CRC tissues. *P<0.05, compared to the miR-NC or anti-miR-NC group.

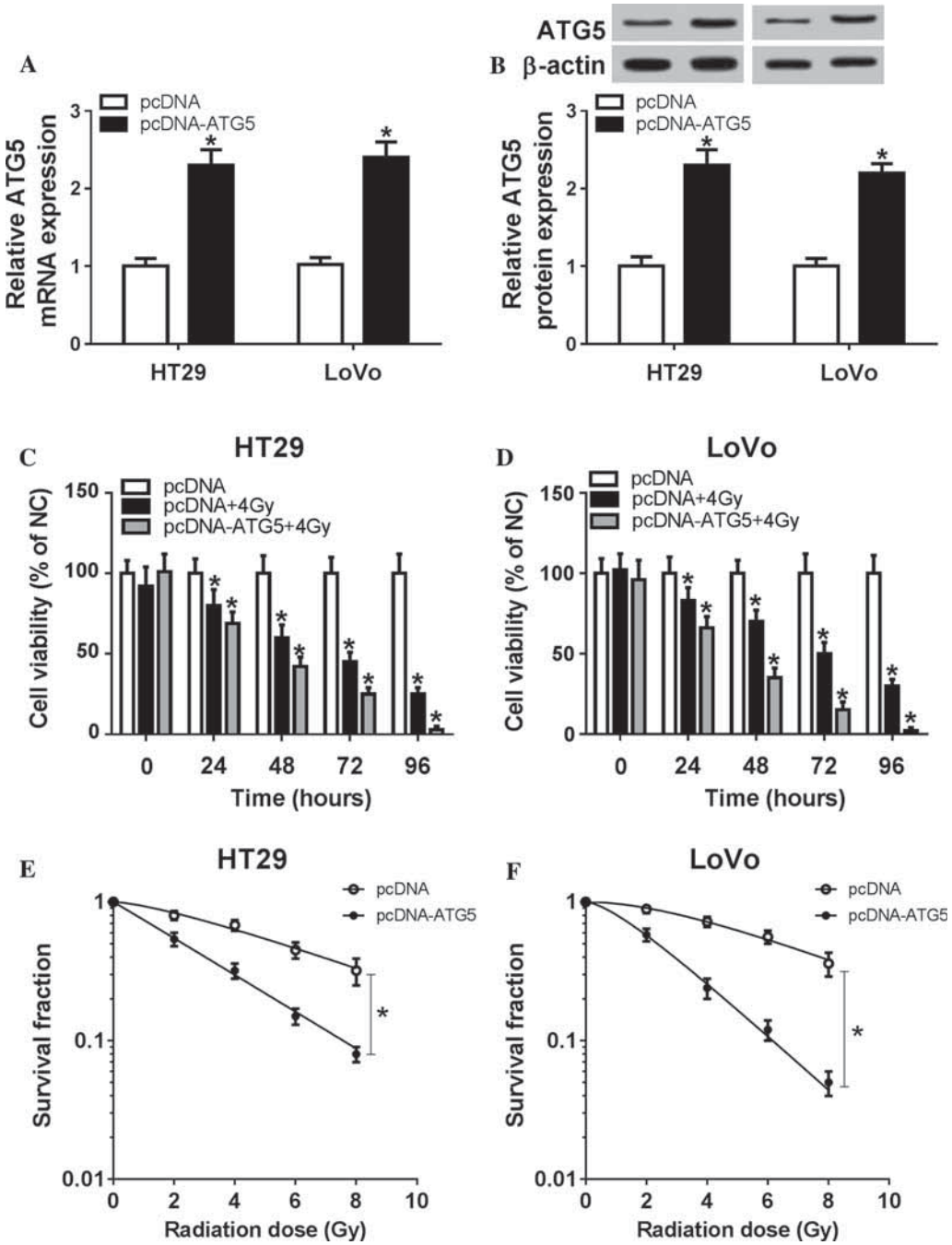

Figure 4. Addition of ATG5 improved the radiosensitivity of CRC cells. (**A** and **B**) ATG5 expression at mRNA and protein levels was tested in pcDNA or pcDNA-ATG5-transfected HT29 and LoVo cells by the qRT-PCR and western blot assays. (**C** and **D**) ATG5-overexpressed HT29 and LoVo cells were exposed to 4 Gy radiation, and then the cell viability was determined by the MTT assay. (**E** and **F**) ATG5-overexpressed HT29 and LoVo cells were treated with different radiation doses, followed by the evaluation of survival fraction of HT29 and LoVo cells by the colony formation assay. *$P<0.05$, compared to the pcDNA group.

inhibitory effect of radiation on cell viability in HT29 and LoVo cells (figure 4C and D). Moreover, the survival fraction in different radiation doses including 2, 4, 6 and 8 Gy was significantly lower in transfected HT29 and LoVo cells with pcDNA-ATG5 compared to the control group (figure 4E and F). These results indicated that the ectopic upregulation of ATG5 improved the sensitivity of CRC cells to radiation.

3.5 *ATG5 was required for miR-183-5p-mediated the radiosensitivity in CRC cells*

Here, rescue experiments displayed that increased miR-183-5p abolished the ATG5-mediated inhibitory effect on cell viability in radiated HT29 and LoVo cells (figure 5A and B). Furthermore, enforced expression of ATG5 induced a

decreased survival fraction, while this effect was markedly relieved after miR-183-5p overexpression in HT29 and LoVo cells following the exposure of different radiation doses (2, 4, 6 and 8 Gy) (figure 5C and D). These findings indicated that miR-183-5p enhanced the radioresistance by interacting with ATG5.

3.6 *MiR-183-5p regulated the sensitivity of CRC to irradiation partly via repression of ATG5 in vivo*

Restoration experiments displayed that re-expression of ATG5 heightened anti-miR-183-5p mediated the inhibitory effect on tumor growth after radiation exposure, as reflected by the decreased tumor volume (figure 6A) and weight (figure 6B). Furthermore, anti-miR-183-5p caused a dramatic downregulation of the miR-183-5p level and a notable upregulation of ATG5 mRNA and protein levels. Moreover, the introduction of pcDNA-ATG5 intensified the effects of anti-miR-183-3p on ATG5 expressions in tumor tissues (figure 6C–E). Taken together, the knockdown of miR-183-5p improved the radiosensitivity of CRC cells partly through negative regulation of ATG5 expression.

4. Discussion

Radiation-based therapy is the primary treatment modality for patients with CRC. However, the acquisition of radioresistance has become a challenging obstacle for radiotherapy failure, leading to tumor recurrence and eventual poor prognosis of CRC patients. Therefore, probing the substantial factors associated with radioresistance and proposing desired strategies to enhance the radiosensitivity of CRC have a vital practical significance for CRC treatment. Numerous miRNAs have been affirmed to play key roles in regulating tumorigenesis and progression from varying aspects, including cell proliferation, apoptosis and radioresistance. Zhang *et al.* reported that enforced miR-630 expression increased the radiosensitivity of CRC cells by directly targeting BCL2L2 and TP53RK genes (Zhang *et al.* 2015b). Hu *et al.* disclosed that the downregulation of miR-214 degraded the sensitivity of CRC cells to radiation through negative modulation of ATG12 expression (Hu *et al.* 2018). By regulating the miR-210/Bcl-2 pathway, HIF-1α lowered the radiosensitivity of CRC cells by activating autophagy (Sun *et al.* 2015b).

Recent studies have highlighted the tumor-suppressive activity of miR-183-5p in several types of human cancers. For example, elevated miR-183-5p is associated with tumor

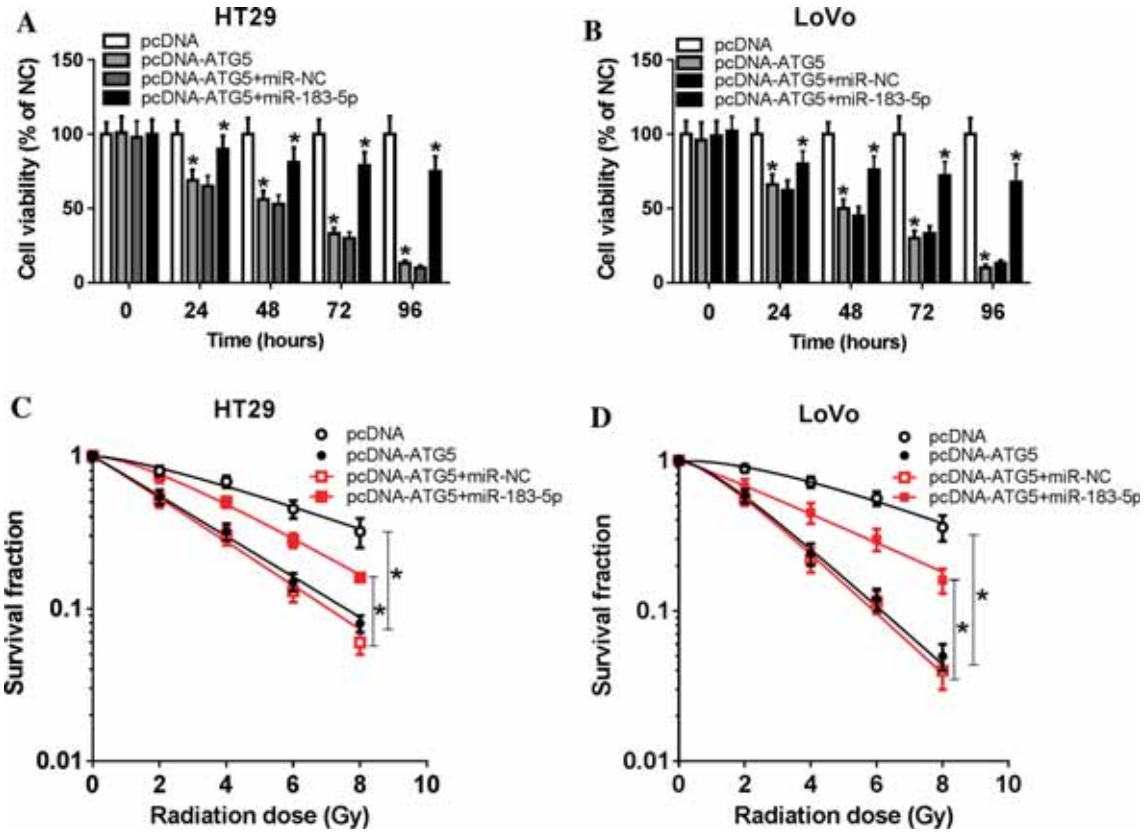

Figure 5. MiR-183-5p attenuated radiosensitivity by targeting ATG5 in CRC cells. (**A** and **B**) ATG5-overexpressed HT29 and LoVo cells were transfected with miR-NC or miR-183-5p mimics. After radiation treatment, the viability of HT29 and LoVo cells at each period was measured by the MTT assay. (**C** and **D**) Cell survival fractions in different radiation doses were assessed by the colony formation assay. **P*<0.05, compared to the pcDNA or pcDNA+miR-NC group.

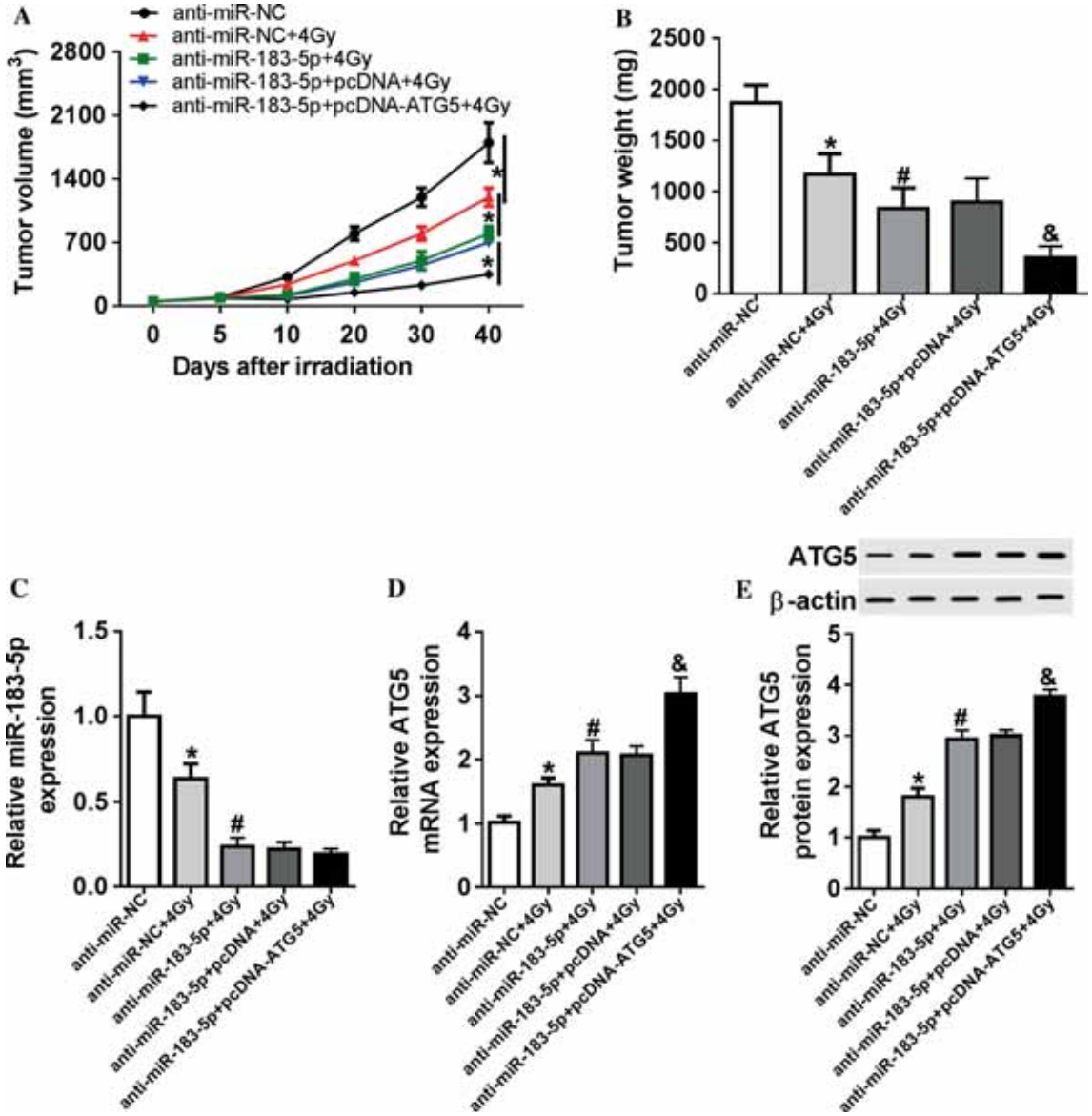

Figure 6. Reduction of miR-183-5p ameliorated radiosensitivity by repressing ATG5 *in vivo*. BABL/c nude mice were injected subcutaneously with anti-miR-NC, anti-miR-183-5p, anti-miR-183-5p+pcDNA or anti-miR-183-5p+pcDNA-ATG5-transfected HT29 cells, followed by the exposure of radiation when the tumor volume was about 60 mm^3. (**A**) Tumor volume was measured at different periods after radiation exposure. (**B**) Tumor weight was detected at the end point. (**C** and **D**) The expressions of miR-183-5p and ATG5 mRNA in tumor tissues were detected by the qRT-PCR. (**E**) ATG5 protein level in tumors was determined by the western blot assay. *P<0.05, compared to anti-miR-NC; $^{\#}P$<0.05, compared to anti-miR-NC+4Gy group; $^{\&}P$<0.05, compared to anti-miR-183-5p+pcDNA+4 Gy group.

progression and commonly signifies a poor prognosis in lung adenocarcinoma (Xu *et al.* 2014). MiR-183 promotes the aggressive phenotypes of pancreatic cancer by negative regulation of PDCD4 (Lu *et al.* 2015). In the present study, we found that miR-183-5p was markedly up-regulated in CRC and was associated with the poor prognosis of patients. Consistent with our findings, a previous study showed that the elevated abundance of miR-183-5p in CRC was implicated in the progression of CRC by interacting with a series of signaling pathways including the mismatch repair pathway and PI3K, Wnt, p53, MAPK, TGF-β and RAS pathways (Falzone *et al.* 2018). miR-183 expression in plasma derived from CRC patients was closely associated with the distant metastasis, advanced TNM stage, lower overall survival and tumor recurrence (Yuan *et al.* 2015). Up-regulated miR-183 functioned as an oncogene to facilitate the cell proliferation and inhibit apoptosis by silencing ABCA1 in colon cancer (Bi *et al.* 2016). In addition, the implication of miR-183-5p in drug/radiation resistance has also been clarified. As reported by Wang *et al.* (2016b), inhibition of miR-183 significantly increases the sensitivity of hepatocellular cells to 5-FU via miR-183-IDH2-HIF-1α

or miR-183-SOCS6-HIF-1α feedback loop. Fan *et al.* (2018) showed that the elevated miR-183 expression promoted the radioresistance of glioblastoma by downregulating LRIG1 and enhancing the EFGR/Akt pathway activity. Consistent with the previous study, our data showed that miR-183-5p knockdown enhanced the sensitivity of the CRC cell response to radiotherapy, which was reflected by a decreased cell viability and reduced survival fraction in the irradiated CRC cells after anti-miR-183-5p transfection.

Bioinformatical and luciferase assay elucidated that ATG5 was a bona fide target of miR-183-5p, and the expression level of ATG5 in CRC tissues was inversely associated with miR-183-5p expression. ATG5, widely known as an autophagy-related gene located on chromosome 6, has been closely associated with numerous processes, such as cell apoptosis, adipogenesis, immunity and cell survival (Yousefi *et al.* 2006; Zhao *et al.* 2008; Baerga *et al.* 2009). Previous findings also proclaimed that ATG5 plays a dichotomous role in mediating radioresistance in a range of cancers via inducing autophagy. On the one hand, the inhibition of ATG5 sensitizes nasopharyngeal carcinoma cells to ionizing radiation therapy by aggravating DNA damage and apoptosis (Mo *et al.* 2014). By hindering irradiation-induced autophagy, including the ATG5 expression and LC3-II/LC3I ratio, the Chk1 inhibitor MK-8776 increases the radiosensitivity of triple-negative breast cancer (Zhou *et al.* 2017). On the other hand, blockade of autophagy with 3-methyladenine or inhibiting autophagy-associated players Atg5 and Beclin-1 results in an increased radiation resistance of cancer cells (Kim *et al.* 2006). Similarly, inducing autophagy by upregulation of ATG5 and Beclin 1 enhanced the radiosensitivity in PTEN null prostate cancer cells (Cao *et al.* 2006). Our study demonstrated that exogenous elevation of ATG5 enhanced the radiosensitivity of CRC, while this effect was abolished by miR-183-5p overexpression. The study by Yuan *et al.* (2018) regarding miR-183-5p that could inhibit autophagy stimulated the authors to hypothesize that miR-183-5p might address the radiosensitivity of CRC cells by regulating ATG5-dependent autophagy, which is needed to be explored in further studies.

5. Conclusion

In conclusion, miR-183-5p was up-regulated in CRC tissues and cell lines and associated with poor prognosis of CRC patients. High abundance of miR-183-5p attenuated the radiosensitivity of CRC cells by interacting with ATG5. These findings may provide a novel therapeutic avenue for CRC patients with radioresistance.

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Corresponding editor: Ullas Kolthur-Seetharam