CANCER BIOLOGY & THERAPY
2023, VOL. 24, NO. 1, 2287128
https://doi.org/10.1080/15384047.2023.2287128

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RESEARCH PAPER

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Aldolase A promotes cervical cancer cell radioresistance by regulating the glycolysis and DNA damage after irradiation

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ABSTRACT

Radioresistance is the major obstacle that affects the efficacy of radiotherapy which is an important treatment for cervical cancer. By analyzing the databases, we found that aldolase A (ALDOA), which is a key enzyme in metabolic reprogramming, has a higher expression in cervical cancer patients and is associated with poor prognosis. We detected the expression of ALDOA in the constructed cervical cancer radioresistance (RR) cells by repetitive irradiation and found that it was upregulated compared to the control cells. Functional assays were conducted and the results showed that the knockdown of ALDOA in cervical cancer RR cells inhibited the proliferation, migration, and clonogenic abilities by regulating the cell glycolysis. In addition, downregulation of ALDOA enhanced radiation-induced apoptosis and DNA damage by causing G2/M phase arrest and further promoted radiosensitivity of cervical cancer cells. The functions of ALDOA in regulating tumor radiosensitivity were also verified by the mouse tumor transplantation model *in vivo*. Therefore, our study provides new insights into the functions of ALDOA in regulating the efficacy of radiotherapy and indicates that ALDOA might be a promising target for enhancing radiosensitivity in treating cervical cancer patients.

ARTICLE HISTORY
Received 16 September 2023
Revised 19 November 2023
Accepted 20 November 2023

KEYWORDS
ALDOA; cervical cancer; radiotherapy; radioresistance; glycolysis; DNA damage

Introduction

As the fourth most common female malignancy, cervical cancer (CC) threatens women's health especially in developing countries with high mortality rate.[1,2] It is estimated that approximately 604,127 cases of CC and 341,831 deaths from CC occurs worldwide in 2020.[3] The major treatment for CC includes surgery, radiotherapy (RT), chemotherapy, and immunotherapy,[4–6] among which RT is an important treatment for advanced CC.[7,8] However, radioresistance (RR) is a major issue that leads to failure of treatment and poor prognosis of patients with advanced CC.[9–11] Therefore, exploring the mechanism of RR is critical to promote the sensitivity of RT and obtain better therapeutic effects.

The mechanism of RR in tumor cells is very complex, and it is currently believed that the high DNA repair ability of tumor cells, the strong self-healing ability of cancer stem cells and the alteration of the tumor microenvironment are important reasons for RR.[12] RT causes DNA double-strand breaks.[13] However, the high DNA repair capacity of tumor cells often leads to RR, such as Poly (ADP-ribose) polymerase – 1 (PARP1), which is involved in single-stranded DNA repair, leading to diminished radiosensitivity in CC.[14] Meanwhile, activation of the Warburg effect in tumor cells implies enhanced metabolism,[15] and metabolic reprogramming also plays an important role in the development of RR.[16] It has been demonstrated that AKT-mediated alterations in the glucose metabolism pathway cause liver cancer cells and CC cells to develop acquired RR.[17] Abnormal activation of enzymes in the glycolytic metabolic pathway leads to lactate accumulation, which is also critical for the development of RR.[12]

Aldolase family members are catalytic enzymes in the fourth step of glycolysis that convert fructose 1, 6-diphosphate to glyceraldehyde-3-phosphate (GAP) and dihydroxyacetone phosphate (DHAP).[18,19] The three subtypes in this family are ALDOA, ALDOB, and ALDOC.[20] ALDOA is highly expressed in a variety of cancers, such as lung cancer, kidney cancer, colorectal cancer, and pancreatic cancer, which is closely related to the development of cancer.[20–23] It has been reported that high expression of ALDOA can enhance the resistance of colorectal cancer cells to RT.[24] In our previous study, ALDOA was found to be upregulated in prostate cancer RR cell lines and inhibition of ALDOA enhanced the radiosensitivity of prostate cancer RR cells.[25] However, whether ALDOA plays a role in regulating radiosensitivity in CC has not been defined yet. In this study, we also found that ALDOA was highly

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/15384047.2023.2287128

expressed in CC patients by bioinformatics analysis. The expression of ALDOA was also detected in our constructed CC RR cell lines by repetitive radiation, which showed higher expression compared to those control cells. Thus, functions and the potential mechanisms of how ALDOA regulates the radiosensitivity of CC were investigated in this study.

Here, we first established the correlation between ALDOA and CC prognosis by analyzing the TCGA databases. We established two pairs of CC RR cell lines and examined the expression of ALDOA. The malignant biological behavior, glycolytic changes and radiosensitivity were further detected in our constructed cells. We also verified the regulatory functions of ALDOA on radiosensitivity in the mouse tumor model. Taken together, our results revealed that the high expression of ALDOA in CC RR cells could promote cell proliferation, migration, and anti-apoptosis. The potential mechanism that ALDOA contributes to RR is via the regulation of glycolysis. Therefore, targeting ALDOA may be a promising strategy to increase radiosensitivity in CC treatment.

Results

Expression of ALDOA in CC is associated with the prognosis

To investigate the clinical significance of ALDOA in CC, we searched and analyzed the TCGA database to compare the differential expression of ALDOA in CC and found that the expression of ALDOA in CC patients was significantly upregulated compared with that in normal controls (Figure 1a). Further analysis showed that the overall survival rate of CC patients with higher expression of ALDOA was poorer (Figure 1b). We further used the ROC curve to evaluate the diagnostic value of ALDOA in CC and the AUC was 0.862 (Figure 1c), suggesting that the prediction effect is very good. The results showed a strong correlation between the expression of ALDOA and the prognosis of CC. We established SiHa-RR and C33A-RR cells as described in the method part for *in vitro* analysis. Clonogenic experiments were used to confirm the successful construction of RR cells. The survival rates of SiHa-RR and C33A-RR cells were higher at 2, 4, and 6 Gy compared to the control non-irradiated CC cells (Figure 1d), suggesting higher RR in constructed irradiated cells. The expression of ALDOA was detected by qRT-PCR and western blot analysis in the constructed cells. The expression of ALDOA in SiHa-RR and C33A-RR cells is higher compared to SiHa and C33A cells (Figure 1e, 1f). These results indicate that ALDOA is overexpressed in CC RR cells.

ALDOA knockdown affected biological activities of CC RR cells

To explore the functions of ALDOA in CC RR cells, we constructed ALDOA knockdown cell models in SiHa-RR and C33A-RR cells with two different shRNA1 or shRNA2. qRT-PCR and western blot analysis were conducted to confirm the knockdown of ALDOA in SiHa-RR and C33A-RR cells (Figures 2a, 2b). The effect of ALDOA on cell colony formation was detected in the transfected cells. Compared with the control group, the colony formation ability of ALDOA knockdown groups was significantly reduced (Figure 2c). CCK-8 was used to evaluate the proliferation ability of ALDOA in CC cells. We found that knockdown of ALDOA significantly inhibited cell proliferation (Figure 2d). In addition, we tested the cell migration by transwell assay and found that the migration ability of SiHa-RR and C33A-RR ALDOA knockdown cells was significantly reduced (Figure 2e, 2f). In conclusion, the knockdown of ALDOA in SiHa-RR and C33A-RR cells inhibits clone formation, cell proliferation, and migration.

ALDOA knockdown promotes cells apoptosis and aggravates DNA damage induced by RT

To verify the role of ALDOA in the radiosensitivity of CC, we evaluated the effect of knockdown ALDOA on CC RR cells by flow cytometry. Compared with the control group, ALDOA knockdown increased the number of apoptotic cells, and the apoptotic rate of the ALDOA knockdown group after RT further increased (Figures 3a, 3b). The percentage of the G2/M phase of CC cells in the ALDOA knockdown group increased. After irradiation, the G2/M phase arrest in the ALDOA knockdown group was further enhanced (Figures 3c, 3d). Irradiation destroys the molecular structure of cells and directly causes DNA damage. γ-H2AX reflects the degree of DNA damage. To detect the mechanism of ALDOA's influence on cells under irradiation, we measured the number of γ-H2AX foci in the stably transformed cell line 24 hours after irradiation by immunofluorescence. Compared with the control group, the formation of γ-H2AX foci in the ALDOA knockdown group increased. After irradiation, the content of γ-H2AX in the ALDOA knockdown group further increased (Figures 3e, 3f). These data indicate that targeting ALDOA can promote irradiation-induced apoptosis, cell G2/M phase arrest, and aggravate radiation-induced DNA damage.

ALDOA knockdown increases the radiosensitivity of CC RR cells in mouse xenotransplantation model

To evaluate the effect of ALDOA on the radiosensitivity of CC *in vivo*, we constructed tumor transplantation in mice. BALB/c nude mice were subcutaneously injected with stably transfected SiHa-RR cells. According to the data, compared with mice injected with SiHa-RR shRNA NC cells, mice injected with SiHa-RR shRNA ALDOA cells had slower xenograft tissue growth and smaller tumor volume and weight (Figure 4a-c). ALDOA knockdown combined with RT further inhibited tumor growth, and the tumor growth rate and volume were significantly lower than those in the control group (Figure 4a-c). We performed immunohistochemical staining on the obtained tumor tissues, and the results showed that the protein representing proliferation of irradiated tumors was significantly reduced, while the protein representing DNA damage was significantly increased (Figures 4d, 4e). These results indicate that ALDOA knockdown can significantly inhibit tumor cell proliferation and improve radiosensitivity.

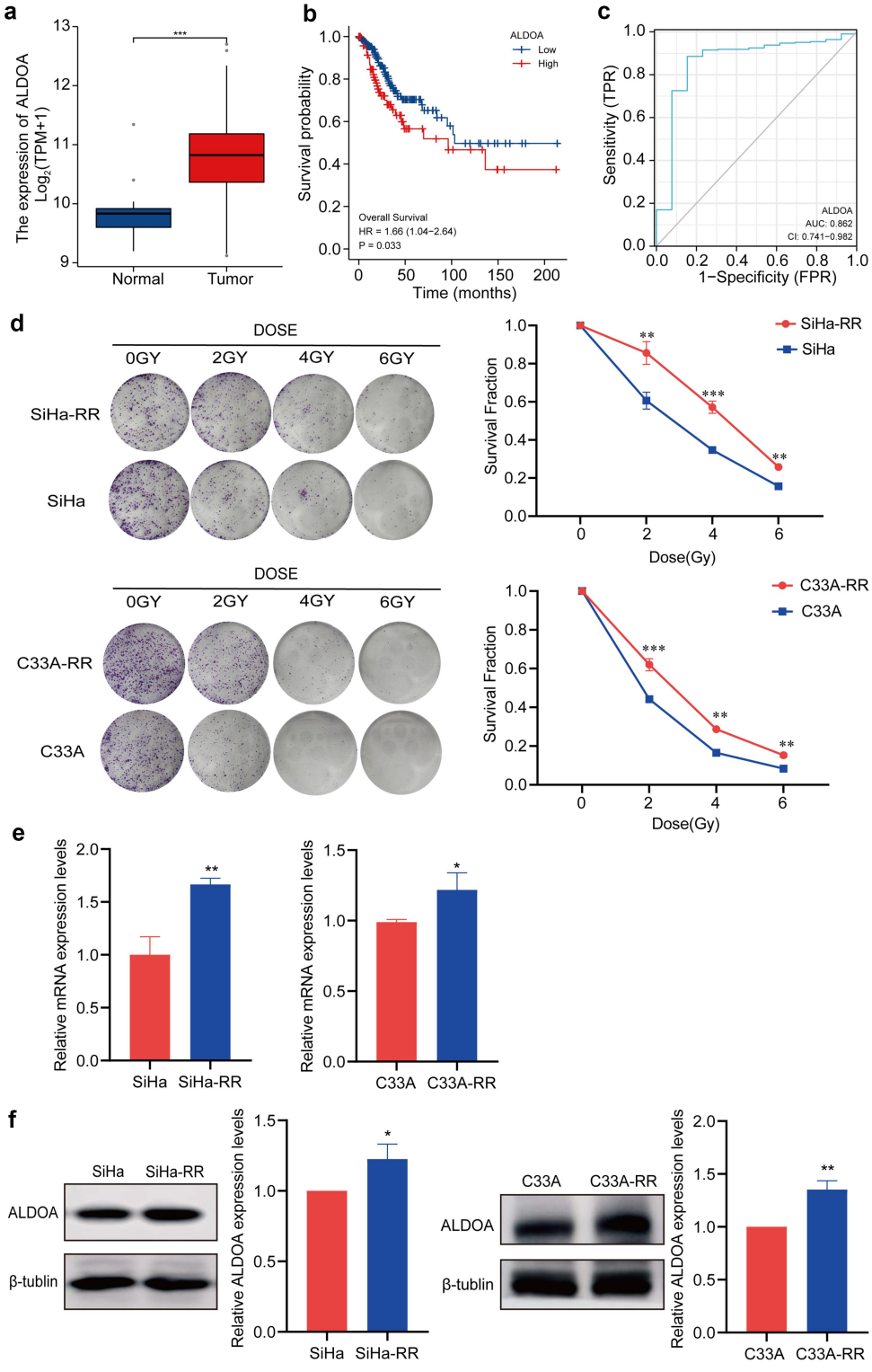

Figure 1. ALDOA is overexpressed in CC RR cell lines and correlated with prognosis. (a) By analyzing the UCSC XENA database (https://xenabrowser.Net/datapages/), ALDOA expression was significantly increased in CC tissues compared with normal cervical tissues (***$P < .001$). (b) According to the TCGA database (https://portal.Gdc.cancer.gov/), CC patients with higher ALDOA expression have a lower overall survival rate (*$P < .05$). (c) According to the AUC curve of the UCSC XENA database (https://xenabrowser.Net/datapages/), ALDOA can be used as a better diagnostic standard for CC (AUC = 86.20% with 95% CI = 74.1–98.2%). (d) Colony formation assay showed that the CC RR cells (SiHa-RR and C33A-RR) had stronger resistance to RT. (e) The expression of ALDOA in CC cell lines SiHa, SiHa-RR, C33A and C33A-RR was measured by qRT-PCR. (f) The protein expression level of ALDOA in SiHa, SiHa-RR, C33A and C33A-RR was detected by western blot. *$P < .05$, **$P < .01$, ***$P < .001$.

ALDOA promotes glycolysis in CC RR cells

Targeting tumor glucose metabolism alters the tumor microenvironment and modulates the radiosensitivity of solid tumors.[26] It is well known that cancer cells mainly obtain energy through the glycolytic pathway, and several glycolysis-related proteins are involved in the regulation of cancer cells' RR.[17,27] Since ALDOA is a key enzyme in the glucose metabolic pathway, we wondered whether ALDOA affected

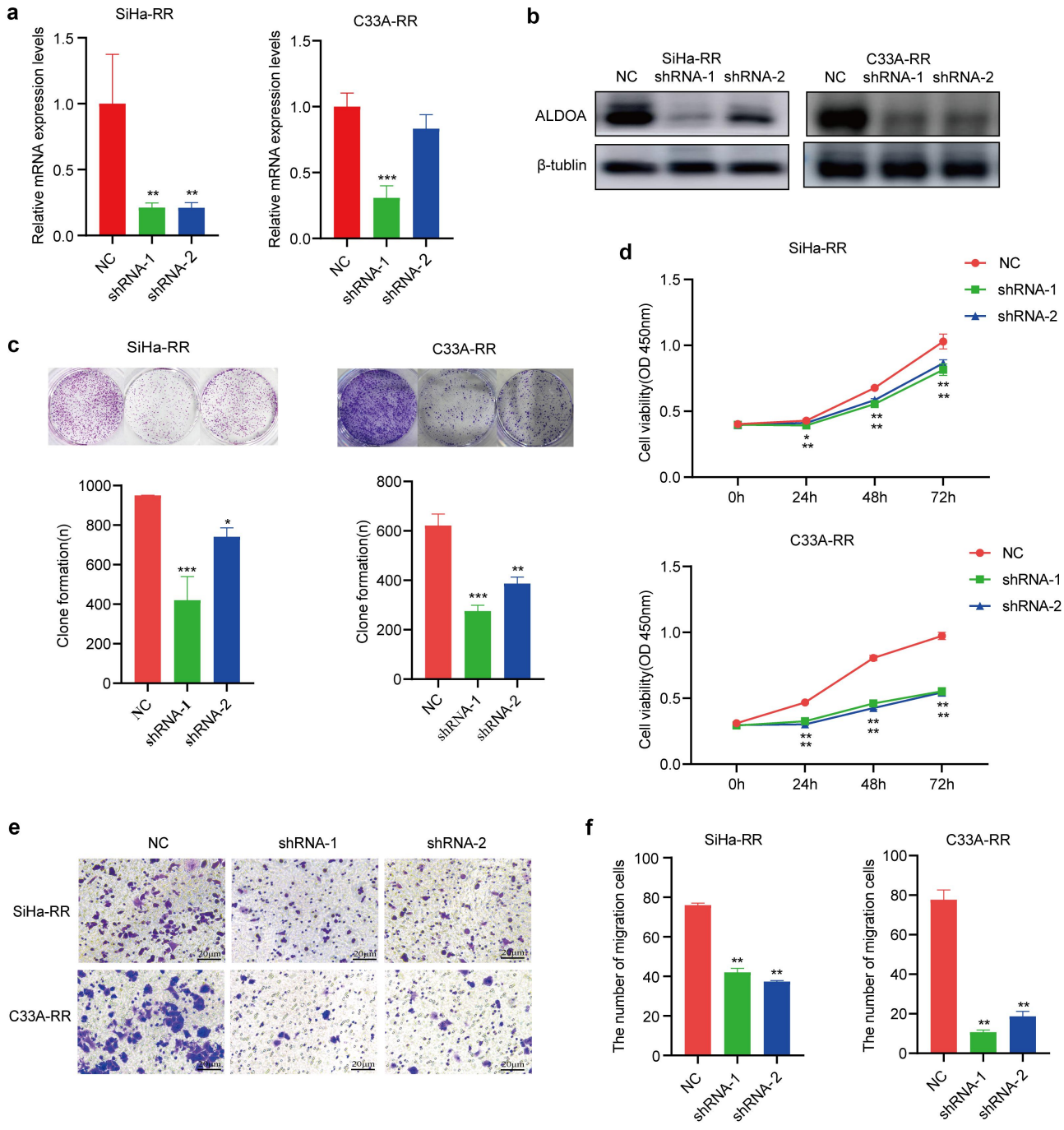

Figure 2. Knockdown ALDOA inhibits cell clonal formation, proliferation, and migration. (a) The mRNA expression of ALDOA in transfected SiHa-RR or C33A-RR cells was measured by qRT-PCR. (b) The protein expression level of ALDOA in transfected SiHa-RR, C33A-RR cells was measured by western blot. (c) The clonal formation experiment showed that the cloning ability of SiHa-RR and C33A-RR decreased after ALDOA knockdown. (d) Cell proliferation of transfected SiHa-RR or C33A-RR cells was analyzed by CCK-8 assays. OD 450 values at indicated time points were displayed to indicate cell proliferation. (e-f) The transfected cells were used for migration measurement, stained with crystal violet, and counted under the light microscope. *$P < .05$, **$P < .01$, ***$P < .001$.

CC RR cells' biological activities by regulating glycolysis in the cells. By seahorse extracellular flux analysis, we found that the knockdown of ALDOA significantly inhibited ECAR in SiHa-RR and C33A-RR cells (Figure 5a). Metabolic product detection consistently found that the knockdown of ALDOA reduced glucose uptake and lactic acid production in CC RR cells (Figures 5b, 5c). We also detected key enzymes related to glycolysis by western blot analysis. The results showed that the expression of glycolysis-related proteins decreased significantly (Figures 5d, 5e). These results clearly indicate that ALDOA regulates the glycolysis pathway in CC RR cells.

Discussion

RT is known to be a crucial treatment for patients with advanced CC.[28–30] The occurrence of RR severely reduces the efficacy of RT, but the mechanism of RR remains elusive,[31,32] and the

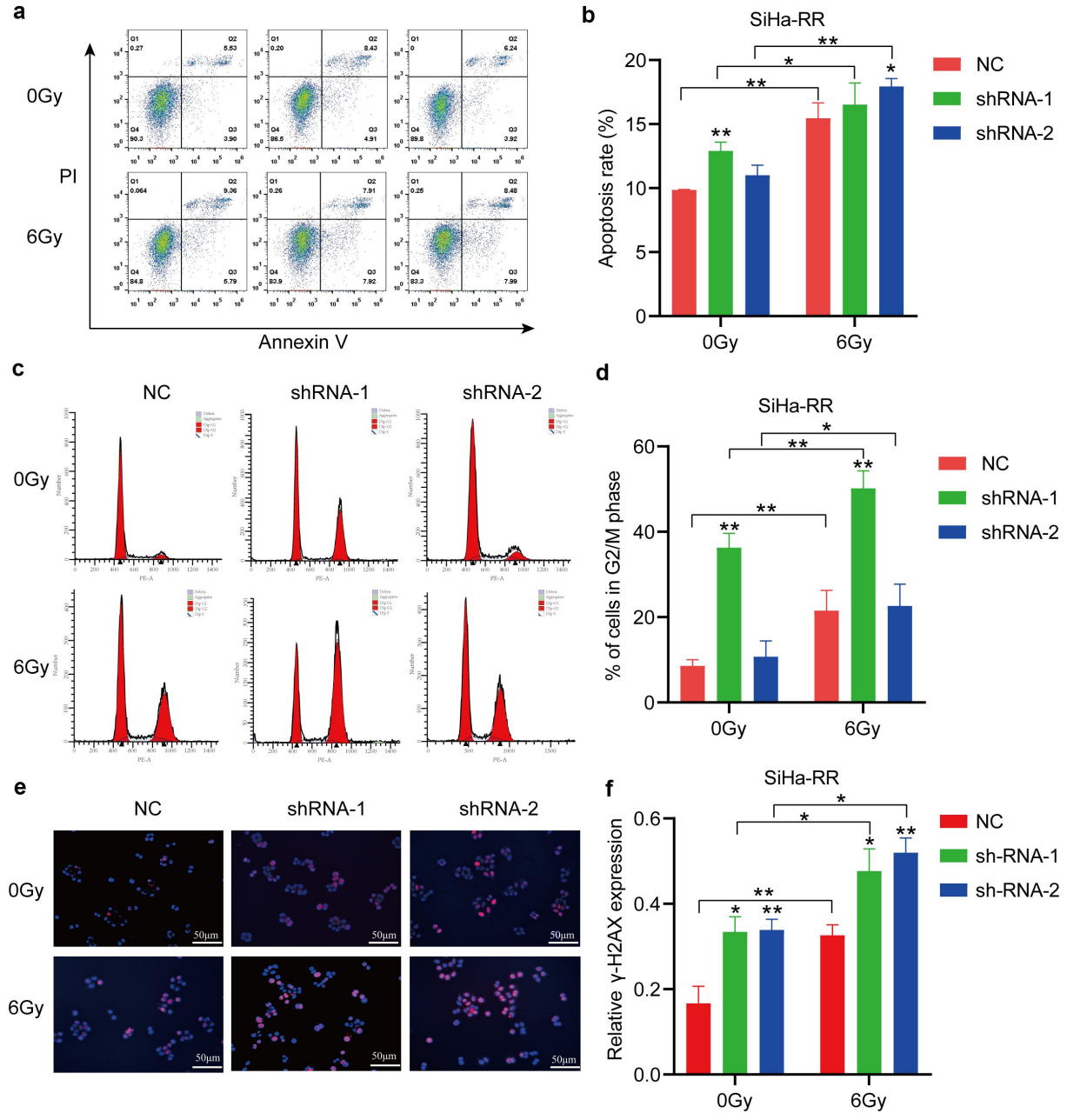

Figure 3. Targeting ALDOA promotes CC cell apoptosis, G2/M phase arrest, and DNA damage induced by irradiation. (a-b) Apoptosis was assessed by flow cytometry in the control group, ALDOA knockdown group, irradiation group and combined treatment group. (c-d) Cell cycle of the control group, ALDOA knockdown group, irradiation group and combined treatment group was measured by flow cytometry. (e-f) Immunofluorescence was used to detect the number of γ-H2AX foci. *$P < .05$, **$P < .01$.

prognosis of patients with advanced CC is still poor.[33] Recent research has revealed that one of the primary causes of RR in cancer is metabolic reprogramming,[34,35] however, the possible mechanism is still unknown. Accumulated evidence shows that cell metabolism affects radiosensitivity and patients with upregulation of glycolysis have poorer prognosis.[36,37] In this study, we found that high ALDOA expression was associated with poor prognosis in patients with CC. In addition, we first reported that targeting ALDOA could elevate the apoptosis induced by radiotherapy and enhance the radiosensitivity of CC cells.

It is reported that metabolic reprogramming provides energy for the growth and proliferation precursors of tumor cells, and ALDOA is related to the proliferation and metastasis of cancer cells.[38,39] In this study, we found that the level of ALDOA in established CC RR cells was higher than that in CC cells. Therefore, we made some exploration by detecting the effect of ALDOA knockdown in CC cells. It has shown that ALDOA knockout could inhibit the clone formation, proliferation, and migration of CC cells. These data together show that targeting ALDOA can inhibit the malignant behavior of CC cells.

DNA double-strand breaks in cells after irradiation exerts a direct effect on cell death, but some tumor cells can repair the DNA damage through homologous recombination (HR) and non-homologous end joining (NHEJ).[40,41] However, the exploration of the effects of ALDOA on RR in CC is very limited. Thus, analyzing whether ALDOA affects CC RR and

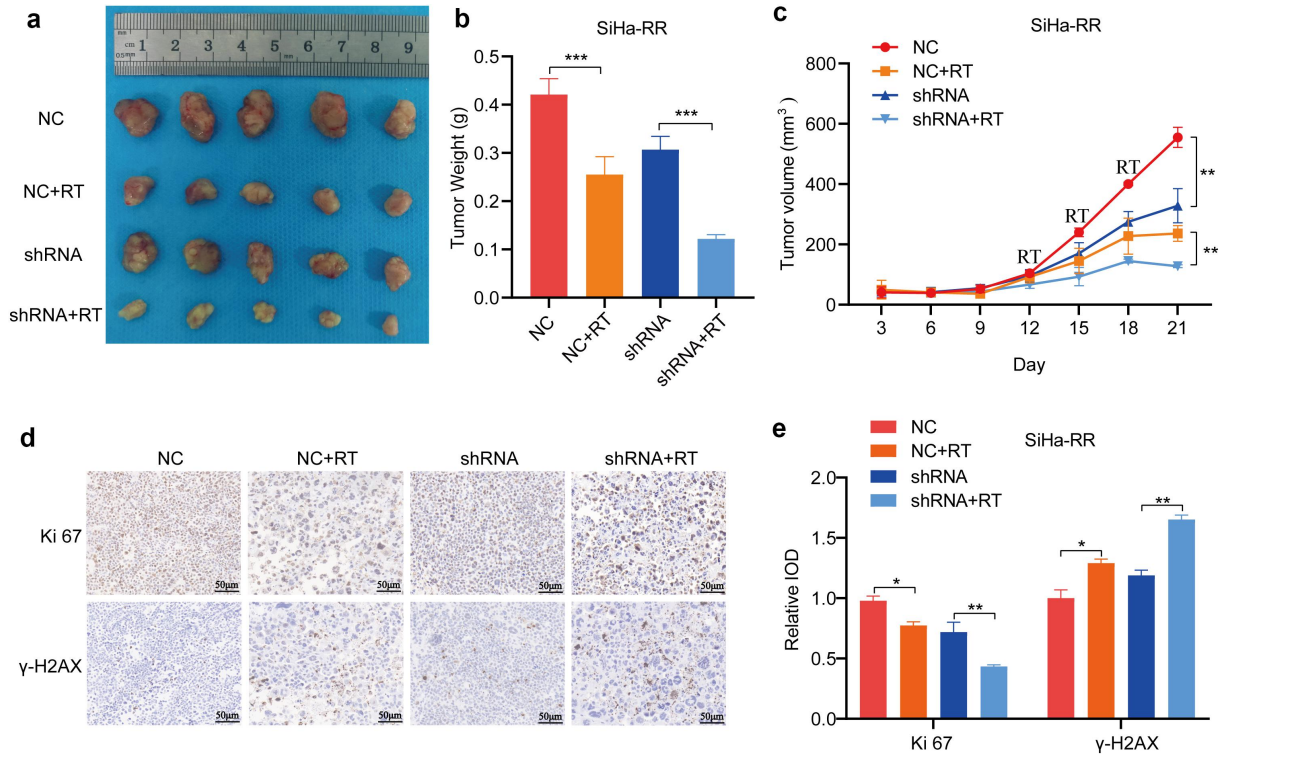

Figure 4. Knockdown ALDOA in CC RR cells combined with irradiation inhibits the growth of CC xenograft *in vivo*. (a) Representative images of xenograft tumors in mice. The animal study was repeated three times ($n = 5$). (b) Tumor weight of mice in different groups. (c) Changes in tumor volume in different groups. (d-e) Representative images and statistical analysis of immunohistochemical staining of tumor tissues. *$P < .05$, **$P < .01$, ***$P < .001$.

its potential mechanism may provide new insights into the related treatment strategies. Our findings showed that the knockdown of ALDOA combined with RT caused G2/M phase arrest of CC cells and inhibited cell proliferation, which was also verified *in vivo*. It indicates that targeting ALDOA aggravates radiation-induced apoptosis. Previous studies have shown that histone H2AX is a key DNA damage signaling protein, and DNA double-strand breaks prompt phosphorylation of Ser 139, and the phosphorylated H2AX is known as γ-H2AX.[42,43] The marker of DNA damage, γ-H2AX, was also up-regulated in the ALDOA knockdown group under irradiation. In addition, ALDOA knockdown combined with RT greatly inhibited the growth of xenograft tumors *in vivo*. Immunohistochemical analysis indicated that the expression of Ki67 was significantly lower while the expression of γ-H2AX was higher in ALDOA knockdown combined with the RT treatment group than in the control group. Therefore, our results strongly support that inhibiting ALDOA can improve the radiosensitivity of CC cells. For the mechanism study, since enhanced glycolysis promotes RR in tumor cells[17,44] and ALDOA plays an important role in glycolysis. Our study demonstrated that targeting ALDOA reduced glucose uptake and lactate production, and inhibited glycolysis in CC RR cells. This suggests that inhibiting glycolysis may be a feasible method for radiosensitization of CC.

To summarize, our research results show that targeting ALDOA can reduce the malignant potential and improve radiosensitivity in CC. Thus, ALDOA can be used as a potential target for radiation sensitization. However, further exploration is still needed to promote clinical transformation and improve the prognosis of CC patients.

Materials and methods

Cell culture

CC cell lines SiHa and C33A were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured in Modified Eagle Medium (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) and 1% penicillin/streptomycin (Procell, Wuhan, China) at 37°C in an incubator containing 5% CO_2.

Establishment of CC RR cells

SiHa and C33A were plated in 25 cm^2 flasks. When cell fusion reached 60%, 1 cm thick compensation was covered on flasks, and the linear accelerator (Siemens, Berlin, Germany) was used to irradiate at a radiation dosage of 2 Gy with a rate of 600 cGy/min. Cells were kept at room temperature for 30 minutes and then transferred to an incubator for incubation for 24 hours before replacement with a fresh medium. To construct stable RR cell lines, cells were irradiated every 2 days subsequently. CC cells SiHa were exposed to a total dose of 76 Gy and C33A was irradiated with 60 Gy. The

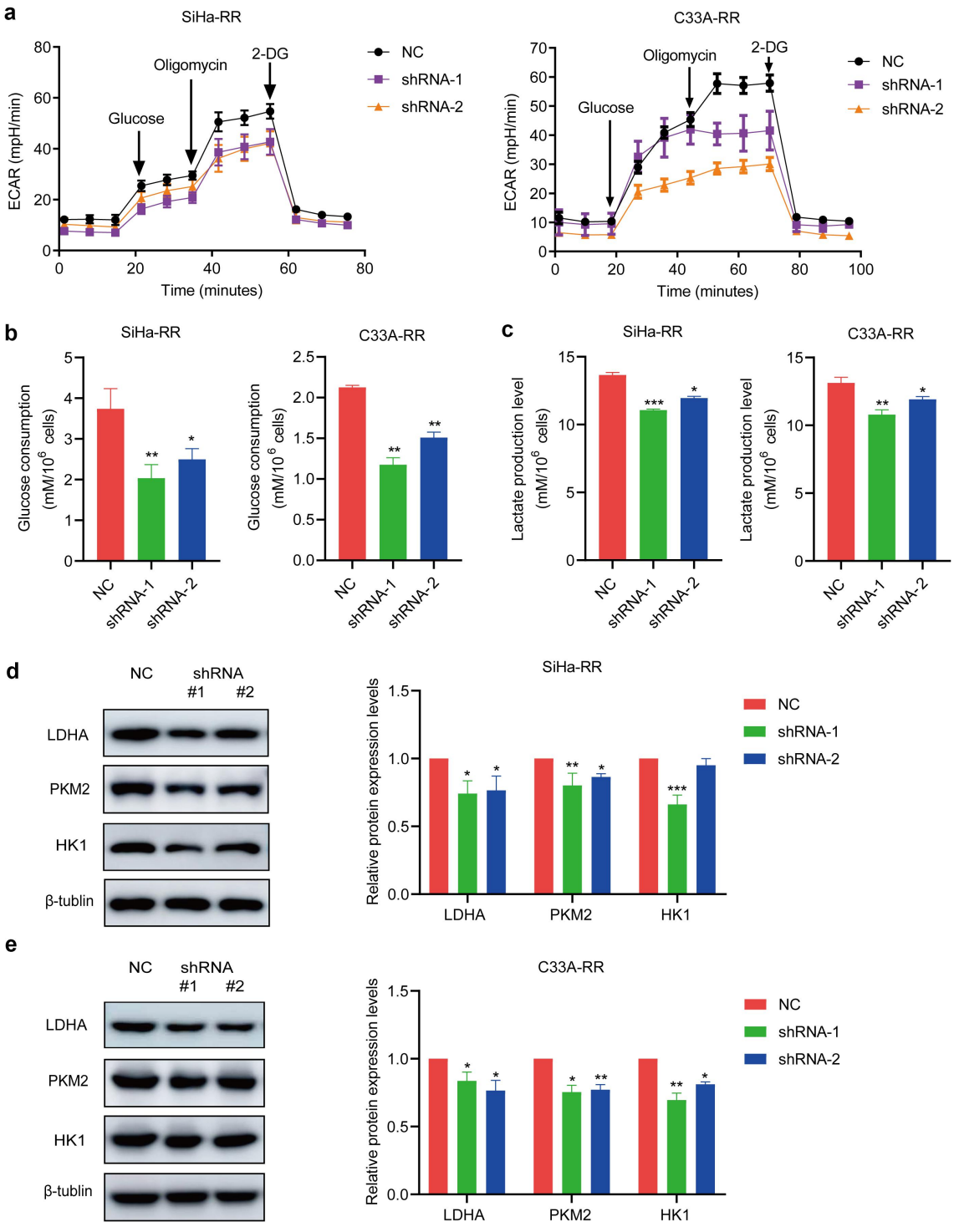

Figure 5. Knockdown ALDOA in CC RR cells inhibits the glycolysis pathway. (a) Seahorse Bioscience XFp analyzer added with the glucose (10 mM), oligomycin (1.0 μM), and 2-deoxy-d-glucose (2-DG, 50 mM) was used to detect the cellular acidification rate of transfected cells. (b) Determination of glucose consumption in SiHa-RR and C33A-RR by stably knockdown ALDOA. (c) Determination of lactic acid production in SiHa-RR and C33A-RR cells by stably knockdown ALDOA. (d-e) The expression levels of glycolytic proteins LDHA, PKM2, and HK1 in transfected SiHa-RR, C33A-RR cells were measured by western blot. *$P < .05$, **$P < .01$, ***$P < .001$.

clonogenesis assay was used to determine the establishment of SiHa-RR and C33A-RR cells.

Cell transfection

The pEX-3 vector was used to construct the short hairpin RNA targeting ALDOA (shRNA ALDOA) accompanied by a negative control sequence (shRNA NC). The shRNA plasmids (GenePharma, Shanghai, China) were purchased and used to knock down ALDOA in SiHa-RR and C33A-RR according to the manufacturer's instructions (GenePharma, Shanghai, China). After 24 hours of transfection, the cells were subcultured into the selective medium (Opti-MEM^{TM}, Thermo Fisher Scientific, Waltham, MA, USA) at the ratio of

1:10. After 1–2 weeks of culture, most of the cells were killed. The target cells stably integrated into the cell genome could survive and grow in the selective medium. Stable transfection was established and cells were collected for further experiments. The sequences of shRNA ALDOA1: 5′-GCCAATGTTCTGGCCCGTTAT-3′, shRNA ALDOA2: 5′-GCCTTGCCTGTCAAGGAAAGT-3′.

Quantitative real-time PCR (qRT-PCR)

Cells were collected and total RNA was extracted by the trizol reagent (Invitrogen, MA, USA). Complementary DNA (cDNA) was obtained by reverse transcription according to the instruction manual of the RNA reverse transcription kit (Takara company, Dalian, China). The cDNA was diluted with diethyl pyrocarbonate (DEPC) water. According to the instructions of the quantitative SYBR Green PCR kit (Takara company, Dalian, China), each gene was set with three repeats, and qRT-PCR was performed with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal reference. Primer sequences are shown in Supplementary Table 1. The experiment was repeated three times with 3 repetitions each time.

Western blotting analysis

Western blot analysis was conducted as previously reported.[45] In brief, cells were lysed by the enhanced RIPA Lysis Buffer (EpiZyme, Shanghai, China). Protein extracts were separated by SDS-PAGE electrophoresis, which were transferred to a polyvinylidene fluoride (PVDF) membrane. The expression of proteins was detected by the incubation of the corresponding antibodies. The antibodies used are listed in Supplementary Table 2. Image J software is used for quantitative analysis. The experiment was repeated three times.

Cell proliferation assay

Cell counting Kit-8 (CCK-8) assay was used for the detection of cell proliferation ability. 4000 cells per well were placed in 96-well plates and incubated in an incubator at 37°C with 5% CO_2 for 24 hours. After adding CCK-8 solution (Dojindo, Kumamoto, Japan) for 2 hours, the absorbance value of each well was determined at 450 nm by an enzyme labeling instrument (BioTek Instruments, Inc., Winooski, VT, USA).

Colony formation assay

2000 stably transfected cells were inoculated in a six-well culture dish. After 2 weeks of culture, the cells on the control plate can form colonies. Take out the medium and wash the cells with 2 ml Phosphate Buffer Saline (PBS). The cells were fixed with 4% paraformaldehyde (Leagene, Beijing, China) at room temperature and stained with 0.1% crystal violet solution (EpiZyme, Shanghai, China). Then rinse the crystal violet with tap water and place the petri dish on the table at room temperature. The cell colonies were imaged and counted.

Cell cycle analysis

Flow cytometry was performed to detect the cell cycle. Cells were irradiated with 6 Gy and cultured for 24 hours, digested with trypsin and washed with PBS, and then fixed with pre-cooled 75% ethanol overnight. Cells were stained with a DNA staining solution for 30 minutes in the dark at room temperature, and analyzed by flow cytometry to determine cell cycle.

Cell apoptosis analysis

Transfected cells were irradiated with 6 Gy and cultured for 24 hours. The cells were digested with trypsin and washed with ice-cold PBS before centrifugation and collection. Cells were double stained with FITC-annexin V and PI (Keygen Biotech, Jiangsu, China), and apoptosis was subsequently analyzed by flow cytometry.

Cell migration assay

The migration capabilities of CC cells were determined by transwell assay. The upper compartment was inoculated with an amount of 1×10^5 cells/well, and 500 μL MEM (Procell, Wuhan, China) containing 20% FBS was added to the lower compartment. After 24 hours of culture, fix it with methanol for 30 minutes, and dye it with crystal violet for 15 minutes. Wipe the cells on the upper layer of the microporous membrane with a cotton swab and count the cells under the upper layer with the microscope (Carl Zeiss, Oberkochen, Germany). Three repetitions were set for each group and the experiment was repeated three times.

Glucose consumption and lactate production

Stably transfected CC cells (1×10^6 cells/well) were inoculated into a six-well plate overnight, the medium was changed and cell supernatant was collected 24 hours later. The glucose and lactic acid contents in the supernatant were detected using the glucose detection kit (Abcam, Milpitas, USA) and the lactic acid detection kit (Abcam, Milpitas, USA) according to the manufacturer's protocol.

Seahorse metabolic measurements

Stably transfected cells were cultured in a conditioned medium and calibrated for 1 hour under CO_2-free conditions. The glucose, oligomycin and 2-DG were added into the XF cell culture microplate, and the extracellular acidification rate (ECAR) in XF base medium containing 1 mM glutamine (pH = 7.4) was measured with the XFp analyzer (Agilent, Santa Clara, CA, USA). The Wave software (version 2.6.1) was used to normalize the original data.

Cell immunofluorescence staining

After irradiation for 24 hours, cells were washed with PBS and then fixed with 4% paraformaldehyde for 30 minutes. Cells were permeated by treatment with 0.3%Triton X-100, then blocked with 5% goat serum for 30 minutes and incubated

overnight with anti-γ-H2AX as the primary antibody (Abcam, Hong Kong, China). The next day, cells were washed with PBS and incubated with anti-rabbit IgG Alexa Fluor 594 Conjugate antibody (Cell Signaling Technology, Boston, USA) for 1 hour at 37°C in the dark before staining with DAPI. Finally, images were captured using a fluorescent inverted microscope (magnification, ×200).

Tumor xenograft model

The experimental protocol was evaluated and approved by the ethics committee of the First Affiliated Hospital of Zhengzhou University (2019-KY-0026-001). We carried out the experiment in strict accordance with the declaration of Helsinki and approved guidelines. 6–8 weeks female BALB/c athymic nude mice were randomly divided into two groups. 5×10^6 stably transfected cells were injected subcutaneously into nude mice. The tumor size (volume = length × width^2/2) was measured after injecting cells with vernier caliper every 3 days. When the average tumor volume reached 100 mm^3, RT was carried out. Each irradiation is 6 Gy, and every other day for 3 times in total. When the tumor length of the control group reached 1.5 cm, the mice were euthanized. Tumor tissues were collected, measured, and embedded in paraffin. Immunohistochemical staining was conducted for anti-γ-H2AX (Cell Signaling Technology, Boston, MA, USA) and anti-Ki67 antibodies (Cell Signaling Technology, Boston, MA, USA) as previously described.[45]

Statistical analysis

SPSS 22.0 and GraphPad Prism 9 software were used for statistical analysis. Student's t-test was used to analyze the differences between the two groups of data, and one-way analysis of variance (ANOVA) was used to analyze the differences between the three groups and more than three groups of data. The measured data are expressed in the form of mean ± SEM. When $^*P < .05$, $^{**}P < .01$, $^{***}P < .001$, it is considered to be statistically significant.

Abbreviations

CC: cervical cancer; ALDOA: aldolase A; RT: radiotherapy; qRT-PCR: quantitative; Real-Time PCR; RR: radioresistance; ATCC: American type culture collection; GAP: glyceraldehyde-3-phosphate; DHAP: dihydroxyacetone phosphate; FBS: fetal bovine serum; cDNA: complementary DNA; DEPC: diethyl pyrocarbonate; PBS: Phosphate Buffer Saline; ECAR: extracellular acidification rate; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; PVDF: poly vinylidene fluoride; CCK-8: cell counting Kit-8; ANOVA: one-way analysis of variance.

Acknowledgments

We acknowledge assistance with the access of analytic instruments from the Translational Medical Center at The First Affiliated Hospital of Zhengzhou University.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by Henan Province Colleges and Universities Innovative Talent Support Program (No. 21HASTIT044), Young and middle-aged subject leader of Henan Provincial Health Commission (No. HNSWJW-2022001), Henan Province Young and Middle-aged Health Science and Technology Innovative Talent Training Project (No. YXKC2020039), Central Plains Youth Top Talent Project (No. ZYYCYU202012169), High-level Talents Return to China for Research Funding Project, Henan Province University Major Research Project (No. 22A320055), Henan Medical Education Research Project (No. Wjlx2020062), Zhengzhou University clinical medicine first-class discipline talent training program (Outstanding young talents) and National Natural Science Foundation of China (No. 82303343)..

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Author contributions

Junying Zhou: Methodology; Software; Data curation; Writing-original draft. **Ningjing Lei**: Project administration; Methodology; Visualization; Data curation; Resources. **Bo Qin**: Software. **Mengyu Chen**: Validation. **Shuai Gong**: Validation. **Hao Sun**: Writing-review and editing. **Luojie Qiu**: Data curation. **Fengling Wu**: Formal analysis. **Ruixia Guo**: Project administration. **Qian Ma:** Validation. **Yong Li**: Supervision; Writing-

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Data availability statement

All data used or analyzed during the current study are within the article and its supplementary materials.

Ethics approval and consent to participate

The study about nude mouse xenograft model was approved by the Ethics Committee of First Affiliated Hospital of Zhengzhou University, with the following ethics batch number: 2019-KY-0026-001.

References

1. Gapare CR, El-Zein M, Patel H, Tope P, Franco EL. 2022. Ecologic analysis of correlates of cervical cancer morbidity and mortality in sub-saharan Africa. Cancer Epidemiol Biomarkers Prev. 31 (9):1804–1811. doi:10.1158/1055-9965.EPI-22-0193.
2. Castle PE, Einstein MH, Sahasrabuddhe VV. 2021. Cervical cancer prevention and control in women living with human immunodeficiency virus. CA Cancer J Clin. 71(6):505–526. doi:10.3322/caac.21696.
3. Singh D, Vignat J, Lorenzoni V, Eslahi M, Ginsburg O, Lauby-Secretan B, Arbyn M, Basu P, Bray F, Vaccarella S, et al. 2023. Global estimates of incidence and mortality of cervical cancer in 2020: a baseline analysis of the WHO global cervical cancer elimination initiative. Lancet Glob Health. 11(2):e197–e206. doi:10.1016/S2214-109X(22)00501-0.
4. Monk BJ, Enomoto T, Kast WM, McCormack M, Tan DSP, Wu X, González-Martín, A, et al. 2022. Integration of immunotherapy into treatment of cervical cancer: recent data and ongoing trials. Cancer Treat Rev. 106:102385. doi:10.1016/j.ctrv.2022.102385.
5. Shoaib S, Islam N, Yusuf N. 2022. Phytocompounds from the medicinal and dietary plants: multi-target agents for cervical cancer prevention and therapy. Curr Med Chem. 29(26):4481–4506. doi:10.2174/0929867329666220301114251.
6. Yang X, Ren H, Fu J. 2022. Combinations of radiotherapy with immunotherapy in cervical cancer. J Cancer. 13(5):1480–1489. doi:10.7150/jca.65074.
7. Shelley CE, Barraclough LH, Nelder CL, Otter SJ, Stewart AJ. 2021. Adaptive radiotherapy in the management of cervical cancer: review of strategies and clinical implementation. Clin Oncol (R Coll Radiol). 33(9):579–590. doi:10.1016/j.clon.2021.06.007.
8. Ni M, Li J, Zhao H, Xu F, Cheng J, Yu M, Ke G, Wu X. 2021. BRD4 inhibition sensitizes cervical cancer to radiotherapy by attenuating DNA repair. Oncogene. 40(15):2711–2724. doi:10.1038/s41388-021-01735-3.
9. DeBoer RJ, Umutoni V, Bazzett-Matabele L, Katznelson E, Nguyen C, Umwizerwa A, Bigirimana JB, Paciorek A, Nsabimana N, Ruhangaza D, et al. 2022. Cervical cancer treatment in Rwanda: resource-driven adaptations, quality indicators, and patient outcomes. Gynecol Oncol. 164(2):370–378. doi:10.1016/j.ygyno.2021.12.002.
10. Jiao X, Zhang S, Jiao J, Zhang T, Qu W, Muloye GM, Kong B, Zhang Q, Cui B. 2019. Promoter methylation of SEPT9 as a potential biomarker for early detection of cervical cancer and its overexpression predicts radioresistance. Clin Epigenetics. 11 (1):120. doi:10.1186/s13148-019-0719-9.
11. Feng Y, Wang Z, Yang N, Liu S, Yan J, Song J, Yang S, Zhang Y. 2021. Identification of biomarkers for cervical cancer radiotherapy resistance based on RNA sequencing data. Front Cell Dev Biol. 9:724172. doi:10.3389/fcell.2021.724172.
12. Zhou J, Lei N, Tian W, Guo R, Chen M, Qiu L, Wu, F, Li, Y, Chang, L, et al. 2022. Recent progress of the tumor microenvironmental metabolism in cervical cancer radioresistance. Front Oncol. 12:999643. doi:10.3389/fonc.2022.999643.
13. Centurione L, Aiello FB. 2016. DNA repair and cytokines: TGF-beta, IL-6, and Thrombopoietin as different biomarkers of radioresistance. Front Oncol. 6:175. doi:10.3389/fonc.2016.00175.
14. Raspaglio G, Buttarelli M, Filippetti F, Battaglia A, Buzzonetti A, Scambia G, Gallo D. 2021. Stat1 confers sensitivity to radiation in cervical cancer cells by controlling Parp1 levels: a new perspective for Parp1 inhibition. Cell Death Disease. 12(10):933. doi:10.1038/s41419-021-04229-y.
15. Hsu PP, Sabatini DM. 2008. Cancer cell metabolism: Warburg and beyond. Cell. 134(5):703–707. doi:10.1016/j.cell.2008.08.021.
16. Rashmi R, Huang X, Floberg JM, Elhammali AE, McCormick ML, Patti GJ, Spitz DR, Schwarz JK. 2018. Radioresistant cervical cancers are sensitive to inhibition of glycolysis and redox metabolism. Cancer Research. 78(6):1392–1403. doi:10.1158/0008-5472.CAN-17-2367.
17. Shimura T, Noma N, Sano Y, Ochiai Y, Oikawa T, Fukumoto M, Kunugita N. 2014. AKT-mediated enhanced aerobic glycolysis causes acquired radioresistance by human tumor cells. Radiotherapy And Oncology. 112(2):302–307. doi:10.1016/j.radonc.2014.07.015.
18. Chang YC, Chiou J, Yang YF, Su CY, Lin YF, Yang CN, Lu P-J, Huang M-S, Yang C-J, Hsiao M, et al. 2019. Therapeutic targeting of aldolase a interactions inhibits lung cancer metastasis and prolongs survival. Cancer Res. 79(18):4754–4766. doi:10.1158/0008-5472.CAN-18-4080.
19. Chang YC, Chan YC, Chang WM, Lin YF, Yang CJ, Su CY, Huang, M.S, Wu, A.T, Hsiao, M et al. 2017. Feedback regulation of ALDOA activates the HIF-1alpha/MMP9 axis to promote lung cancer progression. Cancer Lett. 403:28–36. doi:10.1016/j.canlet.2017.06.001.
20. Ji S, Zhang B, Liu J, Qin Y, Liang C, Shi S, Jin K, Liang D, Xu W, Xu H, et al. 2016. ALDOA functions as an oncogene in the highly metastatic pancreatic cancer. Cancer Lett. 374(1):127–135. doi:10.1016/j.canlet.2016.01.054.
21. Bidkhori G, Benfeitas R, Klevstig M, Zhang C, Nielsen J, Uhlen M, Boren J, Mardinoglu A. 2018. Metabolic network-based stratification of hepatocellular carcinoma reveals three distinct tumor subtypes. Proc Natl Acad Sci USA. 115(50):E11874–E83. doi:10.1073/pnas.1807305115.
22. Huang Z, Hua Y, Tian Y, Qin C, Qian J, Bao M, Liu Y, Wang S, Cao Q, Ju X, et al. 2018. High expression of fructose-bisphosphate aldolaseï¿½A induces progression of renal cell carcinoma. Oncol Rep. 39:2996–3006. doi:10.3892/or.2018.6378.
23. Zhang F, Lin JD, Zuo XY, Zhuang YX, Hong CQ, Zhang GJ, Cui X-J, Cui Y-K. 2017. Elevated transcriptional levels of aldolase a (ALDOA) associates with cell cycle-related genes in patients with NSCLC and several solid tumors. BioData Mining. 10(1):6. doi:10.1186/s13040-016-0122-4.
24. Kawai K, Uemura M, Munakata K, Takahashi H, Haraguchi N, Nishimura J, Hata T, Matsuda C, Ikenaga M, Murata K, et al. 2017. Fructose-bisphosphate aldolase a is a key regulator of hypoxic adaptation in colorectal cancer cells and involved in treatment resistance and poor prognosis. Int J Oncol. 50(2):525–534. doi:10.3892/ijo.2016.3814.
25. Chang L, Ni J, Beretov J, Wasinger VC, Hao J, Bucci J, Malouf D, Gillatt D, Graham PH, Li Y, et al. 2017. Identification of protein biomarkers and signaling pathways associated with prostate cancer radioresistance using label-free LC-MS/MS proteomic approach. Sci Rep. 7(1):41834. doi:10.1038/srep41834.
26. Meijer TW, Kaanders JH, Span PN, Bussink J. 2012. Targeting hypoxia, HIF-1, and tumor glucose metabolism to improve radiotherapy efficacy. Clin Cancer Res. 18(20):5585–5594. doi:10.1158/1078-0432.CCR-12-0858.
27. Liu Y, He D, Xiao M, Zhu Y, Zhou J, Cao K. 2021. Long noncoding RNA LINC00518 induces radioresistance by regulating glycolysis through an miR-33a-3p/HIF-1α negative feedback loop in melanoma. Cell Death Disease. 12(3):245. doi:10.1038/s41419-021-03523-z.

28. Zeng X, Wan L, Wang Y, Xue J, Yang H, Zhu Y. 2020. Effect of low dose of berberine on the radioresistance of cervical cancer cells via a PI3K/HIF-1 pathway under nutrient-deprived conditions. Int J Radiat Biol. 96(8):1060–1067. doi:10.1080/09553002.2020.1770358.
29. Vojtisek R, Hosek P, Sukovska E, Kovarova P, Baxa J, Ferda J, Fínek J. 2022. Treatment outcomes of MRI-guided adaptive brachytherapy in patients with locally advanced cervical cancer: institutional experiences. Strahlenther Onkol. 198(9):783–791. doi:10.1007/s00066-021-01887-x.
30. Li H, Wang S, Liu Y, Wang T, Jin S, Liu Z. 2022. Prophylactic extended-field irradiation for locally advanced cervical cancer. Gynecol Oncol. 166(3):606–613. doi:10.1016/j.ygyno.2022.07.009.
31. Chuang PC, Chen PT, Wang CC, Su WH, Chen YH, Huang EY. 2022. MicroRNA-29a manifests multifaceted features to intensify radiosensitivity, escalate apoptosis, and revoke cell migration for palliating radioresistance-enhanced cervical cancer progression. Int J Mol Sci. 23(10):5524. doi:10.3390/ijms23105524.
32. Yao T, Yao Y, Chen Z, Peng Y, Zhong G, Huang C, Li J, Li R. 2022. CircCASC15-miR-100-mTOR may influence the cervical cancer radioresistance. Cancer Cell Int. 22(1):165. doi:10.1186/s12935-022-02573-3.
33. Gennigens C, Jerusalem G, Lapaille L, De Cuypere M, Streel S, Kridelka F, Ray-Coquard, I et al . 2022. Recurrent or primary metastatic cervical cancer: current and future treatments. ESMO Open. 7(5):100579. doi:10.1016/j.esmoop.2022.100579.
34. Lin J, Xia L, Liang J, Han Y, Wang H, Oyang L, Tan S, Tian Y, Rao S, Chen X, et al. 2019. The roles of glucose metabolic reprogramming in chemo- and radio-resistance. J Exp Clin Cancer Res. 38(1):218. doi:10.1186/s13046-019-1214-z.
35. Nagao A, Kobayashi M, Koyasu S, Chow CCT, Harada H. 2019. HIF-1-Dependent reprogramming of glucose metabolic pathway of cancer cells and its therapeutic significance. Int J Mol Sci. 20 (2):20. doi:10.3390/ijms20020238.
36. de Mey S, Dufait I, De Ridder M. 2021. Radioresistance of human cancers: clinical implications of genetic expression signatures. Front Oncol. 11:761901. doi:10.3389/fonc.2021.761901.
37. Wei X, Sun K, Li S, Lin C, Wei Z. 2023. PSME3 induces radioresistance and enhances aerobic glycolysis in cervical cancer by regulating PARP1. Tissue Cell. 83:102151. doi:10.1016/j.tice.2023.102151.
38. Fu H, Gao H, Qi X, Zhao L, Wu D, Bai Y, Li H, Liu X, Hu J, Shao S. 2018. Aldolase a promotes proliferation and G1/S transition via the EGFR/MAPK pathway in non-small cell lung cancer. Cancer Commun (Lond). 38(1):1–15. doi:10.1186/s40880-018-0290-3.
39. Ortmayr K, Dubuis S, Zampieri M. 2019. Metabolic profiling of cancer cells reveals genome-wide crosstalk between transcriptional regulators and metabolism. Nat Commun. 10(1):1841. doi:10.1038/s41467-019-09695-9.
40. Zhao Y, Chen S. 2019. Targeting DNA Double-strand Break (DSB) repair to counteract tumor radio-resistance. Curr Drug Targets. 20 (9):891–902. doi:10.2174/1389450120666190222181857.
41. Fu S, Jin L, Gong T, Pan S, Zheng S, Zhang X, Yang T, Sun Y, Wang Y, Guo J, et al. 2018. Effect of sinomenine hydrochloride on radiosensitivity of esophageal squamous cell carcinoma cells. Oncol Rep. 39:1601–1608. doi:10.3892/or.2018.6228.
42. Salguero I, Belotserkovskaya R, Coates J, Sczaniecka-Clift M, Demir M, Jhujh S, Wilson MD, Jackson SP. 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.
43. Hoen L, Rudisch C, Wick M, Indenbirken D, Grundhoff A, Wegwitz F, Kalkhof S, Hildebrand J. 2022. Osmotic stress interferes with DNA damage response and H2AX phosphorylation in human keratinocytes. Cells. 11(6):11. doi:10.3390/cells11060959.
44. Cao K, Li J, Chen J, Qian L, Wang A, Chen X, Xiong W, Tang J, Tang S, Chen Y, et al. 2017. microRNA-33a-5p increases radiosensitivity by inhibiting glycolysis in melanoma. Oncotarget. 8 (48):83660–83672. doi:10.18632/oncotarget.19014.
45. Tian W, Lei N, Guo R, Yuan Z, Chang L. 2020. Long non-coding RNA DANCR promotes cervical cancer growth via activation of the Wnt/β-catenin signaling pathway. Cancer Cell Int. 20(1):61. doi:10.1186/s12935-020-1139-9.