Am J Cancer Res 2026;16(3):1231-1241
www.ajcr.us /ISSN:2156-6976/ajcr0171199

Original Article

Carbon-ion beam irradiation combined with miR-17-5p/miR-17-3p inhibitors effectively kill osteosarcoma cells

Eun Ho Kim[1*], Won Seok Lee[1*], Sei Sai[2]

[1]Department of Biochemistry, School of Medicine, Daegu Catholic University, Nam-gu, Daegu 42472, South Korea; [2]Department of Charged Particle Therapy Research, QST Hospital, National Institutes for Quantum Science and Technology, Chiba, Japan. [*]Equal contributors.

Received January 4, 2026; Accepted February 25, 2026; Epub March 25, 2026; Published March 30, 2026

Abstract: Osteosarcoma (OS) is the most common type of bone cancer and is highly resistant to conventional photon beam radiotherapy; however, carbon-ion radiotherapy (CIRT) is effective in treating OS. In this study, to investigate whether miR-17-5p/miR-17-3p inhibitors act as radiosensitizers for CIRT, U2OS and MG63 OS cells were treated with carbon-ion beam irradiation (IR) alone, X-ray IR alone, or with one of the IR treatments in combination with miR-17-3p inhibitors. Cell death and invasive and migratory abilities were analyzed using cell viability and cell Transwell migration and invasion assays. Apoptosis and autophagy-related protein expression and DNA double-strand break (DSB) induction was determined using western blotting and immunofluorescence staining. We found that carbon-ion beam IR combined with miR-17-5p/miR-17-3p inhibitors significantly inhibited OS cell proliferation, migration, and invasion and markedly increased apoptosis-related cleaved-caspase 3, cleaved-PARP expression compared to carbon-ion beam IR and X-ray IR alone. Furthermore, carbon-ion beam IR combined with miR-17-5p/miR-17-3p inhibitors markedly promoted autophagy induction. In addition, combination treatment with miR-17-5p/miR-17-3p inhibitors and carbon-ion beam IR significantly increased the number of γH2AX foci as well as its phosphorylation. Taken together, miR-17-5p/miR-17-3p inhibitors enhanced the carbon-ion beam radiosensitivity of OS cells, presenting a novel strategy for the development of carbon-ion beam combination therapy.

Keywords: Carbon-ion beam, miR-17 inhibitors, osteosarcoma

Introduction

Osteosarcoma (OS) is an aggressive primary bone tumor [1, 2]. Approximately 30 to 40% of patients who are treated exhibit curative intent relapses, with a survival rate of less than 20% [3, 4]. OS is highly resistant to conventional radiotherapy, but high-LET carbon ion radiotherapy (CIRT) is safe and effective, with promising results in terms of local control and overall survival [5-7]. However, OS prognosis in patients treated with CIRT alone remains unsatisfactory [8-13]. To further improve the effectiveness of CIRT, it is necessary to explore drug combination therapies and identify drugs and combinations that can produce optimal synergistic effects [14].

MicroRNAs (miRNAs, miRs) may improve the radiosensitivity of cancers in humans to RT [15, 16]. miRs are non-coding RNA and are involved in controlling the development and progression of OS [17, 18]. Previously, we reported that several miRs such as miR-200c, miR-34 and miR-29b mimics significantly increased the radiosensitivity of pancreatic cancer, chondrosarcoma and OS cells including cancer stem cells to carbon-ion beam IR [19-21]. Here, we focused on miR-17, a member of the miR-17-92 cluster, because it has attracted research attention owing to its pleiotropic functions in tumorigenesis and because its miR-17-92 cluster is associated with OS tumor progression and prognosis [22-24]. Although miR-17-5p and miR-17-3p are different mature miRs derived from the same precursor (pre-miR-17) within the miR-17-92 cluster, they have distinct sequences and target different messenger RNAs (mRNAs), thereby regulating different biological processes. miR-17-5p often promotes cancer/cell proliferation (by targeting tumor suppressors such as PTEN), while miR-17-3p also has oncogenic

effects by downregulation of p21 and Par4 [25, 26], it is therefore associated with tumor cell radioresistance, and that miR-17-5p inhibitors suppress cell proliferation, migration, and invasion in human OS cell lines [27, 28].

In this study, we investigated the effects of both miR-17-5p and miR-17-3p inhibitors in terms of their distinct targets and different biological functions, in combination with CIRT on OS cell viability, migration, and invasion and changes in apoptosis- and autophagy-related gene expression and DNA repair and damage.

Materials and methods

Cell culture

U2OS and MG63 OS cells were obtained from the American Type Culture Collection and cultured in Dulbecco's modified Eagle medium (DMEM) before being supplemented with fetal bovine serum (FBS; WelGene), 1% (v/v) penicillin-streptomycin, and 10% FBS (Gibco®; Thermo Fisher Scientific, Waltham, MA)] and stored in a humidified incubator at 37°C and 5% CO_2.

Reagents

The antibodies used in the present study were poly (ADP-ribose) polymerase (PARP; CST #9542), cleaved PARP (CST #9541), caspase-3 (CST #9662), cleaved caspase-3 (CST #9664), and β-actin (SC-47778).

Irradiation

The cells were irradiated with carbon-ion beams accelerated by a heavy-ion medical accelerator in Chiba at the QST. Details regarding the characteristics of the carbon-ion beams, biological irradiation procedures, and dosimetry have been described elsewhere (24). Briefly, we used 290 MeV/nucleon carbon ion beams with a dose average LET of 50 KeV/μm at the center of spread-out Bragg peak. As a reference, the cells were also irradiated with conventional 200 kVp X-rays (TITAN-320, GE, USA).

miRNA and transient transfection

miR-17 mimic, miR-17-5p/miR-17-3p inhibitor, and the negative control were purchased from Bioneer (Daejeon, South Korea). The cells were transfected with 60 nM control or miRNS-17 mimic and miR-17-5p/miR-17-3p inhibitors for 24 h using G-fectin miRNA transfection reagent according to the manufacturer's instructions.

Cell viability assay

OS cells were seeded in 96-well plates at a density of 5000 cells/well and incubated for the indicated time points. To quantify cell viability, a culture medium containing an equal amount of EZ-Cytox reagent was added to the cells and incubated for 4 h. Then, the cells were treated with carbon-ion beam IR alone, X-ray IR alone, or in combination with miR-17-5p/miR-17-3p inhibitors for 24 h. Cell viability was measured after 48 h using a Multiskan EX instrument (Thermo Fisher Scientific) at 450 nm.

Caspase-Glo 3/7 assay

OS cells were treated with carbon-ion beam IR alone, X-ray IR alone, or with one of the IR treatments in combination with miR-17-5p/miR-17-3p inhibitors and incubated for 72 h. Caspase 3/7 activity was detected using a Caspase-Glo 3/7 assay kit (G8091; Promega, Madison, WI, USA) according to the manufacturer's protocol.

Autophagy assay

OS cells were treated with carbon-ion beam IR alone, X-ray IR alone, or with one of the IR treatments in combination with miR-17-5p/miR-17-3p inhibitors for 72 h. Then, the cells were harvested and stained with Cyto-ID® Green detection reagent (Cyto-ID® Autophagy Detection Kit 2.0, Enzo Life Science, Farmingdale, NY, US) and Hoechst 33342 according to the manufacturer's protocols, and autophagy was measured under a confocal laser scanning microscope (LSM 880).

Western blotting

OS cells were treated with carbon-ion beam IR alone, X-ray IR alone, or with one of the IR treatments in combination with miR-17-5p/miR-17-3p inhibitors and incubated for 24 or 48 h. Then, the cells were lysed with RIPA buffer. Next, the proteins were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes. Membranes were blocked with

5% (v/v) skim milk in PBS and 0.1% Tween 20 and incubated with the indicated antibodies (1:1,000), followed by incubation with the secondary antibodies (1:1,000). Finally, the blots were developed using Pierce Enhanced Chemiluminescence Western Blotting Substrate (Thermo Fisher Scientific) and scanned.

Cell transwell migration and invasion assays

The invasive and migratory abilities of OS cells were determined using Transwell chambers (Millipore) according to the manufacturer's instructions. The cells were placed on the upper chamber of the Transwell system, containing 150 μL of medium that was either untreated or treated with carbon-ion beam IR alone, X-ray IR alone, or with one of the IR treatments in combination with miR-17-5p/miR-17-3p inhibitors for 24 h. Cells that migrated through the Matrigel/gelatin-coated membrane were stained using the cell stain solution provided in the Transwell chamber assay kit (Chemicon, Millipore).

Immunofluorescence staining of γH2AX

OS cells were grown on chambered slides for 1 d and then treated with carbon-ion beam IR alone, X-ray IR alone, or with one of the IR treatments in combination with miR-17-5p/miR-17-3p inhibitors for 6 or 24 h. Then, cells were fixed with 4% paraformaldehyde and permeabilized with 0.5% Triton X-100 in PBS. γH2AX foci were detected after blocking the slides with 4% FBS in PBS for 1 h and staining with a 1:100 dilution of primary antibody against γ-H2AX (Millipore, Billerica, MA, USA) and a 1:500 dilution of FITC-conjugated secondary antibody.

Statistical analysis

Statistical significance was determined using Tukey's Honest Significant Difference (HSD) post-hoc test was performed after a one-way ANOVA. Differences were deemed statistically significant when the *P* value was < 0.001 or 0.05 ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$).

Results

Effects of the miR-17 mimic, miR-17-5p/miR-17-3p inhibitors alone, or the inhibitors in combination with carbon-ion beam IR or X-ray IR on OS cell proliferation and viability

To clarify the cell-killing effect of miR-17 on OS cells, we investigated the effects of the miR-17 mimic and its inhibitor, miR-17-5p/miR-17-3p, on the proliferation and viability of OS cells using the MTT assay and trypan blue staining. We found that the miR-17 mimic alone or in combination with 2 Gy of carbon-ion beam or 2 Gy of X-ray IR significantly increased cell numbers compared to the control in both OS cell lines. Meanwhile, miR-17-5p/miR-17-3p inhibitor treatment alone predominantly decreased cell numbers, which were further suppressed after combination of the inhibitor with 2 Gy of carbon-ion beam or X-ray IR. Interestingly, miR-17-3p inhibitor appeared to be more effectively suppressed cell proliferation compared to that miR-17-5p inhibitor alone, and it was further regressed when combined with carbon-ion beam IR in U2OS cells (**Figure 1A**). However, statistical analysis revealed that no significant difference in the inhibition of cell proliferation was observed between the monotherapy with miR-17-3p inhibitor and miR-17-5p inhibitor and the combined therapy with carbon-ion beam IR in both U2OS and MG63 cells (**Figure 1B**), indicating there is no strand-specific advantage. Cell viability was enhanced by the miRNA-17 mimic alone or in combination with 2 Gy of carbon-ion beam or 2 Gy of X-ray IR in MG63 cells but not in U2OS cells. The viability of both U2OS and MG63 cells was predominantly decreased after treatment with the miR-17-5p and miR-17-3p inhibitors alone, and it was further reduced in combination with carbon-ion beam IR. However, no significant difference was observed between the combination of miR-17-5p inhibitor and carbon-ion beam IR and the combination of miR-17-3p inhibitor and carbon-ion beam IR in both U2OS and MG63 cells (**Figure 1C**, **1D**).

Induction of apoptosis by the miR-17 mimic or miR-17-5p/miR-17-3p inhibitors alone or their combination with carbon-ion beam IR or X-ray IR

To determine the role of miR-17 in OS cell death, we examined the effects of both the miR-17 mimic and its inhibitor, miR-17-5p/miR-17-3p, on caspase activity as well as phosphorylation of cleaved caspase 3 and cleave-PARP using ELISA and western blotting assays. We found that the miR-17-5p/miR-17-3p inhibitor alone increased caspase3 activity in MG63 cells but not in U2OS cells. The miR-17-5p/miR-17-3p inhibitors combined with 2 Gy of car-

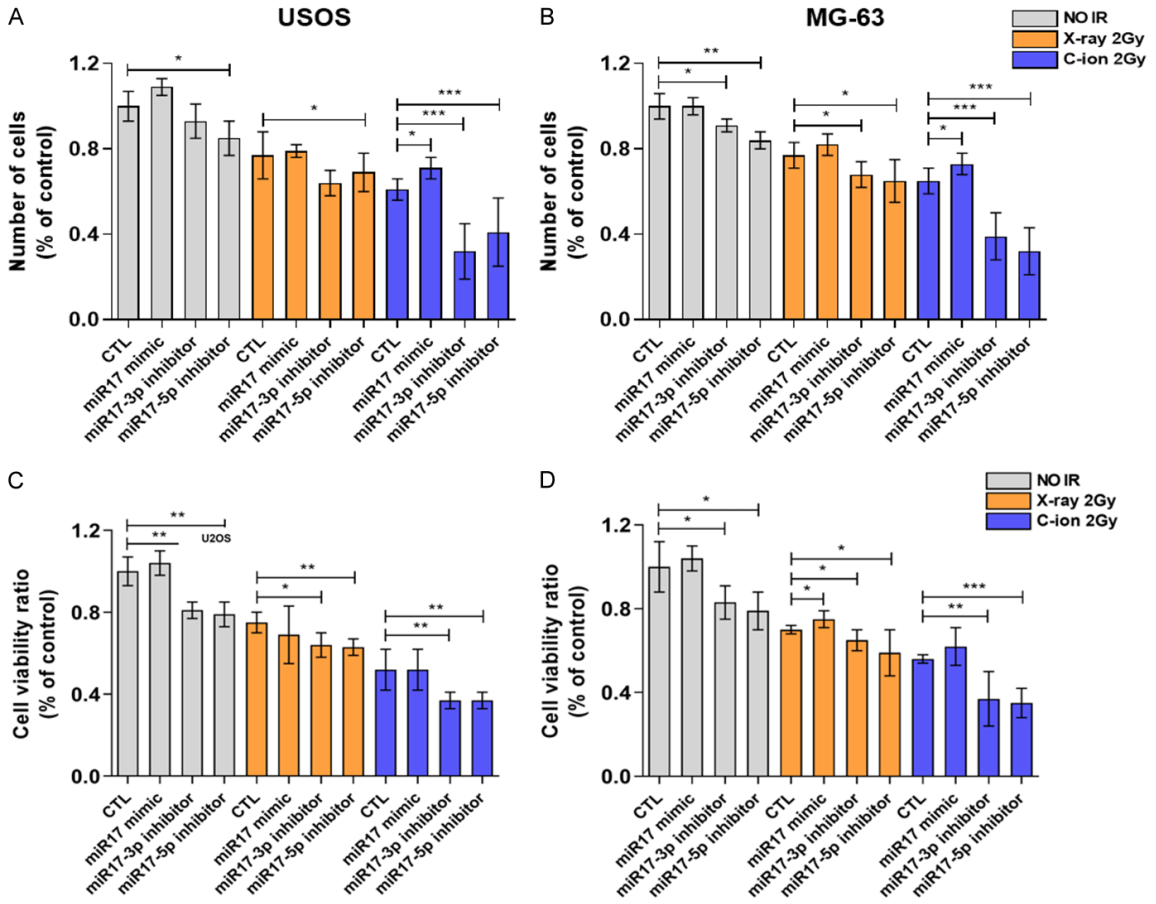

Figure 1. Effect of the miR-17 mimic or miR-17-5p/miR-17-3p inhibitors alone or in combination with carbon-ion beam IR or X-ray IR on U2OS (A) and MG63 cell proliferation (B). U2OS and MG63 cells were treated with themiR-17 mimic or miR-17-5p/miR-17-3p inhibitors alone or in combination with carbon-ion beam IR or X-ray IR for 24 h or 48 h and the cells were counted. Cell viability of U2OS cells (C) and MG63 cell proliferation (D) were determined using a trypan blue staining assay after the treatment. *P < 0.05, **P < 0.01, ***P < 0.001.

bon-ion beam exhibited a remarkably enhanced effect compared with that of their combination with 2 Gy of X-ray IR in both OS cell lines (**Figure 2A**, **2B**). Western blot analysis showed that miR-17-5p/miR-17-3p inhibitors combined with 2 Gy of carbon-ion beam significantly enhanced the expression of cleaved-caspase 3 and cleave-PARP compared with that in combination with 2 Gy of X-ray IR in both OS cell lines (**Figure 2C**, **2D**).

Effects of miR-17 mimic or miR-17-5p/miR-17-3p inhibitors alone or their combination with carbon-ion beam IR, or X-ray IR on OS cell migration and invasion

To investigate the role of miR-17 on OS cell migration and invasion, we estimated the effects of both the miR-17 mimic and its miR-17-5p/miR-17-3p inhibitors alone or in combination with 2 Gy of carbon ion beam or 2 Gy of X-ray IR using a cell transwell migration and invasion assay system. We found that the miR-17 mimic alone or in combination with Gy of carbon-ion beam or 2 Gy of X-ray IR significantly enhanced cell migration and invasion compared with that in the control in both OS cell lines (**Figure 3A-D**). In contrast, miR-17-5p/miR-17-3p inhibitors alone or in combination with 2 Gy of carbon-ion beam or 2 Gy of X-ray IR significantly suppressed cell migration and invasion compared to the control, especially when combined with carbon-ion beam IR (**Figure 3A-D**).

Induction of autophagy following treatment with the miR-17 mimic or miR-17-5p/miR-17-3p inhibitors alone or in combination with carbon-ion beam IR, or X-ray IR

To explore the effects of miR-17 on autophagy induction in OS cells, we used Cyto-ID®

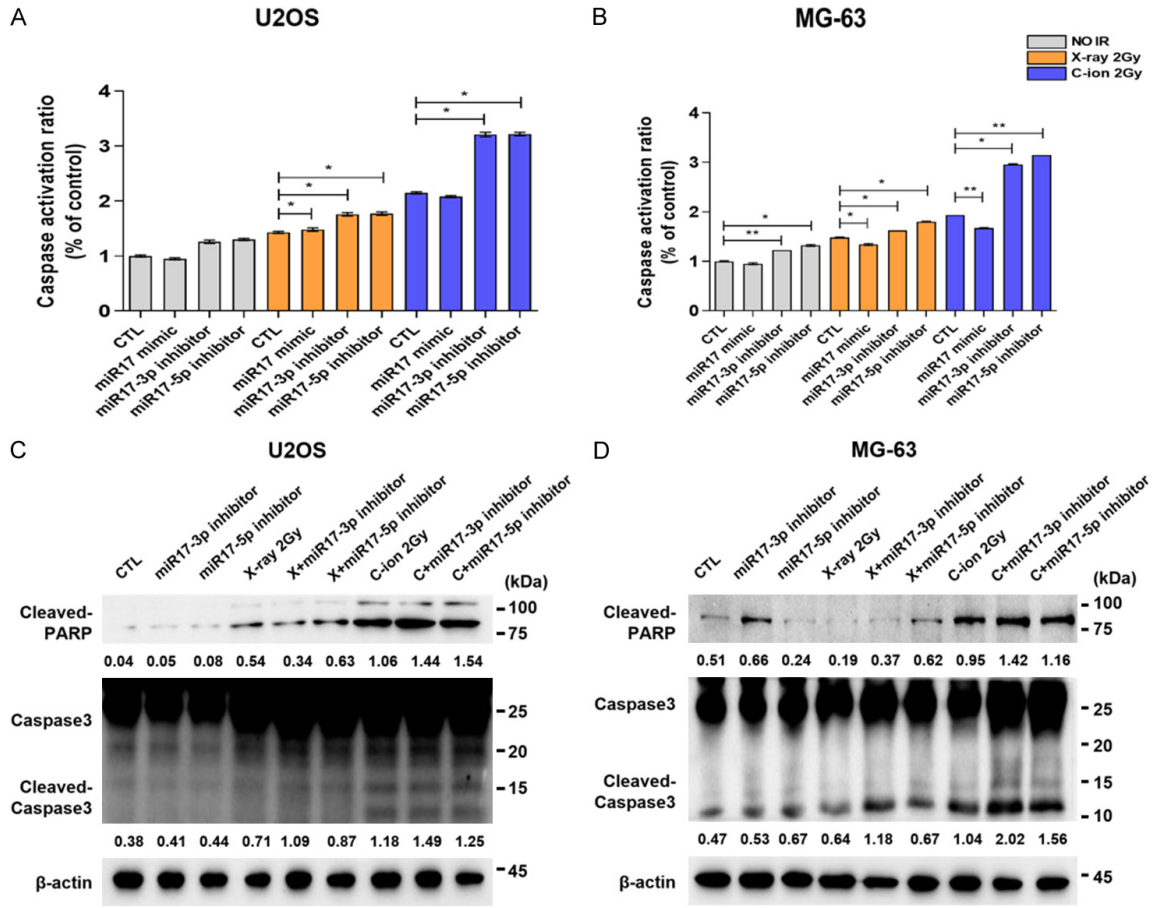

Figure 2. Caspase 3/7 activity after treatment with the miR-17 mimic or miR-17-5p/miRNA-17-3p inhibitors alone or in combination with carbon-ion beam IR or X-ray IR on U2OS (A) and MG63 cells (B) for 48 h. Induction of cleaved caspase 3 and cleaved-PARP after treatment with the miRNA-17 mimic or miR-17-5p/miR-17-3p inhibitors alone or in combination with carbon-ion beam IR or X-ray IR on U2OS (C) and MG63 cells (D) for 48 h.

Autophagy Detection kit to examine induction of pre-autophagosomes, autophagosomes, and autolysosomes following treatment with both the miR-17 mimic and miR-17-5p/miR-17-3p inhibitors alone or in combination with 2 Gy of carbon-ion beam or 2 Gy of X-ray IR using western blotting. We found that treatment with miR-17-5p/miR-17-3p inhibitors combined with 2 Gy of X-ray IR significantly increased autophagy compared with that in the control and or single treatment groups, especially the miR-17-5p/miR-17-3p inhibitors combined with 2 Gy of carbon-ion beam IR dramatically enhanced autophagy induction (**Figure 4A-D**).

Induction of DNA double-strand breaks (DSBs) by miR-17 mimic or miR-17-5p/miR-17-3p inhibitors alone or in combination with carbon-ion beam IR or X-ray IR

To elucidate the induction of DSBs by miR-17 mimic or miR-17-5p/miR-17-3p inhibitors alone or in combination with carbon-ion beam IR or X-ray IR, the levels of the DSB marker γH2AX foci formation and its phosphorylated form were detected using immunofluorescent staining and western blotting. We found that the number of γ H2AX foci was maintained 24 h after combination treatment with miR-17-5p/miR-17-3p inhibitors and 2 Gy of carbon-ion beam IR compared with that under the single treatment or its combination with X-ray IR in both OS cell lines (**Figure 5A**, **5B**). Western blot analysis showed that combination treatment with the miR-17-5p/miR-17-3p inhibitors and 2 Gy of carbon-ion beam IR significantly induced the phosphorylation of γH2AX compared to the treatment alone or its combination with X-ray IR, especially in U2OS cells (**Figure 5C**, **5D**).

Discussion

In this study, our data showed that the miR-17 mimic significantly elevated cell proliferation

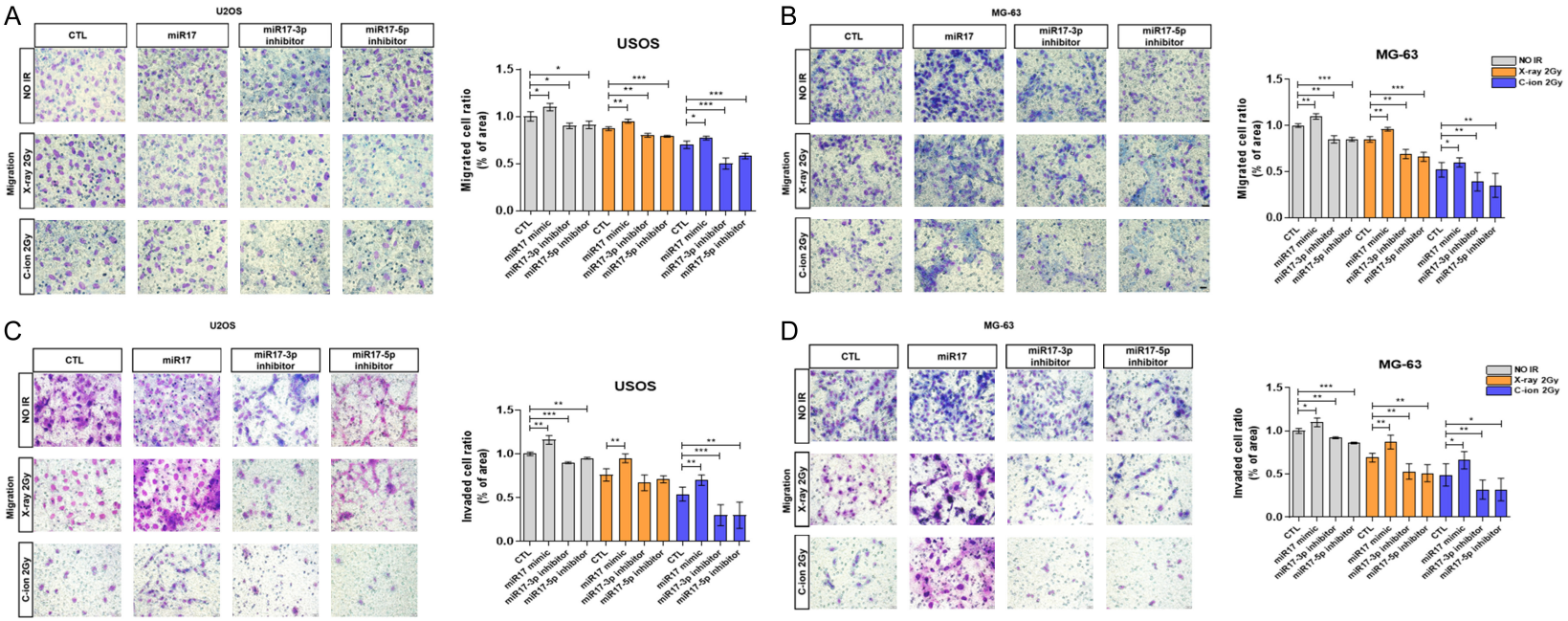

Figure 3. Effect of the miR-17 mimic or miR-17-5p/miR-17-3p inhibitors alone or in combination with carbon-ion beam IR or X-ray IR on U2OS and MG63 cell migration (A, B) and invasion (C, D). The cells were treated with miR-17 mimic or miR-17-5p/miR-17-3p inhibitors alone or in combination with carbon-ion beam IR or X-ray IR for 48 h and the cells were counted. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

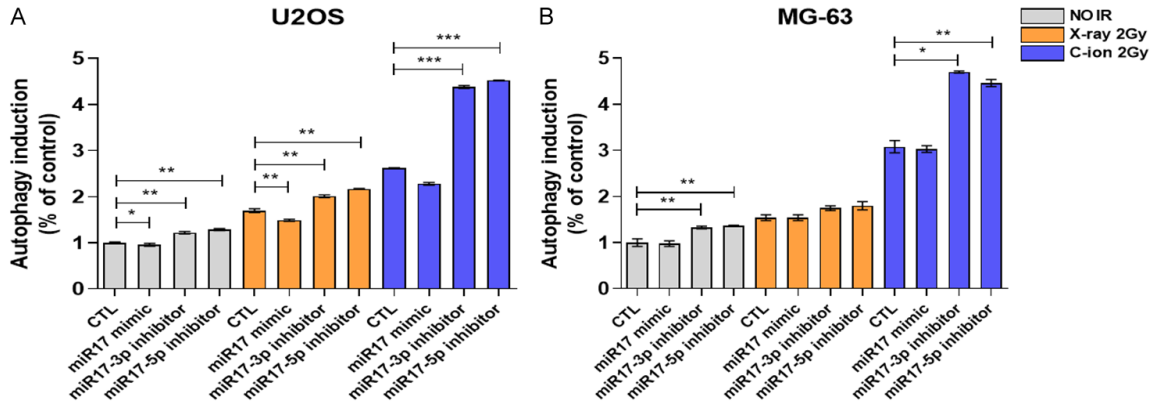

Figure 4. Induction of the autophagy after treatment with the miR-17 mimic or miR-17-5p/miR-17-3p inhibitors alone or in combination with carbon-ion beam IR or X-ray IR on U2OS (A) and MG63 cells (B) for 48 h. *P < 0.05, **P < 0.01, **P < 0.001.

and viability and reduced the growth inhibitory effects of carbon-ion beam and X-ray IR. This result is consistent with the findings of previous reports that the miR-17-92 cluster promotes proliferation and tumorigenesis in a wide range of tumor cells and cancer types, including colorectal, lung, breast, pancreatic, and prostate cancer [27-29]. In contrast, miR-17-5p/miR-17-3p inhibitors, alone or in combination with X-ray IR, effectively suppressed the proliferation and viability of OS cells, which was further reduced in combination with carbon-ion beam IR. This suggests that miR-17-5p/miR-17-3p inhibitors enhance the radiosensitivity of OS cells to carbon-ion beam IR. This is partially consistent with the previously reported finding that the miR-17-92 cluster significantly improved radioresistance in human mantle cell lymphoma cells [27].

Regarding the mechanism of the radiosensitizing effect of miR-17-5p/miR-17-3p inhibitors, we found that combining miR-17-5p/miR-17-3p inhibitors with 2 Gy of carbon-ion beam IR significantly promoted not only caspase 3/7 activity but also the phosphorylation of cleaved caspase 3 and cleaved PARP in both OS cell lines compared with the miR-17-5p/miR-17-3p inhibitors in combination with 2 Gy of X-ray IR. The carbon-ion beam IR combination treatment also enhanced the radiosensitivity of OS cells to carbon-ion beams by inducing apoptosis, which is partially consistent with the previous finding that the inhibition of miR-17-5p suppresses cell proliferation and promotes cell apoptosis in laryngeal squamous cell carcinoma [30]. Furthermore, we found that combining the miR-17-5p/miR-17-3p inhibitor with carbon-ion beam IR dramatically induced autophagy compared to single treatment or combination with X-ray IR, suggesting that both apoptosis and autophagy induction may be involved in the radiosensitization of OS cells to carbon-ion beam IR. It has been reported that high-LET carbon-ion beam IR induces autophagy in tumor cells pro-survival mechanism to manage damage, and inhibiting autophagy (using inhibitors such as 3-MA or chloroquine) increased the radiosensitivity of cancer cells to carbon-ion beam IR and promotes cell death [31, 32]. Based on the above reports, we speculate that carbon-on beam IR alone and in combination with miR-17-3p and -5p inhibitor-induced autophagy may act protectively, but this needs to be clarified using several autophagy inhibitors in the future. Besides, for the other possible cell death molecular mechanisms, including such as pyroptosis and cuproptosis also need to be investigated [33, 34].

Next, we elucidated the role of miRNA-17 in OS cell migration and invasion. The results showed that the miR-17 mimic had positive effects on OS cell migration and invasion. As expected, treatment with miR-17-5p/miRNA-17-3p inhibitors alone suppressed cell migration and invasion, and the suppressive effects were significantly enhanced when the inhibitors were combined with 2 Gy X-ray IR and especially when combined with carbon-ion beam IR, indicating that miR-17-3p inhibitors can improve the anti-migration and anti-invasion effects of CIRT. This is consistent with reports that miR-17-5p promotes migration and

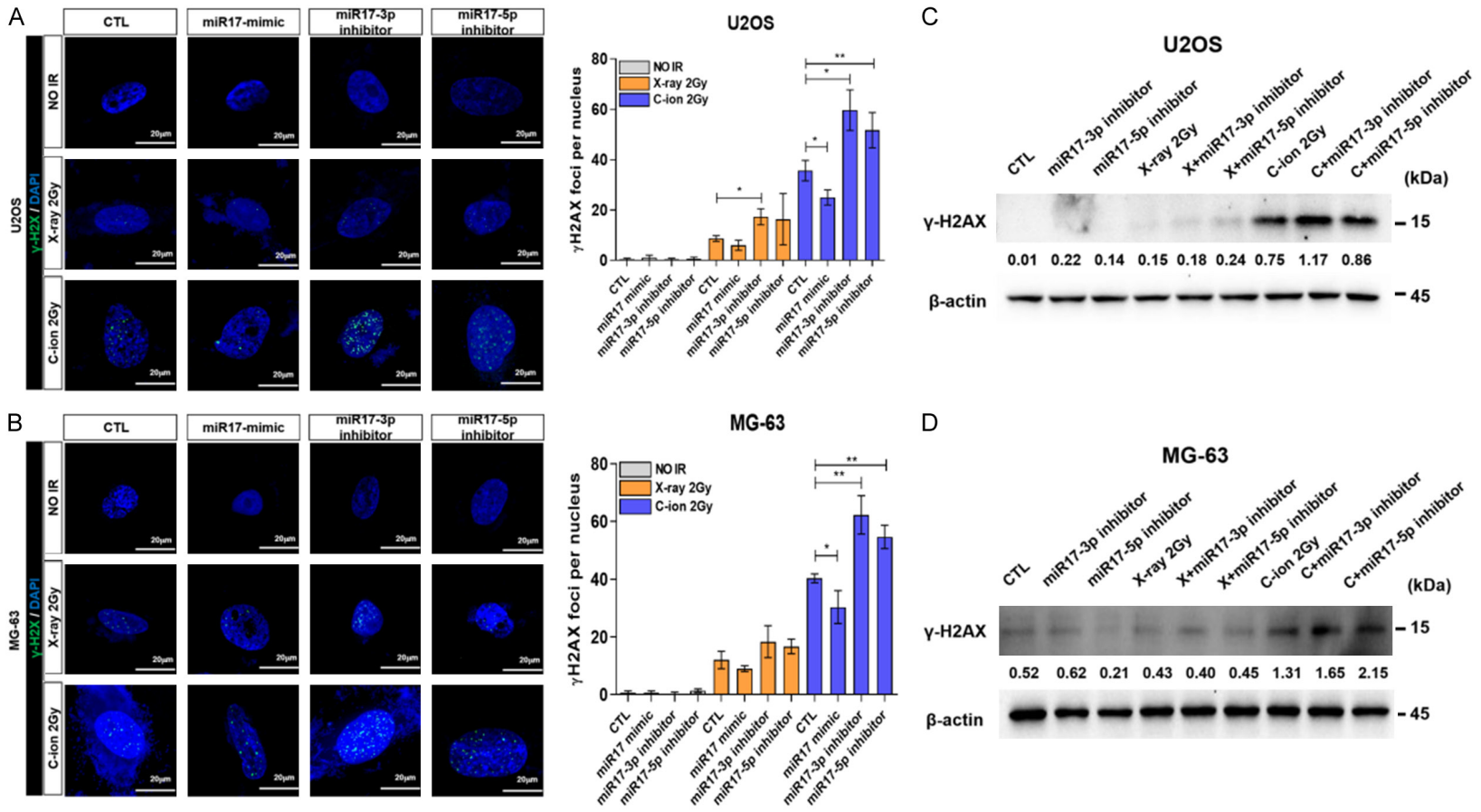

Figure 5. DNA double-strand break (DSB) detection using γH2AX foci immunofluorescence staining after treatment with the miR-17 mimic miR-17-5p/miR-17-3p inhibitors alone or in combination with carbon-ion beam IR or X-ray IR on U2OS (A) and MG63 cells (B) for 24 h. Phosphorylation of γH2AX was detected in U2OS (C) and MG63 cells (D) using western blotting.

invasion, whereas miR-17-5p inhibition has the opposite effect in colorectal and breast cancer cells [30, 35].

Finally, we verified that combination therapy with an miR-17-5p/miR-17-3p inhibitor and carbon-ion beam IR significantly induced γ H2AX foci and their phosphorylation in OS cells compared to monotherapy or combination therapy with X-ray IR, suggesting that severe DSB induction may also contribute to the increased killing effect of OS cells by carbon-ion beam IR, which is partially consistent with a previous finding that some miRNAs, such as miR-138, can regulate the DNA damage response [36].

In summary, miR-17-5p/miR-17-3p inhibitors effectively triggered carbon ion beam radiosensitivity in OS cells via the induction of apoptosis and autophagy accompanied by severe DSB. This combination treatment may provide improved therapeutic effects and overcome radioresistance in OS. Although both miR-17-5p and miR-17-3p inhibitors enhanced radiosensitivity to carbon-ion beam IR, the two strands are unlikely to be functionally redundant. Because of the different seed sequences, they likely regulate different target genes [22-26], even though similar phenotypic effects were observed in our experiments. The comparable radiosensitizing effects may reflect convergence on common stress and DNA damage response pathways rather than identical molecular mechanisms. Further research is needed into the specific molecular mechanisms involved, including the signalling pathways involved in OS cell death, that are induced by the combination of carbon-ion beam radiotherapy and miR-17-5p/miR-17-3p inhibitors. Furthermore, *in vivo* animal studies are required to validate the present *in vitro* results and optimize therapeutic strategies to improve OS treatment.

Acknowledgements

This study was supported by a grant from the Catholic University of Daegu (2023) and a National Research Foundation of Korea (NRF) grant (No. RS-2025-24683068). We would like to express our sincere gratitude to Mrs. Akiko Uzawa for her kind technical support.

Disclosure of conflict of interest

None.

Abbreviations

OS, Osteosarcoma; CIRT, carbon-ion radiotherapy; miRs, microRNAs; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; HIMAC, Heavy-ion Medical Accelerator in Chiba; QST, National Institutes for Quantum Science and Technology.

Address correspondence to: Dr. Sei Sai, Department of Charged Particle Therapy Research, QST Hospital, National Institutes for Quantum and Radiological Science and Technology, 4-9-1 Anagawa Inage-ku, Chiba 263-8555, Japan. Tel: 043-206-3231; Fax: 043-206-3231; E-mail: sai.sei@qst.go.jp; Dr. Eun Ho Kim, Department of Biochemistry, School of Medicine, Daegu Catholic University, Nam-gu, Daegu 42472, South Korea. E-mail: eh140149@cu.ac.kr

References

[1] Pakos EE, Nearchou AD, Grimer RJ, Koumoullis HD, Abudu A, Bramer JA, Jeys LM, Franchi A, Scoccianti G, Campanacci D, Capanna R, Aparicio J, Tabone MD, Holzer G, Abdolvahab F, Funovics P, Dominkus M, Ilhan I, Berrak SG, Patino-Garcia A, Sierrasesumaga L, San-Julian M, Garraus M, Petrilli AS, Filho RJ, Macedo CR, Alves MT, Seiwerth S, Nagarajan R, Cripe TP and Ioannidis JP. Prognostic factors and outcomes for osteosarcoma: an international collaboration. Eur J Cancer 2009; 45: 2367-2375.

[2] Jaffe N, Puri A and Gelderblom H. Osteosarcoma: evolution of treatment paradigms. Sarcoma 2013; 2013: 203531.

[3] Raymond AK and Jaffe N. Osteosarcoma multidisciplinary approach to the management from the pathologist's perspective. Cancer Treat Res 2009; 152: 63-84.

[4] Kager L, Zoubek A, Potschger U, Kastner U, Flege S, Kempf-Bielack B, Branscheid D, Kotz R, Salzer-Kuntschik M, Winkelmann W, Jundt G, Kabisch H, Reichardt P, Jurgens H, Gadner H and Bielack SS; Cooperative German-Austrian-Swiss Osteosarcoma Study Group. Primary metastatic osteosarcoma: presentation and outcome of patients treated on neoadjuvant cooperative osteosarcoma study group protocols. J Clin Oncol 2003; 21: 2011-2018.

[5] Schwarz R, Bruland O, Cassoni A, Schomberg P and Bielack S. The role of radiotherapy in osteosarcoma. Cancer Treat Res 2009; 152: 147-164.

[6] DeLaney TF, Park L, Goldberg SI, Hug EB, Liebsch NJ, Munzenrider JE and Suit HD. Radiotherapy for local control of osteosarcoma. Int J Radiat Oncol Biol Phys 2005; 61: 492-498.

[7] Dong M, Liu R, Zhang Q, Luo H, Wang D, Wang Y, Chen J, Ou Y and Wang X. Efficacy and safety of carbon ion radiotherapy for bone sarcomas: a systematic review and meta-analysis. Radiat Oncol 2022; 17: 172.
[8] Kamada T, Tsujii H, Blakely EA, Debus J, De Neve W, Durante M, Jakel O, Mayer R, Orecchia R, Potter R, Vatnitsky S and Chu WT. Carbon ion radiotherapy in Japan: an assessment of 20 years of clinical experience. Lancet Oncol 2015; 16: e93-e100.
[9] Shiba S, Okamoto M, Kiyohara H, Okazaki S, Kaminuma T, Shibuya K, Kohama I, Saito K, Yanagawa T, Chikuda H, Nakano T and Ohno T. Impact of carbon ion radiotherapy on inoperable bone sarcoma. Cancers (Basel) 2021; 13: 1099.
[10] Demizu Y, Jin D, Sulaiman NS, Nagano F, Terashima K, Tokumaru S, Akagi T, Fujii O, Daimon T, Sasaki R, Fuwa N and Okimoto T. Particle therapy using protons or carbon ions for unresectable or incompletely resected bone and soft tissue sarcomas of the pelvis. Int J Radiat Oncol Biol Phys 2017; 98: 367-374.
[11] Imai R, Kamada T, Tsuji H, Tsujii H, Tsuburai Y and Tatezaki S; Working Group for Bone and Soft Tissue Sarcomas. Cervical spine osteosarcoma treated with carbon-ion radiotherapy. Lancet Oncol 2006; 7: 1034-1035.
[12] Matsunobu A, Imai R, Kamada T, Imaizumi T, Tsuji H, Tsujii H, Shioyama Y, Honda H and Tatezaki S; Working Group for Bone and Soft Tissue Sarcomas. Impact of carbon ion radiotherapy for unresectable osteosarcoma of the trunk. Cancer 2012; 118: 4555-4563.
[13] Sugahara S, Kamada T, Imai R, Tsuji H, Kameda N, Okada T, Tsujii H and Tatezaki S; Working Group for the Bone and Soft Tissue Sarcomas. Carbon ion radiotherapy for localized primary sarcoma of the extremities: results of a phase I/II trial. Radiother Oncol 2012; 105: 226-231.
[14] Sai S, Koto M and Yamada S. Basic and translational research on carbon-ion radiobiology. Am J Cancer Res 2023; 13: 1-24.
[15] Kraemer A, Anastasov N, Angermeier M, Winkler K, Atkinson MJ and Moertl S. MicroRNA-mediated processes are essential for the cellular radiation response. Radiat Res 2011; 176: 575-586.
[16] Metheetrairut C and Slack FJ. MicroRNAs in the ionizing radiation response and in radiotherapy. Curr Opin Genet Dev 2013; 23: 12-19.
[17] Sampson VB, Yoo S, Kumar A, Vetter NS and Kolb EA. MicroRNAs and potential targets in osteosarcoma: review. Front Pediatr 2015; 3: 69.
[18] Chang L, Shrestha S, LaChaud G, Scott MA and James AW. Review of microRNA in osteosarcoma and chondrosarcoma. Med Oncol 2015; 32: 613.
[19] Kim EH, Kim JY, Kim MS, Vares G, Ohno T, Takahashi A, Uzawa A, Seo SJ and Sai S. Molecular mechanisms underlying the enhancement of carbon ion beam radiosensitivity of osteosarcoma cells by miR-29b. Am J Cancer Res 2020; 10: 4357-4371.
[20] Sai S, Kim EH, Koom WS, Vares G, Suzuki M, Yamada S and Hayashi M. Carbon-ion beam irradiation and the miR-200c mimic effectively eradicate pancreatic cancer stem cells under in vitro and in vivo conditions. Onco Targets Ther 2021; 14: 4749-4760.
[21] Vares G, Ahire V, Sunada S, Ho Kim E, Sai S, Chevalier F, Romeo PH, Yamamoto T, Nakajima T and Saintigny Y. A multimodal treatment of carbon ions irradiation, miRNA-34 and mTOR inhibitor specifically control high-grade chondrosarcoma cancer stem cells. Radiother Oncol 2020; 150: 253-261.
[22] Huang G, Nishimoto K, Zhou Z, Hughes D and Kleinerman ES. miR-20a encoded by the miR-17-92 cluster increases the metastatic potential of osteosarcoma cells by regulating Fas expression. Cancer Res 2012; 72: 908-916.
[23] Zhao X, Xu Y, Sun X, Ma Y, Zhang Y, Wang Y, Guan H, Jia Z, Li Y and Wang Y. miR-17-5p promotes proliferation and epithelial-mesenchymal transition in human osteosarcoma cells by targeting SRC kinase signaling inhibitor 1. J Cell Biochem 2019; 120: 5495-5504.
[24] Li X, Yang H, Tian Q, Liu Y and Weng Y. Upregulation of microRNA-17-92 cluster associates with tumor progression and prognosis in osteosarcoma. Neoplasma 2014; 61: 453-460.
[25] Xiang P, Yeung YT, Wang J, Wu Q, Du R, Huang C, Jia X, Gao Y, Zhi Y, Guo F, Wei H, Zhang D, Liu Y, Liu L, Liang L, Wang J, Song Y, Liu K and Fang B. miR-17-3p promotes the proliferation of multiple myeloma cells by downregulating P21 expression through LMLN inhibition. Int J Cancer 2021; 148: 3071-3085.
[26] Lu D, Tang L, Zhuang Y and Zhao P. miR-17-3P regulates the proliferation and survival of colon cancer cells by targeting Par4. Mol Med Rep 2018; 17: 618-623.
[27] Hu Z, Zhou S, Luo H, Ji M, Zheng J, Huang F and Wang F. miRNA-17 promotes nasopharyngeal carcinoma radioresistance by targeting PTEN/AKT. Int J Clin Exp Pathol 2019; 12: 229-240.
[28] Gao Y, Luo LH, Li S and Yang C. miR-17 inhibitor suppressed osteosarcoma tumor growth and metastasis via increasing PTEN expression. Biochem Biophys Res Commun 2014; 444: 230-234.
[29] Jiang P, Rao EY, Meng N, Zhao Y and Wang JJ. MicroRNA-17-92 significantly enhances radio-

resistance in human mantle cell lymphoma cells. Radiat Oncol 2010; 5: 100.
[30] Wang JX, Jia XJ, Liu Y, Dong JH, Ren XM, Xu O, Liu SH and Shan CG. Silencing of miR-17-5p suppresses cell proliferation and promotes cell apoptosis by directly targeting PIK3R1 in laryngeal squamous cell carcinoma. Cancer Cell Int 2020; 20: 14.
[31] Jin X, Li F, Zheng X, Liu Y, Hirayama R, Liu X, Li P, Zhao T, Dai Z and Li Q. Carbon ions induce autophagy effectively through stimulating the unfolded protein response and subsequent inhibiting Akt phosphorylation in tumor cells. Sci Rep 2015; 5: 13815.
[32] Sudo M, Tsutsui H, Hayashi S, Yasuda K, Mitani K, Iwami N, Anzai M, Tsubouchi T, Ishida M, Satoi S, Kanai T, Hirono S, Hatano E and Fujimoto J. Autophagy inhibition increased sensitivity of pancreatic cancer cells to carbon ion radiotherapy. Cell Physiol Biochem 2023; 57: 212-225.
[33] Zhang Z, Wang H, Huang Y, Zhu L, Wang X, Du Y, Zhou G and Zhao Y. Heavy ion radiation induces pyroptosis in skin keratinocytes through a mitogen-activated protein kinase signaling pathway. Int J Radiat Biol 2025; 101: 1142-1150.
[34] Lei G, Sun M, Cheng J, Ye R, Lu Z, Horbath A, Huo D, Wu S, Alapati A, Aggarwal S, Xu Z, Mao C, Yan Y, Yao J, Li Q, Chen X, Lee H, Zhuang L, Jiang D, Pataer A, Roth JA, Navin N, Koong AC, You MJ, Lin SH and Gan B. Radiotherapy promotes cuproptosis and synergizes with cuproptosis inducers to overcome tumor radioresistance. Cancer Cell 2025; 43: 1076-1092, e1075.
[35] Yu W, Wang J, Li C, Xuan M, Han S, Zhang Y, Liu P and Zhao Z. miR-17-5p promotes the invasion and migration of colorectal cancer by regulating HSPB2. J Cancer 2022; 13: 918-931.
[36] Wang Y, Huang JW, Li M, Cavenee WK, Mitchell PS, Zhou X, Tewari M, Furnari FB and Taniguchi T. MicroRNA-138 modulates DNA damage response by repressing histone H2AX expression. Mol Cancer Res 2011; 9: 1100-1111.