Full text open access online (Since 2001)

PRINT: ISSN 0972-3757 ONLINE: ISSN 2456-6330
Int J Hum Genet, 23(1): 75-84 (2023)
DOI: 10.31901/24566322.2023/23.01.845

# Effects of JMJD6 and EYA2 on Modulating Radioresistance of Breast Cancer Cells

**Shuqing Wang[1#], Yufeng Li[2#], Yudong Zhang[3], Dan Li[2], Zhuo Wang[2], Yanrong Zhang[3], Ning Zhang[3] and Jinghua Zhang[4*]**

*[1]Hospital of North China University of Science and Technology, Tangshan, Hebei, 063210, PR China*
*[2]The Cancer Institute, Tangshan People's Hospital, Tangshan, Hebei, 063001, PR China*
*[3]Graduate School of North China University of Science and Technology, Tangshan, Hebei, 063210, PR China*
*[4]Department of Clinical Laboratory, North China University of Science and Technology Affiliated Tangshan Maternal and Child Health Care Hospital, Tangshan, Hebei, 063000, PR China*

**KEYWORDS** Breast Cancer. EYA Transcriptional Coactivator and Phosphatase 2. Jumonji Domain Containing 6. Mediator of DNA Damage Checkpoint 1. Radioresistance

**ABSTRACT** Radiotherapy has been applied for cancer treatment while DNA damage repair (DDR) could increase radioresistance in tumour cells. Therefore, biomarkers that modulated DDR might improve the efficacy of radiotherapy. Mediator of DNA Damage Checkpoint 1 (MDC1) mRNA expressions were assessed by RT-qPCR in MDA-MB-157 cells after irradiation (0, 4, 8, 12, and 16 Gy), showing that it was upregulated in the 4Gy group but suppressed in 16Gy groups. Using CCK-8, viabilities of cells were also inhibited by irradiation dose-dependently. Moreover, flow cytometry was used to evaluate cell apoptosis, showing that overexpression of EYA Transcriptional Coactivator and Phosphatase 2 (EYA2) and Jumonji Domain Containing 6 (JMJD6) suppression both inhibited MDA-MB-157 cell (4Gy) apoptosis and decreased cell viabilities. Hence, JMJD6 and EYA2 might be promising biomarkers for mediating DDR in breast cancer cells.

## INTRODUCTION

Breast cancer, a malignancy that is diagnosed most frequently in women, is the second cause of cancer death among females worldwide (Fahad Ullah 2019). Despite research in the laboratory stage and clinical stage, the incidence of breast cancer is still on the rise. As a heterogeneous disease, breast cancer can be caused by genetic and non-genetic sources (Wagner et al. 2019). Nowadays, therapeutic approaches for treating breast cancer have been improved, in which surgery is a critical method (Kaufman 2019). For high-risk patients, radiotherapy is the first choice after surgery (Haussmann et al. 2020; Shah et al. 2021). Radiation therapy can reduce risks in recurrence and death of breast cancer (Taylor and Kirby 2015). According to previous studies, radiotherapy not only lengthens cancer patients' lives, but also saves economic costs (Atun et al. 2015). Evidence has verified that DNA damage repair (DDR) is a critical process causing tumorigenesis, thereby reducing therapeutic impacts of irradiation on cancers (Jiang et al. 2017). DNA damage can activate biochemical responses against irradiation, in which sensors in DNA damage initiate signalling to trigger DDR (Huang and Zhou 2021). Thus, to figure out roles of those biomarkers that participate in DDR might help improve effects of irradiation.

Mediator of DNA damage checkpoint 1 (MDC1), also named as nuclear factor with BRCT domains 1 (NFBD1), is a scaffolding protein in DDR (Ruff et al. 2020). MDC1 has been detected to magnify phosphorylation of H2A.X (H2A.X variant histone) to form γ-H2A.X, leading to recruitment of MRN complex (MRE11, RAD50 and NBS1) via interactions with γ-H2A.X to facilitate DDR (Lou et al. 2006; Salguero et al. 2019). Previous studies have explored oncogenic effects of MDC1 on cancer development. MDC1 was promoted by liver receptor homolog-1, resulting in increased resistance to cisplatin and Adriamycin by reducing DNA damage (Wang et al. 2018). Additionally, the recruitment of MDC1 was reduced by sulforaphane-upregulated Large Tumour Suppressor Kinase 2, causing elevated sensitivity to radiotherapy in ovarian cancer cells (Wang et al. 2022). MDC1 was downregulated by miRNA-22 in breast cancer cells, causing

*#Authors contribute equally to the study*
**Email address for correspondence:*
*E-mail:*jhzhang_te@163.com

inhibited DDR while upregulated MDC1 protein rescued double-stranded breaks (DSB) repair (Lee et al. 2017). Therefore, biomarkers that interact with MDC1 might be promising targets for improving impacts of irradiation on treating breast cancer.

Jumonji domain-containing protein 6 (JMJD6), a member of Jumonji C domain-containing proteins family, has been found to act as a lysyloxidase and catalyse N-methyl argininyl demethylation (Kwok et al. 2017). High expression of JMJD6 has been associated with increased tumour growth and severe tumour grade (Aprelikova et al. 2016). JMJD6 has been verified to accelerate hydroxylation of p53, thereby suppressing tumour colorectal cancer development (Wang et al. 2014). Moreover, JMJD6 has been discovered to upregulate phosphorylated H2A.X expressions in melanoma cells, implying promoting roles of JMJD6 in DDR (Li et al. 2019). Moreover, JMJD6 was recruited to DNA damage sites and JMJD6 overexpression upregulated γH2A.X after irradiation (Huo et al. 2020). Hence, this study aimed at investigating functions of JMJD6 in modulating DDR in breast cancer cells after irradiation.

Eyes absent transcriptional coactivator and phosphatase 2 (EYA2) is a transcription activator governing organ development (Xu et al. 2019). EYA2 has been detected to dephosphorylate H2A.X after or before irradiation, leading to decreased DDR in colorectal cancer cells (Liu et al. 2016). Despite EYA2 accelerating tumorigenesis in breast cancer cells (Ren et al. 2021), impacts of EYA2 on DDR in breast cancer cells after irradiation were going to be explored in this study.

### Objectives

The present study aimed to investigate MDC1 mRNA expressions in breast cancer cells after irradiation and effects of JMJD6 and EYA2 on modulating breast cancer cell viabilities and apoptosis to figure out their roles in regulating DDR after irradiation.

### Experimental

#### *Main Reagents*

The main reagents used in this study were RPMI-1640 medium (Gibco, USA), penicillin/streptomycin (Gibco), foetal bovine serum (FBS, Gibco), Lipofectamine 3000 (Invitrogen, USA), pcDNA3.1 vector (Invitrogen, USA), Trizol (Beyotime, Shanghai, China), iScript Reverse Transcription (Bio-Rad, USA), SYBR Green qPCR Mix (Beyotime), CCK-8 (Beyotime), and Annexin V-FITC (Beyotime).

## METHODOLOGY

### Cell Culture and Irradiation

MDA-MB-175 cells (breast cancer cell line) were obtained from ATCC (USA). Thereafter, RPMI-1640 medium (Gibco, USA) containing one percent penicillin/streptomycin (Gibco) and ten percent foetal bovine serum (FBS, Gibco) was applied for cell cultivation at $37^0$C and five percent $CO_2$. As for radiotherapy, MDA-MB-157 cells were irradiated by X-ray at 0, 4, 8, 12 and 16 Gy through the Pantak HF320S (Japan).

### Cell Transfection

To figure out the effects of EYA2 and JMJD6 on MDA-MB-157 cells, cell transfection was performed. EYA2 overexpression (oeEYA2) was conducted using pcDNA3.1 vector (Invitrogen, USA) and its control was called oeNC. Small interfering RNA of JMJD6 (siJMJD6) was provided by GenePharma (Shanghai, China) while its control was named siNC. Sequences of siJMJD6 were listed, which was siJMJD6, 5'- GGCAUGUUGUCCUCAAUCUTTAGAUUGAGGACAACAUGCCTT-3'. Meanwhile, MDA-MB-157 cells ($1\times10^5$ cells/well) were cultivated using a six-well plate. After the confluence reached seventy percent, cell transfection was performed to transfect siNC, siJMJD6, oeNC and oeEYA2 into MDA-MB-157 cells using Lipofectamine 3000 (Invitrogen, USA). RAN expressions were examined using RT-qPCR.

### RT-qPCR

To analyse MDC1 mRNA expression after irradiation, total RNA was first segregated from MDA-MB-157 cells using Trizol (Beyotime, Shanghai, China) after X-ray irradiation (0, 4, 8, 12 and 16Gy) for 24 hours followed by cDNA synthesis using iScript Reverse Transcription (Bio-Rad, USA). Thereafter, PCR was performed using SYBR Green qPCR Mix (Beyotime) and CFX Opus 384 RT-PCR

System (Bio-Rad, USA). Primers sequences were listed, which were, MDC1, forward, 5'-AGCAACCCCAGTTGTCATTC-3' and reverse, 5'-AGCGCTGCTGAGACTTCTTC-3' (Wang et al. 2018), EYA2, forward, 5'-CAGCACAAGCCTATGGAATCC-3' and reverse, 5'-GCTGAGGAATCCACTCTGGC-3'; JMJD6, forward, 5'-GCGCAGGAGAAATGGACTCT-3' and reverse, 5'-AGGGTGTTCACCATAGCTGC-3' and GAPDH, forward, 5'-TTTAACTCTGGTAAAGTGGA-3' and reverse, 5'-GAATCATATTGGAACATGTA-3' (Zheng et al. 2019). Conditions of PCR were also listed as pre-denaturation, at $95^0$C for 5 minutes and 35 cycles of $95^0$C for 30 seconds, $60^0$C for 30 seconds and $72^0$C for 30 seconds in denaturation, annealing and extension, respectively. MDC1 mRNA expressions were normalised to GAPDH and calculated the using $2^{-\Delta\Delta Ct}$ method.

### CCK-8

To evaluate viabilities of MDA-MB-157 cells after irradiation and cell transfection, MDA-MB-157 cells were first incubated in a 96-well plate ($510^3$ cells/well) with five percent $CO_2$ at $37^0$C. Thereafter, cells were irradiated by X-ray (0, 4, 8, 12 and 16Gy) for 12 hours, 24 hours, 36 hours, 48 hours and 60 hours. Afterwards, CCK-8 (Beyotime) was added and incubated with cells for another 2 hours. The absorbance of cells was examined using Model 680 Microplate Reader (Bio-Rad, USA) at 450 nm.

### Flow Cytometry

To investigate impacts of JMJD6 and EYA2 on modulating apoptosis of MDA-MB-157 cells, flow cytometry was applied using Annexin V-FITC (Beyotime). First, after transfection, MDA-MB-157 cells ($110^5$) were first resuspended by Annexin V-FITC buffer. Later, 5μl Annexin V-FITC and 10μl propidium iodide (PI) were added and cultured with cells for 15 minutes in darkness. Finally, cell apoptosis was examined using BD Accuri™ C6 Plus flow cytometer (BD Biosciences, USA).

### Statistical Analysis

All data were expressed by mean SD and analysed using SPSS 17.0 (USA). Experiments were all run in a triplicate. Student's t-test was used for differences between two groups. One-way ANOVA was applied for comparing differences between more than two groups. $P<0.05$ was considered to indicate a statistical difference.

## RESULTS

X-ray irradiation suppressed MDA-MB-157 cell viabilities and downregulated MDC1 mRNA expressions.

To verify DNA damage with irradiation, mediator of DNA damage checkpoint protein 1 (MDC1) mRNA expressions in MDA-MB-157 cells were examined, showing that 4Gy of X-ray upregulated MDC1 mRNA expressions while the irradiation of X-ray in 16Gy downregulated MDC1 mRNA expressions (Fig. 1A). Moreover, viabilities of MDA-MB-157 cells were evaluated after irradiation by X-ray (0, 4, 8, 12 and 16Gy), indicating that the cell viabilities were decreased dose-dependently and the difference was not significant in the 12Gy and the 16Gy group (Fig. 1B). Based on these results, MDA-MB-157 cells treated by 4Gy of X-ray were selected for the following experiments.

### EYA2 Overexpression Facilitated MDA-MB-157 Cell Apoptosis

To confirm effects of EYA2 on MDA-MB-157 cells, cell apoptosis rate of MDA-MB-157 cells were examined. Results of flow cytometry indicated that EYA2 overexpression promoted the apoptosis rate of X-ray (4Gy)-irradiated MDA-MB-157 cells (Fig. 2). Hence, EYA2 might promote breast cancer cell apoptosis after irradiation.

### Downregulated JMJD6 Accelerated MDA-MB-157 Apoptosis Rate

Afterwards, impacts of JMJD6 on modulating apoptosis of irradiated MDA-MB-157 cells were evaluated, revealing that suppressed JMJD6 elevated apoptosis rate of MDA-MB-157 cells were irradiated by 4Gy of X-ray (Fig. 3). Therefore, JMJD6 might restrain apoptosis of X-ray-irradiated breast cancer cells.

### EYA2 and JMJD6 Regulated MDA-MB-157 Cell Viabilities

After apoptosis of MDA-MB-157 cells with EYA2 overexpression or JMJD6 suppression was examined, viabilities of MDA-MB-157 cells were

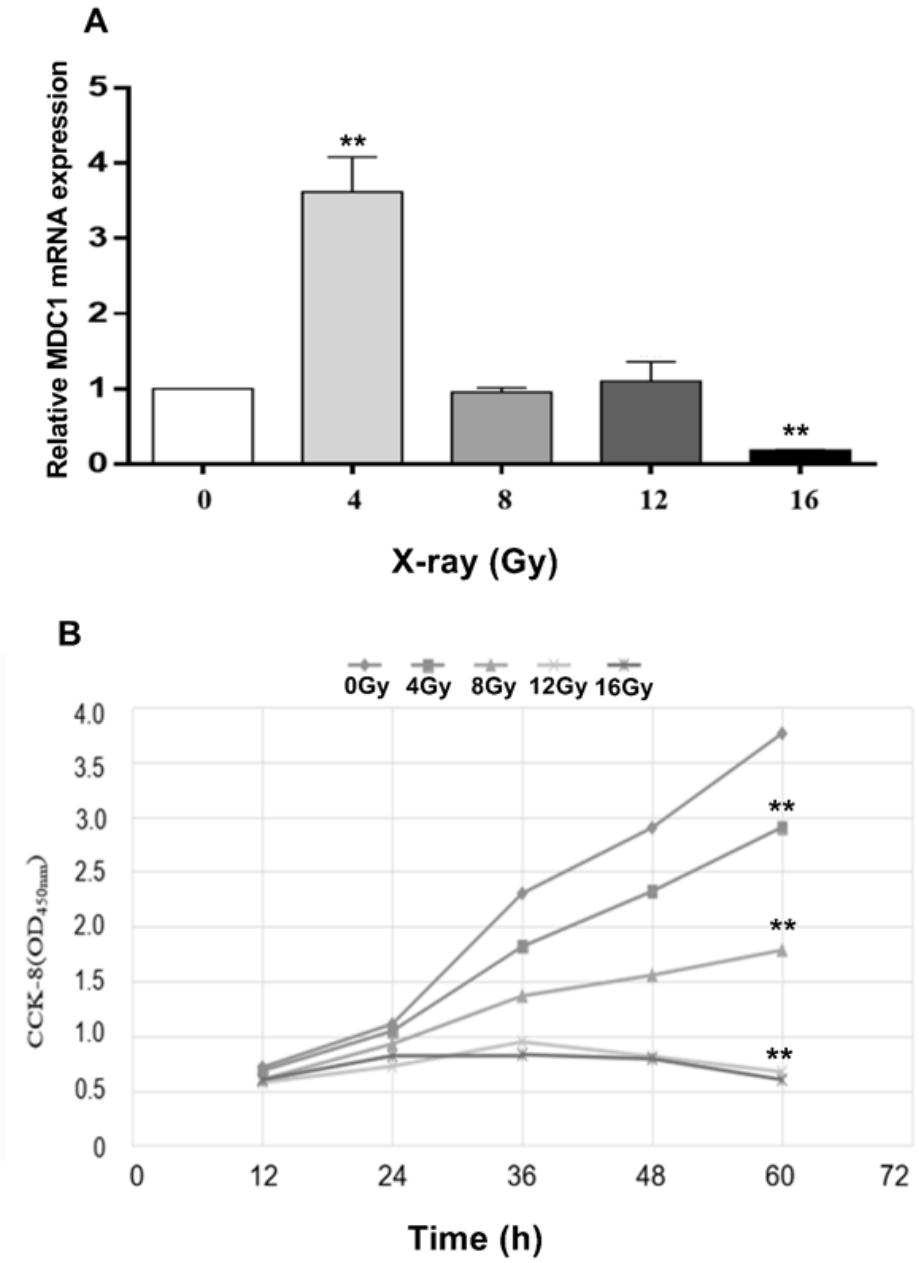


**Fig. 1. X-ray irradiation suppressed MDA-MB-157 cell viabilities and downregulated MDC1 protein expressions. A: MDC1 mRNA expressions in MDA-MB-157 cells with X-ray (0, 4, 8, 12 and 16Gy) were examined suing RT-qPCR, $^{**}P<0.05$. B: MDA-MB-157 cell viabilities were evaluated using CCK-8 after X-ray irradiation (0, 4, 8, 12 and 16Gy), $^{**}P<0.05$**

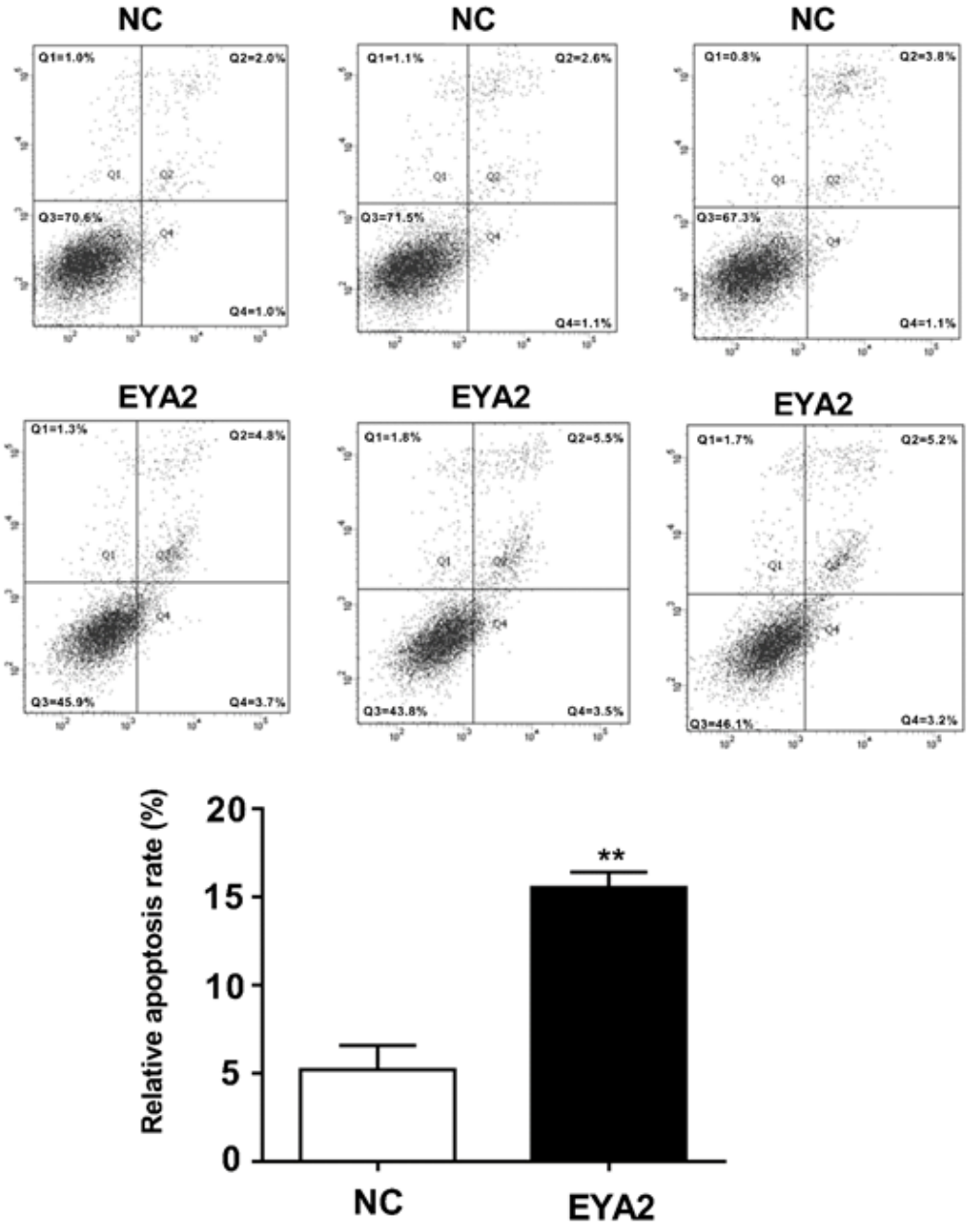


**Fig. 2. EYA2 overexpression facilitated MDA-MB-157 cell apoptosis. Apoptosis rate of X-ray-irradiated MDA-MB-157 cells (4Gy) with NC or overexpressed EYA2 were validated using flow cytometry, $^{**}P<0.05$**

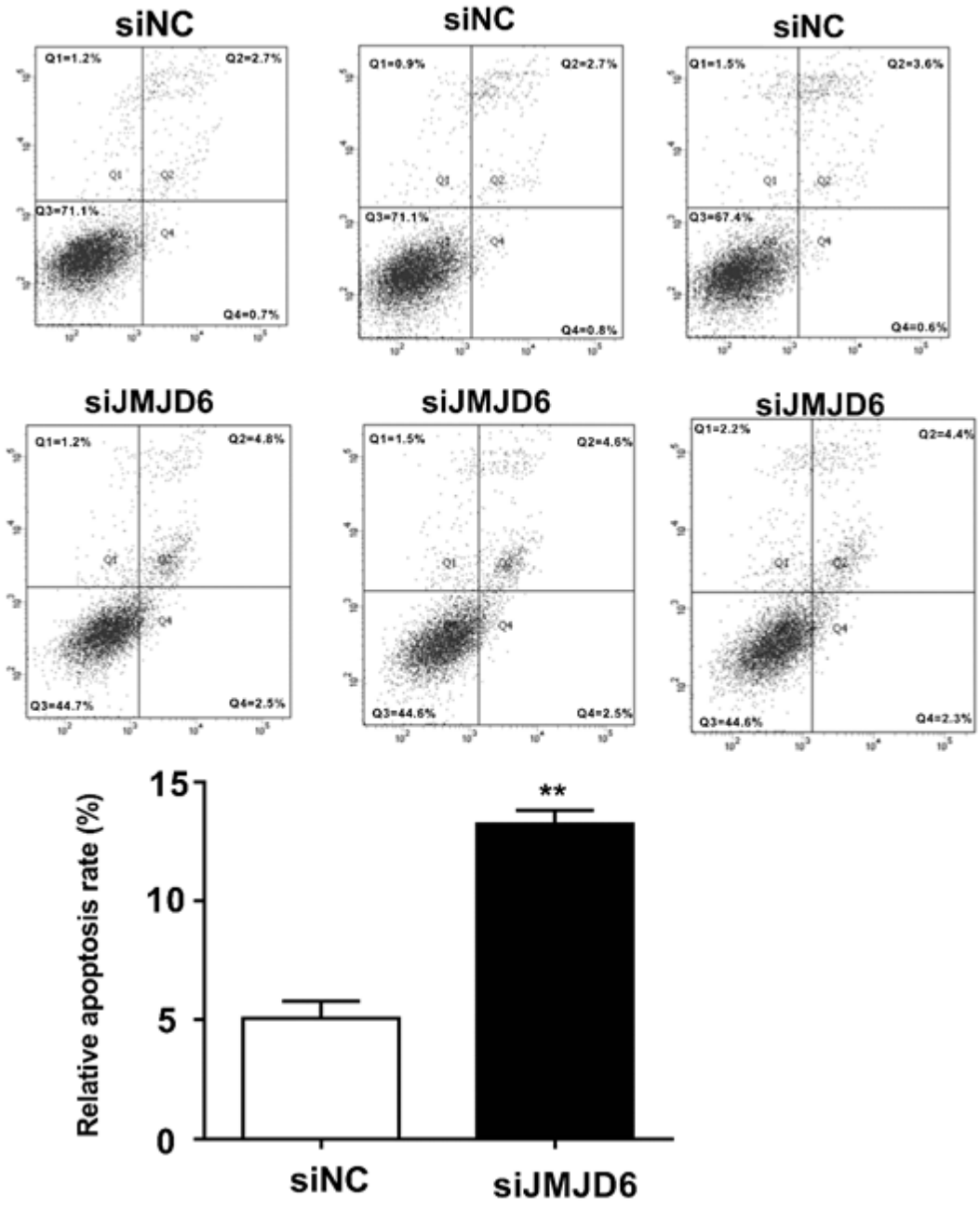


**Fig. 3. Downregulated JMJD6 accelerated MDA-MB-157 apoptosis rate. Apoptosis rate of X-ray-irradiated MDA-MB-157 cells (4Gy) with siNC or suppressed JMJD6 were validated using flow cytometry, $^{**}P<0.05$**

analysed. CCK-8 results indicated that EYA2 overexpression and JMJD6 downregulation could both restrain MDA-MB-157 cell viabilities with the irradiation of 4Gy of X-ray (Fig. 4A, B). Herein, JMJD6 and EYA2 might act oppositely in breast cancer cells.

## DISCUSSION

Dysregulated DNA damage repair (DDR) has been verified to restrain efficacy of DNA damaging anticancer therapies due to promoted DDR-caused elevated resistance to chemotherapy or radiotherapy (Broustas and Lieberman 2014). Hence, targeting on DDR inhibition might be a promising approach to increase sensitivity of cancer cells to these treatments. Chen et al. (2020) have reported that leucine-rich repeat-containing protein 31 elevated radiosensitivity in breast cancer brain metastasis via suppressing DDR. Moreover, epithelial membrane protein 3 restrained DDR in breast cancer cells, causing elevated sensitivity to Adriamycin, a DNA-damaging medicine (Zhou et al. 2021). To amplify the efficacy of radiotherapy, it is necessary to discover new biomarkers that are related to DDR in breast cancer. In this study, re-

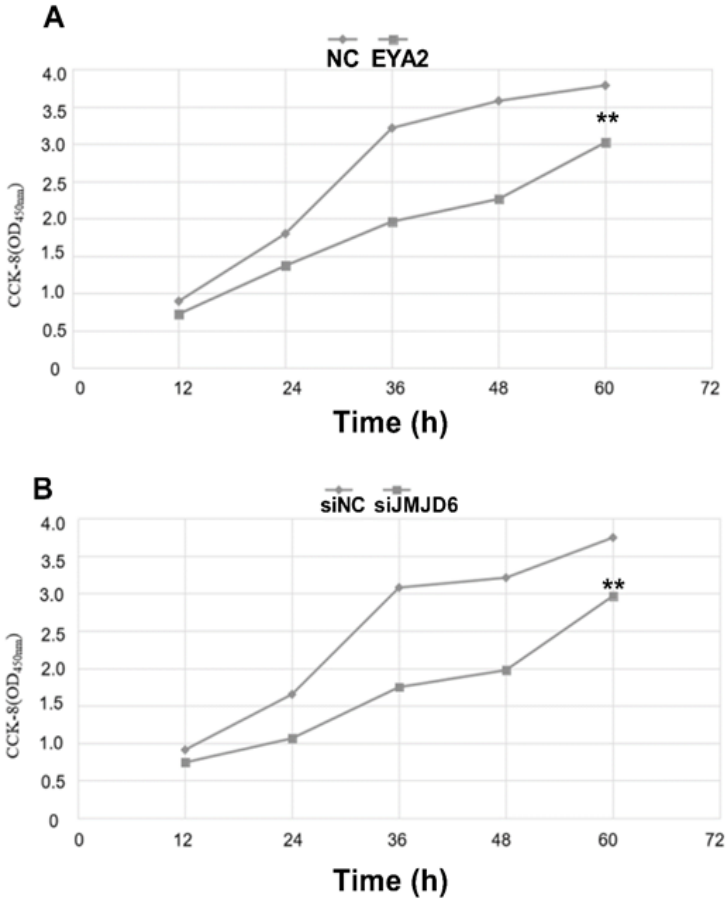


**Fig. 4. EYA2 and JMJD6 regulated MDA-MB-157 cell viabilities. A: X-ray-irradiated (4Gy) MDA-MB-157 cell viabilities with NC or overexpressed EYA2 were detected by CCK-8, $^{**}P<0.05$. B: X-ray-irradiated (4Gy) MDA-MB-157 cell viabilities with siNC or JMJD6 suppression were detected by CCK-8, $^{**}P<0.05$**

searchers have demonstrated that JMJD6 restrained irradiation-induced breast cancer cell apoptosis and promoted cell viabilities while EYA2 facilitated breast cancer cell apoptosis after irradiation but suppressed cell viability.

Radioresistance has been defined as reduced therapeutic effects of irradiation on treating cancer (Steinbichler et al. 2019) and become the main obstacle in tumour treatment. Radioresistance can damage tumour-adjacent normal tissues, accelerate tumour recurrence, and cause poor prognosis and bad treatment response (Huang and Zhou 2020). Suppressing radiotherapy resistance has been verified to be a promising approach to improve impacts of irradiation on cancer treatment. Linc00312 has been found to bind DNA-PKcs, resulting in inhibited DDR and increased radiosensitivity in nasopharyngeal carcinoma cells (Guo et al. 2021). In this study, researchers have examined MDC1 mRNA expressions to analyse DDR after irradiation. According to results of RT-qPCR, 4Gy of X-ray upregulated MDC1 mRNA expression while 8, 12 and 16Gy of X-ray reduced MDC1 mRNA expressions. Based on the detections, DDR of MDA-MB-157 cells was accelerated in 4Gy of X-ray group.

According to previous evidence, EYA2 has been reported to be upregulated in breast cancer cells and accelerate cell proliferation and tumorigenesis (Liang et al. 2017; Xu et al. 2019). Nevertheless, Liu et al. (2016) have reported suppressive effects of EYA2 on DDR and proliferation of breast cancer cells via dephosphorylating H2A.X Tyr39. Moreover, in this study, EYA2 overexpression has been detected to accelerate MDA-MB-157 cell apoptosis after irradiation and suppress cell viabilities. Therefore, EYA2 might act as a cancer suppressor via restraining DDR in breast cancer cells.

JMJD6 has been reported to play oncogenic impacts on breast cancer development. JMJD6 has been demonstrated to be co-expressed with Enhancer of Zeste homolog 2, inducing upregulation of DNA replication and repair genes and causing shorter survival of breast cancer patients (Biswas et al. 2020). In the study of Liu et al. (2019) JMJD6 facilitated phosphorylation of Y39 of histone H2A.X, leading to autophagy in triple negative breast cancer. Beyond that, researchers also examined the roles of JMJD6 in MDA-MB-157 cells, showing that JMJD6 suppression had the same effects as overexpressed EYA2. Hence, JMJD6 might accelerate breast cancer cell progression via accelerating DDR.

## CONCLUSION

X-ray irradiation suppressed MDA-MB-157 cell while MDC1 mRNA expressions were first upregulated in low dose of X-ray (4Gy) and suppressed in high dose of X-ray (16 Gy). Additionally, EYA2 inhibited X-ray-irradiated MDA-MB-157 cell viabilities and accelerated the apoptosis while JMJD6 facilitated X-ray-irradiated MDA-MB-157 cell viabilities but restrained cell apoptosis. Hence, EYA2 and JMJD6 might be promising biomarkers for regulating DNA damage repair of breast cancer cells.

## RECOMMENDATIONS

MDC1 mRNA expressions were first upregulated in 4Gy of X-ray irradiation but suppressed in the 16 Gy of X-ray. Moreover, irradiated MDA-MB-157 cell viabilities were decreased with irradiation dose-dependently. Thereafter, EYA2 overexpression and JMJD6 downregulation both facilitated irradiated MDA-MB-157 cell apoptosis and restrained cell viabilities. Herein, EYA2 and JMJD6 might be underlying mRNAs to increase effects of radiotherapy on breast cancer treatment by modulating DDR. However, deep detections about mechanisms of EYA2 and JMJD6 in DDR needs to be investigated.

## FUNDING

This work was supported by the Natural Science Foundation of Hebei Province (grant number H2019105034); the Program for Innovation Research Team (in Science and Technology) of Tangshan (20130202D), Hebei Key Laboratory of Molecular Oncology (grant no. SZX2020043) and the National Natural Science Foundation of China (grant number 82173065).

## AUTHOR CONTRIBUTION

Shuqing Wang and Yufeng Li contribute to the article equally as the first author.

## ABBREVIATION LIST

JMJD6: Jumonji Domain Containing 6
EYA2: EYA Transcriptional Coactivator and Phosphatase 2

MDC1: Mediator of DNA Damage Checkpoint 1
DDR: DNA Damage Repair
RT-qPCR: Real Time Quantitative Polymerase Chain Reaction
DSB: DNA Double-strand
NFBD1: Nuclear Factor with BRCT Domains 1
H2A.X: H2A Histone Family Member X
FBS: Foetal Bovine Serum

## REFERENCES


Aprelikova O, Chen K, El Touny LH et al. 2016. The epigenetic modifier jmjd6 is amplified in mammary tumors and cooperates with c-myc to enhance cellular transformation, tumor progression, and metastasis. *Clin Epigenetics*, 8: 38. doi: 10.1186/s13148-016-0205-6.

Atun R, Jaffray DA, Barton MB et al. 2015. Expanding global access to radiotherapy. *Lancet Oncol*, 16(10): 1153-1186. doi: 10.1016/S1470-2045(15)00222-3.

Biswas A, Mukherjee G, Kondaiah P, Desai KV 2020. Both ezh2 and jmjd6 regulate cell cycle genes in breast cancer. *BMC Cancer*, 20(1): 1159. doi: 10.1186/s12885-020-07531-8.

Broustas CG, Lieberman HB 2014. DNA damage response genes and the development of cancer metastasis. *Radiat Res*, 181(2): 111-130. doi: 10.1667/RR13515.1.

Chen Y, Jiang T, Zhang H, Gou X, Han C et al. 2020. Lrrc31 inhibits DNA repair and sensitizes breast cancer brain metastasis to radiation therapy. *Nat Cell Biol*, 22(10): 1276-1285. doi: 10.1038/s41556-020-00586-6.

Fahad Ullah M 2019. Breast cancer: Current perspectives on the disease status. *Adv Exp Med Biol*, 1152: 51-64. doi: 10.1007/978-3-030-20301-6_4.

Guo Z, Wang YH, Xu H, Yuan CS et al. 2021. Lncrna linc00312 suppresses radiotherapy resistance by targeting DNA-pkcs and impairing DNA damage repair in nasopharyngeal carcinoma. *Cell Death Dis*, 12(1): 69. doi: 10.1038/s41419-020-03302-2.

Haussmann J, Corradini S, Nestle-Kraemling C et al. 2020. Recent advances in radiotherapy of breast cancer. *Radiat Oncol*, 15(1): 71. doi: 10.1186/s13014-020-01501-x.

Huang R, Zhou PK 2021. DNA damage repair: Historical perspectives, mechanistic pathways and clinical translation for targeted cancer therapy. *Signal Transduct Target Ther*, 6(1): 254. doi: 10.1038/s41392-021-00648-7.

Huang RX, Zhou PK 2020. DNA damage response signaling pathways and targets for radiotherapy sensitization in cancer. *Signal Transduct Target Ther*, 5(1): 60. doi: 10.1038/s41392-020-0150-x.

Huo D, Chen H, Cheng Y, Song X et al. 2020. Jmjd6 modulates DNA damage response through downregulating h4k16ac independently of its enzymatic activity. *Cell Death Differ*, 27(3): 1052-1066. doi: 10.1038/s41418-019-0397-3.

Jiang Y, Liu Y, Hu H 2017. Studies on DNA damage repair and precision radiotherapy for breast cancer. *Adv Exp Med Biol*, 1026: 105-123. doi: 10.1007/978-981-10-6020-5_5.

Kaufman CS 2019. Increasing role of oncoplastic surgery for breast cancer. *Curr Oncol Rep*, 21(12): 111. doi: 10.1007/s11912-019-0860-9.

Kwok J, O'shea M, Hume DA, Lengeling A 2017. Jmjd6, a jmjc dioxygenase with many interaction partners and pleiotropic functions. *Front Genet*, 8: 32. doi: 10.3389/fgene.2017.00032.

Lee JH, Park SJ, Kim SW et al. 2017. C-fos-dependent mir-22 targets mdc1 and regulates DNA repair in terminally differentiated cells. *Oncotarget*, 8(29): 48204-48221. doi: 10.18632/oncotarget.18389.

Li Y, Yu P, Zou Y, Cai W, Sun W, Han N 2019. Kras-erk signalling promotes the onset and maintenance of uveal melanoma through regulating jmjd6-mediated h2a.X phosphorylation at tyrosine 39. *Artif Cells Nanomed Biotechnol*, 47(1): 4257-4265. doi: 10.1080/21691401.2019.1673764.

Liang Y, Xu X, Wang T, Li Y et al. 2017. The egfr/mir-338-3p/eya2 axis controls breast tumor growth and lung metastasis. *Cell Death Dis*, 8(7): 1-12. doi: 10.1038/cddis.2017.325.

Liu Y, Long YH, Wang SQ, Li YF, Zhang JH 2016. Phosphorylation of h2a.X(t)(yr39) positively regulates DNA damage response and is linked to cancer progression. *Febs J*, 283(24): 4462-4473. doi: 10.1111/febs.13951.

Liu Y, Long YH, Wang SQ, Zhang YY et al. 2019. Jmjd6 regulates histone h2a.X phosphorylation and promotes autophagy in triple-negative breast cancer cells via a novel tyrosine kinase activity. *Oncogene*, 38(7): 980-997. doi: 10.1038/s41388-018-0466-y.

Lou Z, Minter-Dykhouse K, Franco S, Gostissa M et al. 2006. Mdc1 maintains genomic stability by participating in the amplification of atm-dependent DNA damage signals. *Mol Cell*, 21(2): 187-200. doi: 10.1016/j.molcel.2005.11.025.

Ren L, Guo D, Wan X, Qu R 2021. Eya2 upregulates mir-93 to promote tumorigenesis of breast cancer by targeting and inhibiting the sting signaling pathway. *Carcinogenesis*, 1-30. doi: 10.1093/carcin/bgab001.

Ruff SE, Logan SK, Garabedian MJ, Huang TT 2020. Roles for mdc1 in cancer development and treatment. *DNA Repair (Amst)*, 95: 102948. doi: 10.1016/j.dnarep.2020.102948.

Salguero I, Belotserkovskaya R, Coates J, Sczaniecka-Clift M et al. 2019. Mdc1 pst-repeat region promotes histone h2ax-independent chromatin association and DNA damage tolerance. *Nat Commun*, 10(1): 5191. doi: 10.1038/s41467-019-12929-5.

Shah C, Al-Hilli Z, Vicini F 2021. Advances in breast cancer radiotherapy: Implications for current and future practice. *JCO Oncol Pract*, 17(12): 697-706. doi: 10.1200/OP.21.00635.

Steinbichler TB, Dudás J, Skvortsov S, Ganswindt U, Riechelmann H, Skvortsova II 2019. Therapy resistance mediated by exosomes. *Mol Cancer*, 18(1): 58. doi: 10.1186/s12943-019-0970-x.

Taylor CW, Kirby AM 2015. Cardiac side-effects from breast cancer radiotherapy. *Clin Oncol (R Coll Radiol)*, 27(11): 621-629. doi: 10.1016/j.clon.2015 .06.007.

Wagner J, Rapsomaniki MA, Chevrier S, Anzeneder T et al. 2019. A single-cell atlas of the tumor and immune ecosystem of human breast cancer. *Cell*, 177(5): 1330-1345. doi: 10.1016/j.cell.2019.03.005.

Wang F, He L, Huangyang P, Liang J et al. 2014. Jmjd6 promotes colon carcinogenesis through negative regulation of p53 by hydroxylation. *PLoS Biol*, 12(3): 1-18. doi: 10.1371/journal.pbio.1001819.

Wang S, Zou Z, Luo X, Mi Y, Chang H, Xing D 2018. Lrh1 enhances cell resistance to chemotherapy by transcriptionally activating mdc1 expression and attenuating DNA damage in human breast cancer. *Oncogene*, 37(24): 3243-3259. doi: 10.1038/s41388-018-0193-4.

Wang S, Wang Y, Liu X, Yang Y, Wu S, Liu Y 2022. Sfn enhanced the radiosensitivity of cervical cancer cells via activating lats2 and blocking rad51/mdc1 recruitment to DNA damage site. *Cancers (Basel)*, 14(8): 1872. doi: 10.3390/cancers14081872.

Xu H, Jiao Y, Yi M, Zhao W, Wu K 2019. Eya2 correlates with clinico-pathological features of breast cancer, promotes tumor proliferation, and predicts poor survival. *Front Oncol*, 9: 26. doi: 10.3389/fonc.2019.00026.

Zheng H, Tie Y, Fang Z, Wu X et al. 2019. Jumonji domain-containing 6 (jmjd6) identified as a potential therapeutic target in ovarian cancer. *Signal Transduct Target Ther*, 4: 24. doi: 10.1038/s41392-019-0055-8.

Zhou K, Sun Y, Dong D, Zhao C, Wang W 2021. Emp3 negatively modulates breast cancer cell DNA replication, DNA damage repair, and stem-like properties. *Cell Death Dis*, 12(9): 844. doi: 10.1038/s41419-021-04140-6

**Paper received for publication in June, 2022**
**Paper accepted for publication in September, 2022**