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Regular paper

miR-3942-3p Increases the radiosensitivity of nasopharyngeal carcinoma through negatively regulating BARD1

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Nasopharyngeal carcinoma (NPC) has high incidence in China and East and Southeast Asia. The study was performed to investigate the effect of microRNA3942-3p (miR-3942-3p) on the radiosensitivity of NPC. Compared with non-cancer tissue, NPC had significantly lower miR-3942-3p expression. X-irradiation (IR) reduced the expression of miR-3942-3p in a dose-dependent way in NPC cells. Down-regulation of miR-3942-3p using miR-3942-3p inhibitor resulted in significantly increased cell viability, decreased apoptosis of CNE1 cells. Bax decreased and Bcl2 increased after IR. The expression of BARD1, a cancer predisposing gene, was elevated in NPC tissue. It was confirmed to be a target of miR-3942-3p using luciferase reporter assay. Down-regulation of BARD1 using siRNA significantly reduced cell viability and significantly increased apoptosis both before and after IR. The same response was observed when miR-3942-3p mimics was used to transfect BARD1-overexpressing CNE1 cells, suggesting the up-regulation of miR-3942-3p could sensitize CNE1 cells to X-rays *via* BARD1. Our data demonstrate that up-regulation of miR-3942-3p could sensitize NPC to X-rays *via* a downstream target BARD1, offering potential new strategies for radiotherapy of NPC.

Keywords: microRNA, radiosensitivity, apoptosis, cell viability, nasopharyngeal carcinoma, radiotherapy

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Abbreviations: NPC, Nasopharyngeal carcinoma; IR, X-irradiation; miRNA, microRNA; mRNA, messenger RNA; EGFR, epidermal growth factor receptor; BARD1, BRCA1-associated RING domain 1; DMEM, Dulbecco's modified Eagle medium; FBS, fetal bovine serum; OD, optical density; FACS, fluorescence-activated cell sorting; PVDF, polyvinylidene fluoride membrane; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HRP, horseradish peroxidase; ECL, enhanced chemiluminescence; SD, standard deviation; ANOVA, analysis of variance; HIF-1α, hypoxia-inducible factor1-alpha

INTRODUCTION

Nasopharyngeal carcinoma (NPC) is head and neck cancer that has high incidence in southern China, as well as East and Southeast Asia (Chen *et al*., 2019a). The main histological types of the malignant tumor are poorly differentiated and undifferentiated carcinoma derived from the nasopharyngeal epithelium, which are sensitive to chemotherapy and radiotherapy (Lam & Chan, 2018). However, recurrence and distant metastasis after radiotherapy are a challenge in the treatment of advanced NPC, mainly due to acquired resistance to radiotherapy and complications (Lei *et al*., 2020). Therefore, there is an urgent need to discover effective therapeutics and strategies to improve radiosensitivity for better therapeutic outcomes and survival rate of NPC patients. Early studies have shown that NPC could acquire the resistance to radiotherapy *via* cell-matrix and cell-cell crosstalk and αV integrin appears to play a major role in acquiring the radioresistance and blocking αV integrin is shown to increase the radiosensitivity (Ou *et al*., 2012).

Recently, microRNAs (miRNAs) have attracted significant interest in predicting and modifying the outcomes of radiotherapy and chemotherapy in different cancers (Hummel *et al*., 2010). miRNAs are single stranded small (21-22 nt) non-coding RNAs with regulatory functions. They pair with the bases in the untranslated regions of target messenger RNA (mRNA) to block its translation or to facilitate its degeneration to impact various pathophysiological processes, including cell apoptosis, proliferation and differentiation (Rupaimoole & Slack, 2017). Abnormal expression of miRNA has been reported in a variety of human cancers, including NPC (Jiang *et al*., 2020; Zheng *et al*., 2019). They are also found to be associated with radioresistance-associated metastasis in NPC (Zhou *et al*., 2021). In addition, miRNAs have been demonstrated to take part in the occurrence and development of cancers as well as the radiosensitivity of tumor. Previous studies showed that miR-7 could activate the epidermal growth factor receptor (EGFR) pathway to sensitize human cancer cells to radiation (Lee *et al*., 2011). MiR-200c could inhibit the autophagy of breast cancer cells and enhance the radiosensitivity of breast cancer cells by targeting ubiquilin-1 (Sun *et al*., 2015). MiR-95 expression in prostate cancer was upregulated when the cancer was irradiated with ionizing radiation, leading to increased radioresistance by targeting the sphingolipid phosphatase (Huang *et al*., 2013). miR-203 sensitized NPC to radiation by targeting IL8/AKT signaling pathways (Ou *et al*., 2012) and miR-124 enhanced radiosensitivity of NPC by targeting PDCD6 (Zhang *et al*., 2017). Furthermore, the expression of miRNAs has been found changed in radioresistant cancer cells as compared to radiosensitive cells. For instance, the expression of miR-662 is induced by radiation and is upregulated in radioresistant colorectal cancer cells (Ma *et al*., 2015). In addition, long non-coding (lnc) RNA also impacts radiosensitivity of cancer *via* miRNA (Zhong *et al*., 2020)

miR-3942-3p is a newly discovered miRNA that is abnormally expressed in breast cancer and is involved in regulating the development of breast cancer (Zhao *et al*., 2018). It is downregulated in patients with early onset preeclampsia (Lykoudi *et al*., 2018). These findings indicate that miR-3942-3p has biological functions in cancer and pregnancy. However, the expression of miR-3942-3p in NPC has not been investigated and it is not clear if it could impact radiosensitivity of the cancer. Bioinformatic analysis showed that it could pair with BRCA1-associat-

ed RING domain 1(BARD1), a gene encoding a protein which interacts with the N-terminal region of BRCA1. Although *BRCA1* is shown to be a human tumor suppressor gene (Duncan *et al*., 1998; Yoshida & Miki, 2004), the role of *BARD1* in NPC is unclear, although it is considered to be a cancer predisposing gene for breast and ovarian cancers (Yoshida & Miki, 2004).

In this study, we investigated the expression of miR-3942-3p in NPC and its impact on radiosensitivity of the cancer. The findings may offer new clues to develop better therapeutic strategies for NPC.

MATERIAL AND METHODS

Tissue samples

NPC tissue (n=9) and the adjacent normal tissue (n=9) samples were obtained from patients undergoing surgery at our hospital after histopathological confirmation (based on WHO criteria). The specimens were taken from patients who did not receive any radiotherapy or chemotherapy, and were kept in liquid nitrogen before being used in the experiments. This study was approved by the ethics committee of People's Hospital of Guangxi Zhuang Autonomous Region, Nanning, China and written informed consent was obtained from every patient.

Cell culture and irradiation

Human NPC line CNE1(Yao, 1978) was obtained from Jack Strominger (Harvard) and was grown in Dulbecco's modified Eagle medium (DMEM; Thermo Scientific, Waltham, MA) supplemented with 10% fetal bovine serum (FBS; Invitrogen, Grand Island, NY) and 0.5% penicillin-streptomycin (Invitrogen-Gibco). Cells were grown at 37°C in a humidified, 5% CO_2 incubator. For irradiation (IR) treatment, CNE1 cells were cultured in 60-mm Petri dishes at a density of 1×10^7 cells/ml and irradiated with X-rays using an irradiation apparatus (2100 C/D, VARIAN, CA, USA) at a dose rate of 0.25 Gy/min at room temperature.

Transfection

CNE1 cells were cultured to 80% confluency and digested with 0.25% for subculture. The cells in the logarithmic growth period were harvested and transfected with miR-3942-3p mimics (5'-UCGCAUAGUCUCAGAAGUGAUC-3'), miR-3942-3p inhibitor (5'-GCUUACUCAUGUGACUAUGCUA-3'), and scrambled negative controls (NC-mimic, 5'-ACUUUAUCAGUAGUCCUAAUCA-3, NC-inhibitor, 5'-AUUGUACAAUCAUCUAGUUAUC-3') using lipofectamine 2000 according to the manufacture's instruction (Invitrogen, USA) at a final concentration of 100 nM. These miRNAs were synthesized at Genepharma, Shanghai, China. To overexpress BARD1, the coding sequence of *BARD1* was inserted into pcDNA3.1 vector (Invitrogen, USA) to generate an overexpressing vector *pcBARD1*. To knockdown *BARD1*, BARD1-siRNA and siRNA-NC were obtained from Genepharma, Shanghai. 24 h after the transfection, the cells were irradiated with 0, 2, 4 and 8 Gy X-rays and grown for another 24 h before being harvested for assessments.

Cell viability assay

Cells in the logarithmic growth phase were used for viability assay. Diluted cells were seeded in the wells of 96 well plates at 5000 cells per well and grown to 70% confluency at 37°C in a humidified, 5% CO_2 incubator. To assess cell viability, the CCK-8 assay was performed according to the manufacture's instruction (Dojindo, Rockville, MD, USA). The cells were washed twice with PBS, added with 10 µL CCK-8 solution and incubated at 37°C for 4 h. Optical density (OD) values were measured at 595 nm using a microplate reader (Thermo Fisher Scientific Inc., Waltham, MA, USA). The experiments were performed independently three times.

Apoptosis assay

Apoptotic rate was determined using the Annexin V/PI Apoptosis Detection Kit (Keygen Biotech, Nanjing, China) according to the manufacturer's instructions. In brief, approximately 5×10^5 cells were harvested and resuspended in 1×binding buffer, added with 5 µl Annexin V-FITC and incubated at room temperature for 15 min, followed by incubation with 10 µl propidium iodide (PI, 10 mg/ml) in the dark at room temperature for 5 min. Flow cytometry (Bection Dikinson, USA) was used to assess the apoptotic cells using fluorescence-activated cell sorting (FACS) according to the manufacturer's instructions. The quantitation of apoptotic cells was calculated by CellQuest software. The experiments were performed independently three times.

Dual-luciferase reporter assay

Potential target genes of miR3942-3p were predicted using Targetscan (http://www.targetscan.org). Dual luciferase activity assay was performed to confirm that BARD1 is an mRNA target of miR3942-3p. The human BARD1 3'UTRs containing a putative miR3942-3p binding site and its mutant version were inserted into the luciferase reporter vector psiCHECK-2 (Promega, USA) to generate psi-BARD1-wt and psi-BARD1-mut. Before the assays, CNE1 cells were seeded in 24-well plates and transfected with 100 ng vectors, together with miR3942-3p NC and miR-3942-3p mimics using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Luciferase activity was detected using a Dual Luciferase Reporter Assay kit (Promega). The experiments were performed independently three times.

Quantitative real time PCR (RT-qPCR)

Total RNA was extracted from tissues and cells using the Trizol reagent (Invitrogen, Carlsbad, CA, USA). The purity and quantity of extracted RNA were determined using Nanodrop 2000 and RNA was reversely transcripted using TaqMan MicroRNA Array kit (Applied Biosystems, CA, USA) into cDNA for quantification. The relative expression level of miR-3942-3p was quantified using TaqMan Universal Master Mix II (Applied Biosystems) with U6 as the internal reference on 7500 Fast Real-time PCR System (Applied Biosystems, Foster City, California) based on stem-loop primer method (Yang *et al*., 2014). Relative expression levels were calculated using $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001). The primers used were as follows: miR-3942-3p, 5'-TGATTCCAGCTGAATTGCATAGTCGTAAA-3' (forward) and 5'-CGAGGAAGAAGACGGAAGAAT-3' (reverse, stem-loop primer); U6 snRNA, 5'-CCCTTCGGGGACATCCGATA-3' (forward) and 5'-TTTGTGCGTGTCATCCTTGC-3' (reverse). BARD1 was amplified using forward primer: AGTAATATATTTGGTCTGCGG and reverse primer: GCTACTGTGGATTCAAAGAC. The thermocycling condition parameters were 10 min at

96°C, followed by 40 cycles of 1 min at 94°C, 2 min at 62°C, and 1 min at 72°C.

Western blot

Total proteins were extracted using RIPA buffer (Beyotime, Beijing, China) and quantified using BCA protein assay kit (Pierce, France). About 50 µg proteins were separated on a 12% sodium dodecyl sulfate–polyacrylamide gel and transferred to polyvinylidene fluoride membranes (PVDF, Millipore, Bedford, MA, USA). The membranes were blocked with 5% defatted milk in PBS-2% Tween20 at room temperature for 1 h and incubated with rabbit polyclonal antibodies against Bax (ab32503, 1:2000, Abcam, US), Bcl2 (AB692, 1:500, Abcam) or mouse monoclonal antibody against GAPDH (TA-08, 1: 2000, ZSbio, Beijing) at 4°C overnight. The membranes were washed and incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (1:5000, Cell Signaling Technology) at room temperature for 2 h and the immunoreactive bands were visualized using an enhanced chemiluminescence (ECL) detection kit (Pierce). The gray values of reactive bands were analyzed by Quantity One software.

Statistical analysis

Statistical analysis was performed using SPSS 13.0 statistical software and the data were expressed as the means ± standard error (S.D.) from at least three independent experiments. The Student's *t*-test and one-way analysis of variance (ANOVA) were used to compare means between the two groups and among the groups, respectively. Values differences with $P<0.05$ were considered statistically significant.

RESULTS

X-ray radiation up-regulates the expression of miR3942-3p

We first investigated the expression of miR3942-3p in NPC and normal tissue. qRT-PCR analysis showed that the miRNA was significantly down-regulated in the cancer cells as compared to normal tissue ($P<0.01$, Fig. 1A). After IR, expression of miR3942-3p in CNE1 cells was up-regulated as compared to non-irradiated cells ($P<0.01$) and the increase was positively related to the radiation dose (Fig. 1B) in the dose range used.

Down-regulation of miR-3942-3p increases radioresistance

We then examined if up- and down-regulation of miR-3942-3p would influence the radiosensitivity of CNE1 cells. Compared with inhibitor-NC, miR-3942-3p inhibitor significantly knockdowned the expression of miR-3942-3p ($P<0.01$). As a consequence, miR-3942-3p inhibitor-transfected CNE1 cells had significantly increased cell viability ($P<0.05$) and reduced cell apoptosis rate ($P<0.05$) as compared inhibitor-NC. Western blot analysis showed that Bax and Bcl2 expressions were significantly reduced or increased after transfection with miR-3942-3p inhibitor (Fig. 2). Compared with non-irradiated CNE1 cells, miR-3942-3p level were significantly higher in inhibitor-NC- and inhibitor-transfected CNE1 cells after exposed to 6 Gy X-ray ($P<0.01$), while the cell viability was lower ($P<0.05$) and apoptosis was

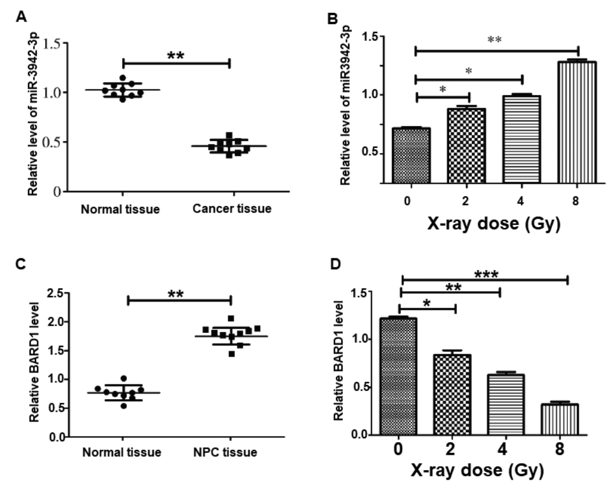

Figure 1. Expression of miR3942-3p and BARD1 in NPC (A, C) and CNE1 cells (B, D) after X-irradiation.
* and ** denote $P<0.01$ and <0.05 *vs*. normal tissue (n = 9) or columns under bars (experiments repeated three times), respectively.

higher ($P<0.05$). The expressions of Bax and Bcl2 were increased or decreased, respectively (Fig. 2).

MiR-3942-3p negatively regulates *BARD1* as a target gene in CNE1 cells

To confirm that BARD1 is a target of miR-3942-3p as predicted using Targetscan, we used luciferase reporter gene assay to investigate their interaction in 293T cells. When miR-3942-3p mimics was co-transfected with

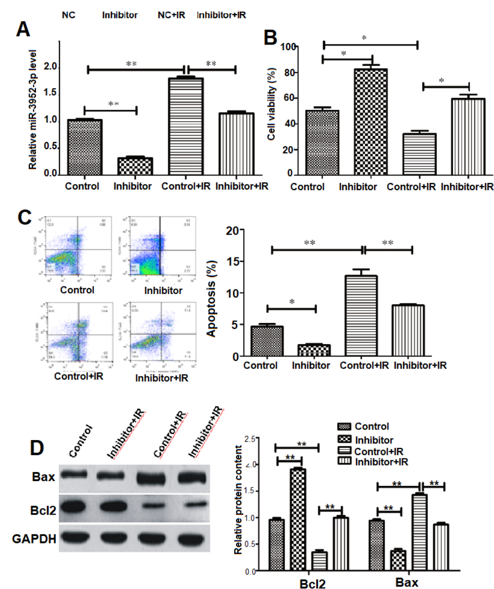

Figure 2. Effect of miR-3942-3p on cell viability, apoptosis and expression of Bax and Bcl2 upon irradiation.
(**A**) miR-3942-3p expression after transfection with miR-3942-3p inhibitor before and after 8 Gy X-irradiation; (**B**) viability of CNE1 cells after transfection with miR-3942-3p inhibitor before and after 8 Gy X-irradiation; (**C**) left panel: flow cytometry results (also see Supplement file Fig. 2-FACS at https://ojs.ptbioch.edu.pl/index.php/abp), right panel: apoptosis rates of CNE1 cells after transfection with miR-3942-3p inhibitor before and after 8 Gy X-irradiation; (**D**) left panel: representative Western blot, right pane: relative expression level of Bax and Bcl2 in CNE1 cells after transfection with miR-3942-3p inhibitor before and after 8 Gy X-irradiation (experiments repeated three times). * and ** denote $P<0.01$ and <0.05 between the columns under the bars, respectively.

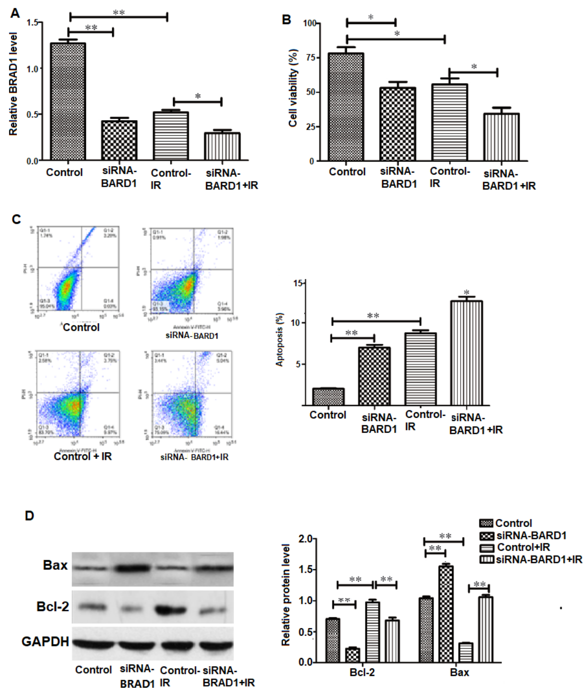

Figure 3. Effect of BARD1on cell viability, apoptosis and expression of Bax and Bcl2 upon irradiation.
(**A**) BARD1 expression after transfection with siRNA before and after 8 Gy X-irradiation; (**B**) viability of CNE1 cells after transfection with siRNA before and after 8 Gy X-irradiation; (**C**) left panel: flow cytometry results (also see Supplement file Fig. 3-FACS at https://ojs.ptbioch.edu.pl/index.php/abp), right panel: apoptosis rates of CNE1 cells after transfection with siRNA before and after 8 Gy X-irradiation; (**D**) left panel: representative Western blot, right pane: relative expression level of Bax and Bcl2 in CNE1 cells after transfection with siRNA before and after 8 Gy X-irradiation (experiments repeated three times). * and ** denote $P<0.01$ and <0.05 between the columns under the bars, respectively.

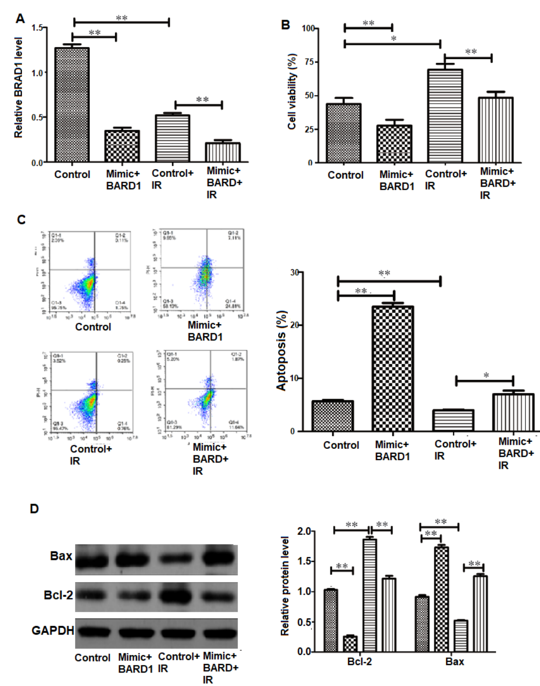

Figure 4. Regulation of miR-3942-3p on BARD1expression, cell viability, apoptosis, Bax and Bcl2 expression upon irradiation.
(**A**) BARD1 expression after co-transfection with miR-3942-3p mimics before and after 8 Gy X-irradiation; (**B**) viability of CNE1 cells after co- transfection with miR-3942-3p mimics before and after 8 Gy X-irradiation; (**C**) left panel: flow cytometry results (also see Supplement file Fig. 4-FACS at https://ojs.ptbioch.edu.pl/index.php/abp), right panel: apoptosis rates of CNE1 cells after co-transfection with miR-3942-3p mimics before and after 8 Gy X-irradiation; (**D**) left panel: representative Western blot, right pane: relative expression level of Bax and Bcl2 in CNE1 cells after co-transfection with miR-3942-3p mimics before and after 8 Gy X-irradiation (experiments repeated three times). * and ** denote $P<0.01$ and <0.05 between the columns under the bars, respectively.

psiCHECK-2-BARD1-wt, the luciferase activity was significantly reduced when compared with co-transfection with miR-3942-3p NC ($P<0.01$) (Supplementary Fig. 1 at https://ojs.ptbioch.edu.pl/index.php/abp). However, such reduction was absent when psiCHECK-2-BARD1-mut was used ($P>0.05$), confirming that BARD1 is a specific target of miR-3942-3p. Furthermore, compared with NC, the cells transfected with miR-3942-3p inhibitor and miR-3942-3p mimics had significantly higher or lower levels of BARD1 ($P<0.01$) (Supplementary Fig. 1, $P<0.01$), indicating that the expression of *BARD1* is negatively regulated by miR-3942-3p.

Expression of *BARD1*

Since *BARD1* was confirmed to a target of miR-3942-3p, we examined its expression in NPC. Compared with normal tissues, the expression of *BARD1* was significantly higher in NPC tissue ($P<0.01$, Fig. 1C). After X-irradiation, the expression level of *BARD1* in CNE1 cells decreased significantly as the dose increased from 0 to 8 Gy ($P<0.05$, <0.01 and <0.001, Fig. 1D) in a dose-dependent manner.

Down-regulation of BARD1 increased radiosensitivity

We then investigated the impact of BARD1 on radiosensitivity of CNE1 cells. For this purpose, the cells were transfected with siRNA-BARD1 to knockdown *BARD1* expression. As shown in Fig. 3, compared with control, siRNA-BARD1 significantly reduced the level of BARD1 ($P<0.01$) and cell viability ($P<0.05$), and significantly increased apoptosis ($P<0.05$). Meanwhile, the levels of Bax and Bcl2 were up- or down-regulated significantly ($P<0.01$), respectively. Similar changes were observed after the cells were irradiated with 8 Gy X-rays (Fig. 3), suggesting that down-regulation of BARD1 increased the radiosensitivity of CNE1 cells.

MiR-3942-3p sensitized the radiosensitivity of CNE1 cells *via* BARD1

We further investigated if miR-3942-3p could impact the radiosensitivity of CNE1 cells *via* BARD1. Compared with co-transformation of control, co-transformation of miR-3942-3p mimic and pcBARD1 significantly reduced the expression of *BARD1* and the viability of CNE1 cells, and significantly increased the apoptosis of CNE1 cells ($P<0.05$ or < 0.01). *Bax* and Bcl2 expressions were up or down-regulated, correspondingly (Fig. 4). Furthermore, upon IR with 8 Gy X-rays, co-transfection of miR-3942-3p mimics and BARD1 expression vector significantly reduced the expression of *BARD1* and cell viability, increased apoptosis with increased *Bax* expression and reduced *Bcl2* expression as compared with co-transformation with miR-3942-3p mimics NC (Fig. 4), suggesting that up-regulation of miR-3942-3p could sensitize CNE1 cells to X-rays *via* BARD1.

DISCUSSION

In the present study, we investigated the role of miR-3942-3p on radiosensitivity of NPC using NPC cell line CNE1. Cell viability, apoptosis and expression of apoptosis-related proteins Bax and Bcl2 were measured after exposing to X-rays and up- and down-regulation of miR-3942-3p using miR-3942-3p inhibitor and mimics. Our results showed up-regulation of miR-3942-3p sensitizes CNE1 cells to X-radiation and this sensitization is likely mediated by BARD1, which is a targeted by miR-3942-3p. These findings could be further explored to improve radiotherapy strategies for NPC.

miRNA has been shown to play important roles in various biological processes by posttranscriptionally regulating signaling molecules, such as growth factors, cytokines and transcription factors. In recent years, many oncogenes and tumor suppressors are found to be regulated by miRNAs during the occurrence and development of cancer (Jiang *et al*., 2020; Zheng *et al*., 2019). They may also modify the radiosensitivity of cancer (Lee *et al*., 2011) and have been explored as an approach to increase radiosensitivity of cancer patients (Zhao *et al*., 2012; Zheng *et al*., 2017b). For example, down-regulation of miR21 was shown to sensitize NPC to X-rays by inhibiting the proliferation of radioresistant NPC cells at the G1 phase of the cell cycle (Zhu *et al*., 2015); miR-33a-5p increases radiosensitivity of melanoma by targeting hypoxia-inducible factor1-alpha (HIF-1α) to inhibit glycolysis (Cao *et al*., 2017); miR-26b increases radiosensitivity of hepatocellular carcinoma by down-regulating the expression of ephrin type-A receptor 2 (Jin *et al*., 2016) and miR153-3p increases radiosensitivity of human glioma by targeting BCL2 (Sun *et al*., 2018). miR-195 enhances the radiosensitivity of colorectal cancer cells by suppressing CARM1(Zheng *et al*., 2017a). Our study showed that miR-3942-3p expression is reduced in NPC tissue and is down-regulated in NPC cells upon radiation. Furthermore, we found that knockdown of miR-3942-3p increases the viability and reduces apoptosis of CNE1 cells upon radiation, suggesting that down-regulation of miR-3942-3p increases the radioresistance of CNE1 cells. It would be interesting to know which part of cell cycle is being affected by the down-regulated miR-3942-3p, leading to the increased viability of CNE1 cells. A deliberation of this cell cycle effect may help use of radiation at the most appropriate cell stage for maximal therapeutic outcomes.

In the study, in addition to cell viability and apoptosis assays, the expression of *Bax* and *Bcl2* was assessed to further deliberate the cellular response at molecular levels. The Bcl2 family proteins are key regulators of apoptosis cell death and Bcl2 is a pro-survival protein that suppresses cell death (Adams & Cory, 1998; Leibowitz & Yu, 2010) and Bax is an pro-apoptotic protein that promotes cell death (Youle & Strasser, 2008). During apoptosis, Bax is often up-regulated and Bcl2 is down-regulated in cancer cells (Naseri *et al*., 2015). It has been shown that a high ratio of Bax to Bcl2 can lead to collapse the potential of mitochondrial membrane, leading to the release of cytochrome c and consequently apoptosis (Boersma *et al*., 1997; Teijido & Dejean, 2010). Previously, radiation was shown to increase the Bcl-xl to Bax protein ratios in human cell sensitive to UVC-radiation (Kita *et al*., 2011) and UV-irradiation increased the expression of *Bax* and increased *Bcl2* expression and apoptotic rates (Jia *et al*., 2012). Similarly, in our study, increased apoptosis was observed to associate with increased *Bax* expression and reduced *Bcl2* expressions, suggesting that Bcl2 family proteins are involved in miR-3942-3p and radiation-related apoptosis.

Since it was unclear how miR-3942-3p modulates the radiosensitivity, we searched potential targets of miRNA using bioinformatic tools and found that BARD1 is a putative target. BARD1 is one of the molecules that are involved in DNA double-stranded break (DSB) repair. When histone H2A variant H2AX is phosphorylated, BARD1 is recruited to repair DSB (Adamovich *et al*., 2019). BARD1 has different functions under different conditions. For example, transcription factor FOXK2 could suppresses the growth of ERα-positive breast cancer cells by destabilizing ERα *via* interacting with involving BRCA1 (Liu *et al*., 2015). Mutation of BARD1 leads to high risk of breast and ovarian cancer. BARD1 is essential for the BRCA1/BARD1 complex to ubiquitylate nucleosomal histone H2A with its RING domains, and for transcriptional regulation of estrogen metabolism genes (Stewart *et al*., 2018). The BRCA1-associated protein BARD1 is conserved as a putative tumor suppressor that may mediate apoptosis because mutation Q564H of BARD1 is defective in apoptosis induction (Irminger-Finger *et al*., 2001). Luciferase reporter assay confirmed that the luciferase activity was specifically reduced when reporter gene with BARD1-wt sequence was co-transfected with miR-3942-3p mimics, but the activity remained unchanged when BARD1-mut sequence was used. Furthermore, RT-qPCR results showed that tBARD1 mRNA level is negatively regulated by miR-3942-3p, suggesting that miR-3942-3p may degrade BARD1 mRNA as observed in other miRNA-mRNA interactions (Rupaimoole & Slack, 2017). The expression of BARD1 was found elevated in NPC compared with normal tissue. This is consistent with earlier results that BARD1 is highly expressed in many cancers such as breast cancer (Chen *et al*., 2019b), lung cancer (Pilyugin *et al*., 2017) and ovarian cancer (Irminger-Finger *et al*., 2001), although its expression in NPC has not been characterized. Upon X-ray irradiation, the expression level of *BARD1* was reduced in a dose-dependent way. Using siRNA approach, we also found that knockdown of *BARD1* results in increased radiosensitivity with reduced cell viability and increased apoptosis. It is well known that radiation may cause various forms of DNA damage, including generation of DSB (Vignard *et al*., 2013), reduced *BARD1* expression might hamper the repair of DNA damage, leading to increased sensitivity to radiation.

Due to the presence of interaction between miR-3942-3p and BARD1, we then investigated if miR-3942-3p would modify the radiosensitivity *via* BARD1. Co-transformation of miR-3942-3p mimic and pcBARD1 reduced the expression level of BARD1, as well as reduced the viability of CNE1 cells and significantly increased the apoptosis of CNE1, suggesting that miR-3942-3p may sensitize CNE1 cells to X-rays *via* down-regulation BARD1. These findings offer new avenue to improve radiotherapy strategies for NPC. For example, miR-3942-3p may be explored as a therapeutic agent to increase the radiosensitivity of NPC patients before radiotherapy for better efficacy. Several strategies have been proposed to deliver miRNA *in vivo* to achieve the therapeutic effect, in which miRNA may be used after chemical modification to enhance stability and efficacy (Li & Rana, 2014; Rezaeian *et al*., 2020). In addition, other targets in miR-3942-3p-related and BARD1-related signaling pathways could be examined as potential targets for the molecular treatment of cancer. Concerning miR-3942-3p down-regulation in other cancers and nor-

mal cells, it needs to be addressed in the future, because the answers to these questions would generalize the findings from this study to other cancers and provide way to protect normal cells.

CONCLUSION

The present study demonstrates that overexpression of miR-3942-3p results in increased radiosensitivity in CNE1 cells. This sensitization is likely mediated *via* BARD1, which is a target of miR-3942-3p and that expression is negatively regulated by miR-3942-3p. The findings could be further explored in animal model and human subjects to develop new radiotherapeutic strategies for NPC.

Declarations

Ethics approval and consent to participate: This study was approved by the ethics committee of People's Hospital of Guangxi Zhuang Autonomous Region, Nanning, China and written informed consent was obtained from every patient.

Consent for publication: N/A.

Availability of data and material: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: none.

Funding: none.

Authors' contributions: LP, JC and HL: project conceptualization, investigation and data analysis. LP, JC, HL, JC, QP, YW and XL: data collection, analysis and methodology development. JC, QP, YW and XL: investigation and methodology development. All authors wrote and approved the final version of the manuscript.

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