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Irradiation Induces Gasdermin E-Triggered Tumor Immunity to Inhibit Esophageal Carcinoma Cell Survival

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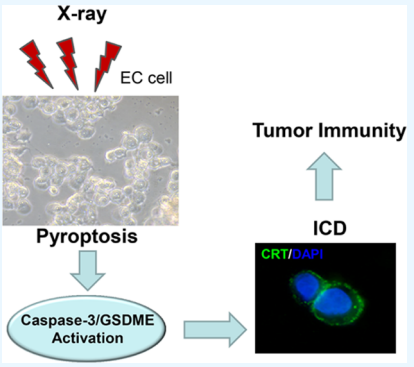
ABSTRACT: Gasdermin E (GSDME), an executor of pyroptosis, can be activated by caspase-3 and has been recognized as a tumor suppressor in various human cancers. In addition, caspase-3/GSDME signal-induced pyroptosis is a form of immunogenic cell death (ICD). In this study, we aimed to understand the association between radiotherapy and caspase-3/GSDME signal-related ICD in esophageal carcinoma (EC) cells. The expression of caspase-3 and GSDME in two EC cell lines, ECA-109 and KYSE-150, was silenced or overexpressed by transfection with specific siRNAs or overexpression vectors. Cells were subjected to 0−8 Gy irradiation, and cell death was evaluated by CCK-8 assay, annexin V-FITC staining, lactate dehydrogenase (LDH) detection kit, Western blotting, and immunofluorescence. Irradiation in both EC cell lines promoted dose-dependent viability loss and apoptosis. More specifically, 8 Gy X-ray increased the apoptosis rate from 4.1 to 12.8% in ECA-109 cells and from 4.6 to 21.1% in KYSE-150 cells. In irradiated EC cells, the levels of LDH release and caspase-3/GSDME cleavage were increased. Caspase-3 silencing inhibited irradiation-induced GSDME cleavage and EC cell death. Furthermore, we identified the death of EC cells suppressed by caspase-3 siRNA, and the levels of CRT, HMGB1, HSP70, and HSP90 were also markedly downregulated by caspase-3 siRNA. Similarly, GSDME silencing diminished irradiation-induced EC cell death and the levels of ICD markers. Overexpression of caspase-3 and GSDME accelerated irradiation-induced ICD. In summary, irradiation in EC cells induces GSDME-mediated pyroptosis and activates ICD to inhibit esophageal carcinoma cell survival.



INTRODUCTION

Esophageal carcinoma (EC) is a common aggressive malignancy of the digestive tract with high morbidity and mortality.[1] In 2012, there were approximately 450,000 new cases of EC and 400,000 EC-related deaths, making EC the eighth most frequent cancer and the sixth leading cause of cancer-related deaths worldwide.[2] EC incidence is higher in men than in women.[3] Smoking, alcohol consumption, low intake of vitamins and minerals, and family history are identified risk factors for EC.[3] Early EC diagnosis is difficult since it is typically asymptomatic; therefore, patients are commonly diagnosed in advanced disease stages.[2]

According to the guidelines of the European Society for Medical Oncology, tumor resection is the standard therapeutic intervention for EC.[4] For patients with early-stage EC, endoscopic resection and ablation alone are the appropriate therapeutic approaches.[5] However, for patients with advanced-stage EC, endoscopic resection is usually followed by chemotherapy, radiotherapy, or both.[6] The abscopal effects of irradiation are effective in killing postoperative residual cells and controlling micrometastases to prevent recurrence.[7] A meta-analysis of 1147 EC patients suggested that preoperative radiotherapy promoted a 3−4% survival improvement.[8]

Immunogenic cell death (ICD) is a specific immunogenic-driven cancer cell death that can be initiated by various stimuli, including radiotherapy.[9] In response to these types of stimuli, the dying tumor cells release damage-associated molecular patterns (DAMPs), namely, high mobility group box 1 (HMGB1), heat shock protein (HSP)70, and HSP90. These factors activate antitumor immunity and result in long-term efficacy of radiotherapy.[10] In a clinical trial with 88 EC patients, T-cell immunity induced by chemoradiotherapy was triggered by ICD and found to be correlated to overall survival.[11] Although ICD has been widely recognized as a form of irradiation-induced tumor cell death,[10,12] further understanding of the mechanisms underlying irradiation-invoked ICD and its related immune responses is required for the continued development of therapeutic approaches.

Pyroptosis is an inflammatory programmed cell death that is critical for regulation of the innate immune response against various stimuli and for maintenance of host homeostasis.[13] Pyroptosis depends on the caspase-induced formation of

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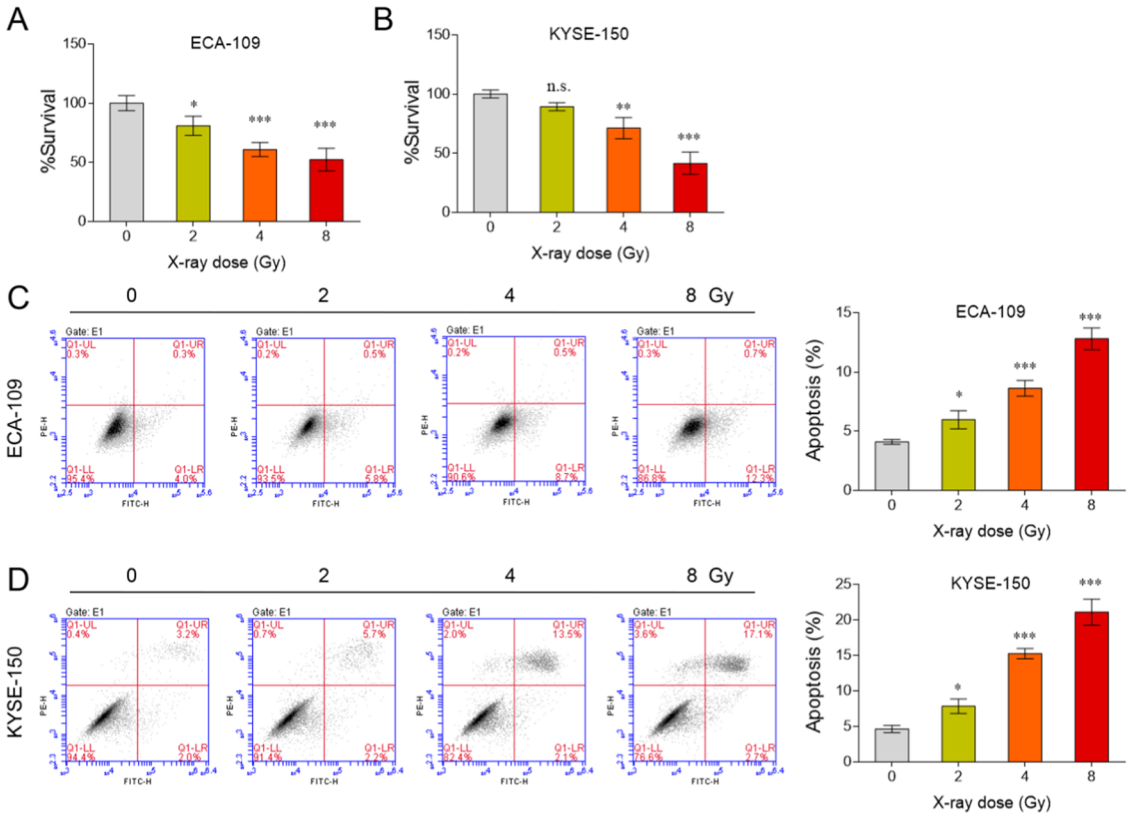

Figure 1. Irradiation in EC cell lines induces cell death. (A–D) ECA-109 and KYSE-150 cells were exposed to 0, 2, 4, and 8 Gy of X-ray. Viability of ECA-109 (A) and KYSE-150 cells (B) was analyzed using a CCK-8 kit. Apoptosis of ECA-109 (C) and KYSE-150 cells (D) was analyzed using an annexin V-FITC/PI detection kit. n.s., not significant; *$p < 0.05$; **$p < 0.01$; and ***$p < 0.001$ vs control.

plasma membrane pores created by gasdermin (GSDM) family members, mainly GSDMD and GSDME.[14] Specifically, GSDMD is rapidly cleaved by activated caspase-1/4/5/11, while GSDME activation depends on activated caspase-3.[15] As an executor of pyroptosis, the tumor suppressive effects of GSDME have been reported in various human cancers, including melanoma, colon, gastric, and breast cancers.[16] Caspase-3/GSDME signaling is associated with radiation-induced tumor cell toxicity, suggesting that radiotherapy can induce pyroptosis.[17] Additionally, caspase-3/GSDME-induced pyroptosis has been identified as a form of ICD,[16] and the production of HMGB1 from GSDME-overexpressing tumors triggers antitumor immunity.[16,18] However, the crosstalk between radiotherapy and caspase-3/GSDME signal-related ICD is still unclear.

This study aims to reveal the effects of radiation on the activation of caspase-3/GSDME signaling and ICD in EC cells. The findings of this study will broaden our understanding of the killing effect of radiotherapy on tumor cells.

MATERIALS AND METHODS

Cell Culture. Esophageal squamous cell carcinoma cell lines, ECA-109 and KYSE-150, were purchased from Procell Science and Technology Co., Ltd. (Wuhan, China). ECA-109 cells were cultured in RPMI-1640 medium (Procell) supplemented with 10% fetal bovine serum (FBS; Hyclone, Logan, UT) and 1% penicillin–streptomycin (Procell). KYSE-150 cells were cultured in 45% RPMI-1640 medium (Procell) and 45% Ham's F12 medium (Procell), supplemented with 10% FBS and 1% penicillin–streptomycin. Cells were cultured split at confluency up to 80% using trypsin/EDTA solution (Sigma-Aldrich, St. Louis, MO). All cell culture procedures were conducted at 37 °C with 5% CO_2.

Cell Irradiation. Irradiation was performed in a logarithmic growth phase in both ECA-109 and KYSE-150 cells. Cells were subjected to 0, 2, 4, or 8 Gy of irradiation by 6 MV X-ray generated from a medical linear accelerator (VARIAN 23EX)with 300 cGy/min rate, 100 cm source-to-surface distance and 180° gantry angle. The field size was set at 25 × 25 cm^2, and the irradiation was provided at 2.5 cm from the edge of the field.

Cell Transfection. The caspase-3 targeted siRNA (5′-GGAAGCGAATCAATGGACTCT-3′), the caspase-3 scrambled control (5′-GAGTCACATCAGGGTCGTAAA-3′), the GSDME targeted siRNA (5′-GAATGACTCTGA-TAAGTTACA-3′), and the GSDME scrambled control (5′-GGTGATATAACCGTACATTAA-3′), were all synthesized by RiboBio (Guangzhou, China). The caspase-3 and GSDME overexpression plasmids as well as the empty vector were provided by Genechem (Shanghai, China). Cell transfection was performed using Lipofectamine 3000 (Invitrogen, Carlsbad, CA) for 48 h, according to the manufacturer's instructions.

Cell Counting Kit (CCK)-8 Assay. Cell viability of ECA-109 and KYSE-150 was evaluated using a CCK-8 kit (Beyotime, Shanghai, China). Cells in 96-well plates at a density of 5000 cells/well were incubated with 10 μL of CCK-8 solution for 2 h at 37 °C, and the optical density was measured at 450 nm using a microplate reader (Bio-Rad, Hercules, CA).

Lactate Dehydrogenase (LDH) Release Assay. ECA-109 and KYSE-150 cells were plated in 96-well plates and

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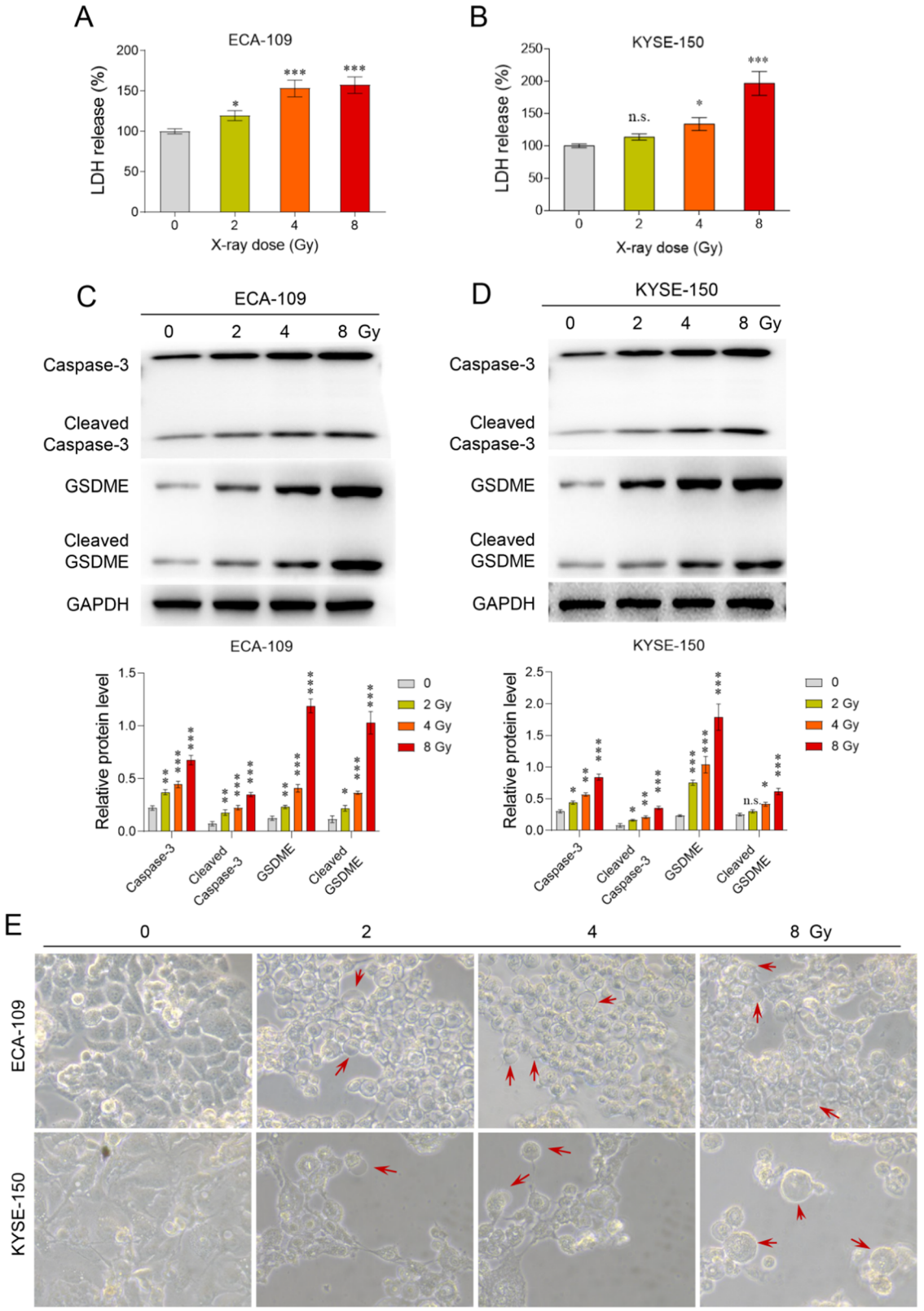

Figure 2. Irradiation in EC cell lines induces caspase-3 and GSDME cleavage. (A–E) ECA-109 and KYSE-150 cells were exposed to 0, 2, 4, and 8 Gy of X-ray. The LDH release of ECA-109 (A) and KYSE-150 cells (B) was analyzed using an LDH detection kit. The expression of caspase-3 and GSDME in ECA-109 (C) and KYSE-150 cells (D) was detected by western immunoblotting. (E) Morphology of ECA-109 and KYSE-150 cells was observed under a light microscope. n.s., not significant; *$p < 0.05$; **$p < 0.01$; and ***$p < 0.001$ vs control.

grown to 70–80% confluence. An LDH cytotoxicity assay kit (Solarbio Life Sciences, Beijing, China) was used to determine the LDH release from cells, according to the manufacturer's instructions.

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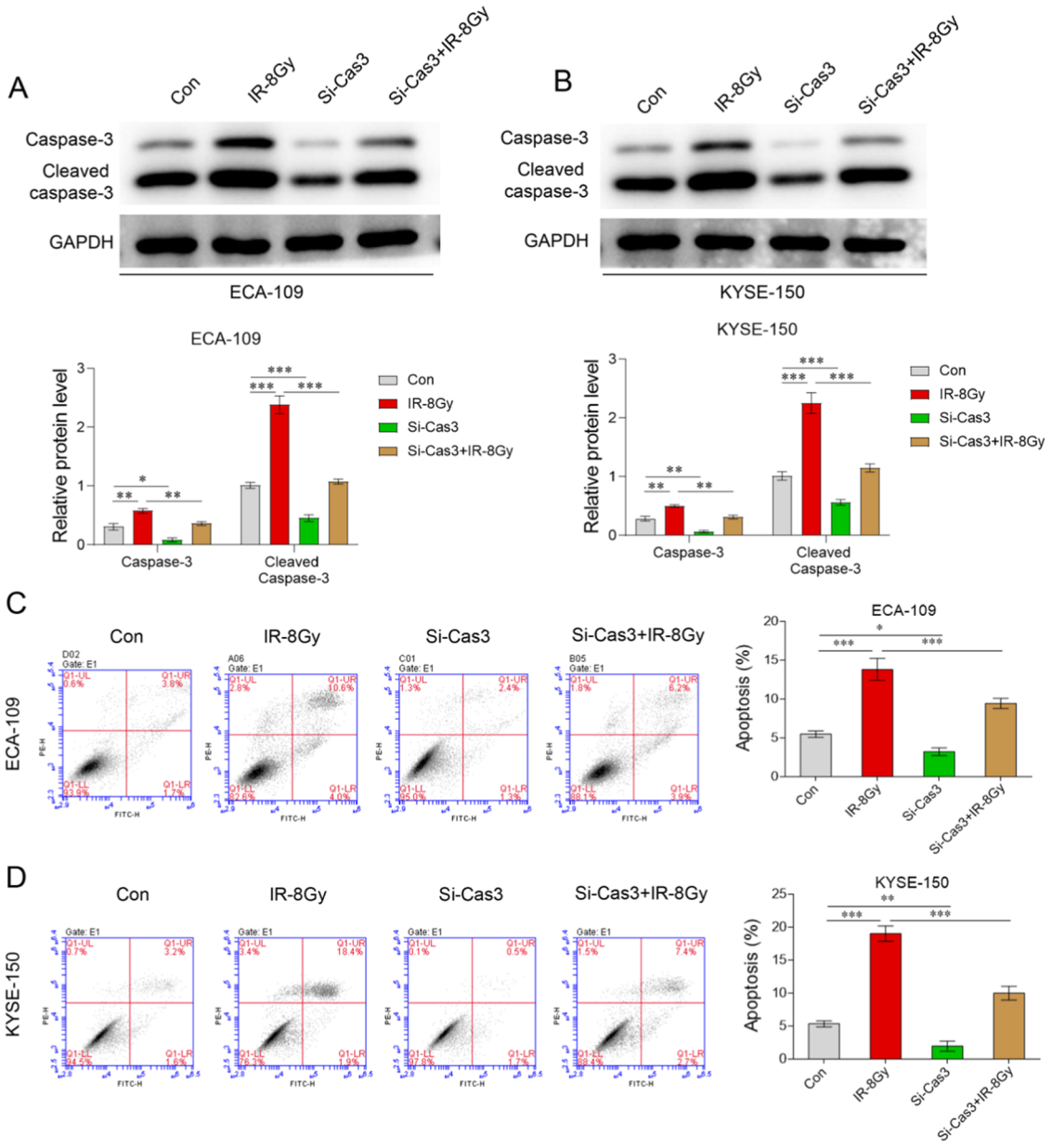

Figure 3. Silencing of caspase-3 reverses irradiation-induced EC cell death. (A–D) ECA-109 and KYSE-150 cells were transfected with caspase-3-specific siRNA and then exposed to 8 Gy of X-ray. Expression of caspase-3 in ECA-109 (A) and KYSE-150 cells (B) was measured by using western immunoblotting. Apoptosis of ECA-109 (C) and KYSE-150 cells (D) was analyzed using an annexin V-FITC/PI detection kit. *$p < 0.05$; **$p < 0.01$; and ***$p < 0.001$ vs the indicated group.

Apoptosis Detection. The apoptosis of ECA-109 and KYSE-150 cells was evaluated by using an Annexin V-FITC/PI detection kit (Sangon Biotech, Shanghai, China). Cells in 24-well plates were collected using a trypsin solution (Sigma-Aldrich) and resuspended in 195 μL binding buffer (5×10^5 cells/mL). Cells were incubated with 5 μL of annexin V-FITC for 15 min in the dark, washed twice with 200 μL of binding buffer, and stained with 10 μL of PI. Annexin V-FITC-positive cells were detected using a flow cytometer (FACSCalibur; BD Biosciences, San Jose, CA).

Western Blotting. Cellular proteins were extracted using RIPA lysis buffer (Thermo Scientific, Rockford, IL), and the protein concentration was quantified using the BCA Protein Assay Kit (Thermo Scientific). Proteins were resolved on SDS-PAGE gels by electrophoresis and transferred onto polyvinylidene fluoride (PVDF; Millipore, Bedford, MA) membranes. After blocking for 2 h with 5% skim milk, the membranes were incubated overnight at 4 °C with the following primary antibodies: caspase-3 (1:1000, 9662S, Cell Signaling Technology, Danvers, MA), GSDME (1:1000, 19453, Cell Signaling Technology), HSP70 (1:1000, ab5439, Abcam, Cambridge, MA), HSP90 (1:500, ab59459, Abcam), and GAPDH (1:2500, ab9485, Abcam). After incubation, membranes were washed and incubated with secondary antibodies conjugated with horseradish peroxidase for 1 h: goat antimouse (1:2500, ab6789, Abcam) or goat antirabbit (1:10000, ab6721, Abcam). Following incubation, membranes were washed, and an ECL chemiluminescence detection kit (Vazyme, Nanjing, China) was used to develop the immunoblotting. Blotting bands were semiquantified using ImageJ software (National Institutes of Health, Bethesda, MD).

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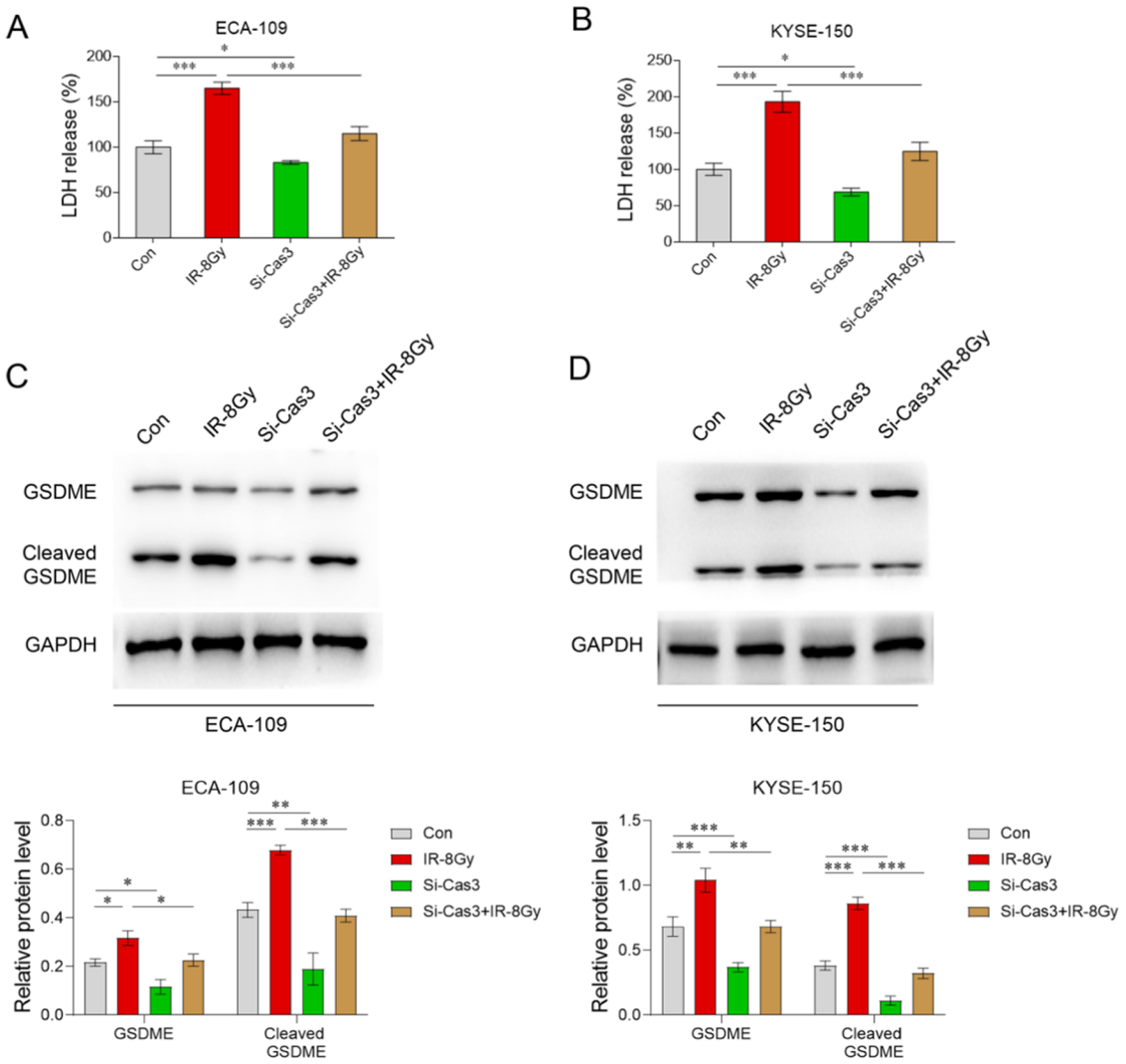

Figure 4. Silencing of caspase-3 reverses irradiation-induced GSDME cleavage. (A–D) ECA-109 and KYSE-150 cells were transfected with caspase-3 specific siRNA and then exposed to 8 Gy of X-ray. The LDH levels in ECA-109 (A) and KYSE-150 cells (B) were measured using an LDH detection kit. The expression of GSDME in ECA-109 (C) and KYSE-150 cells (D) was measured using western immunoblotting. *$p < 0.05$; **$p < 0.01$; and ***$p < 0.001$ vs the indicated group.

Immunofluorescence Assays. ECA-109 and KYSE-150 cells were grown on glass coverslips in 24-well plates. After the indicated treatment, cells were washed twice with PBS, fixed with 4% paraformaldehyde for 15 min, and incubated with 0.5% Triton X-100 for 20 min. The cells were then blocked with normal goat serum (Gibco, Grand Island, NY) at room temperature for 30 min and incubated with CRT monoclonal antibody (1:200, PA5–25922, Invitrogen) at 4 °C overnight. After this procedure, cells were washed and incubated with Alexa Fluor 488 goat antirabbit secondary antibody (1:2000, A27034, Invitrogen) for 1 h at 37 °C. 4′,6-diamidino-2-phenylindole (DAPI) was used for nuclear staining. Immunofluorescence images were captured under a fluorescence microscope (Olympus, Tokyo, Japan), and the fluorescence intensity was quantified using ImageJ software (National Institutes of Health).

Statistical Analysis. Results are presented as the mean ± the standard deviation (standard deviation) from three independent experiments. Statistical analyses were carried out using GraphPad Prism software (version 6.0). One-way analysis of variance followed by Tukey's multiple comparisons test was performed to evaluate statistical differences between groups. A p-value lower than 0.05 was defined as significantly different.

RESULTS

Irradiation in EC Cells Induces Caspase-3 and GSDME Cleavage. We first examined the effect of irradiation on the EC cells. Compared to the control group, ECA-109 and KYSE-150 cells exposed to 2, 4, and 8 Gy X-ray showed significant dose-dependent viability loss ($p < 0.05$, Figure 1A,B). Flow cytometer results also showed an irradiation dose-dependent increase in apoptosis of both cell lines ($p < 0.05$, Figure 1C,D). More specifically, 8 Gy X-ray increased the apoptosis rate from 4.1 to 12.8% in ECA-109 cells and from 4.6 to 21.1% in KYSE-150 cells.

LDH is a key feature of cell death; it is rapidly released into the cell culture supernatant when the plasma membrane is damaged. Here, the LDH level was measured to confirm the irradiation-induced cell death. Compared to the control group, irradiation significantly increased LDH levels in a dose-dependent manner ($p < 0.05$, Figure 2A,B) in the irradiated

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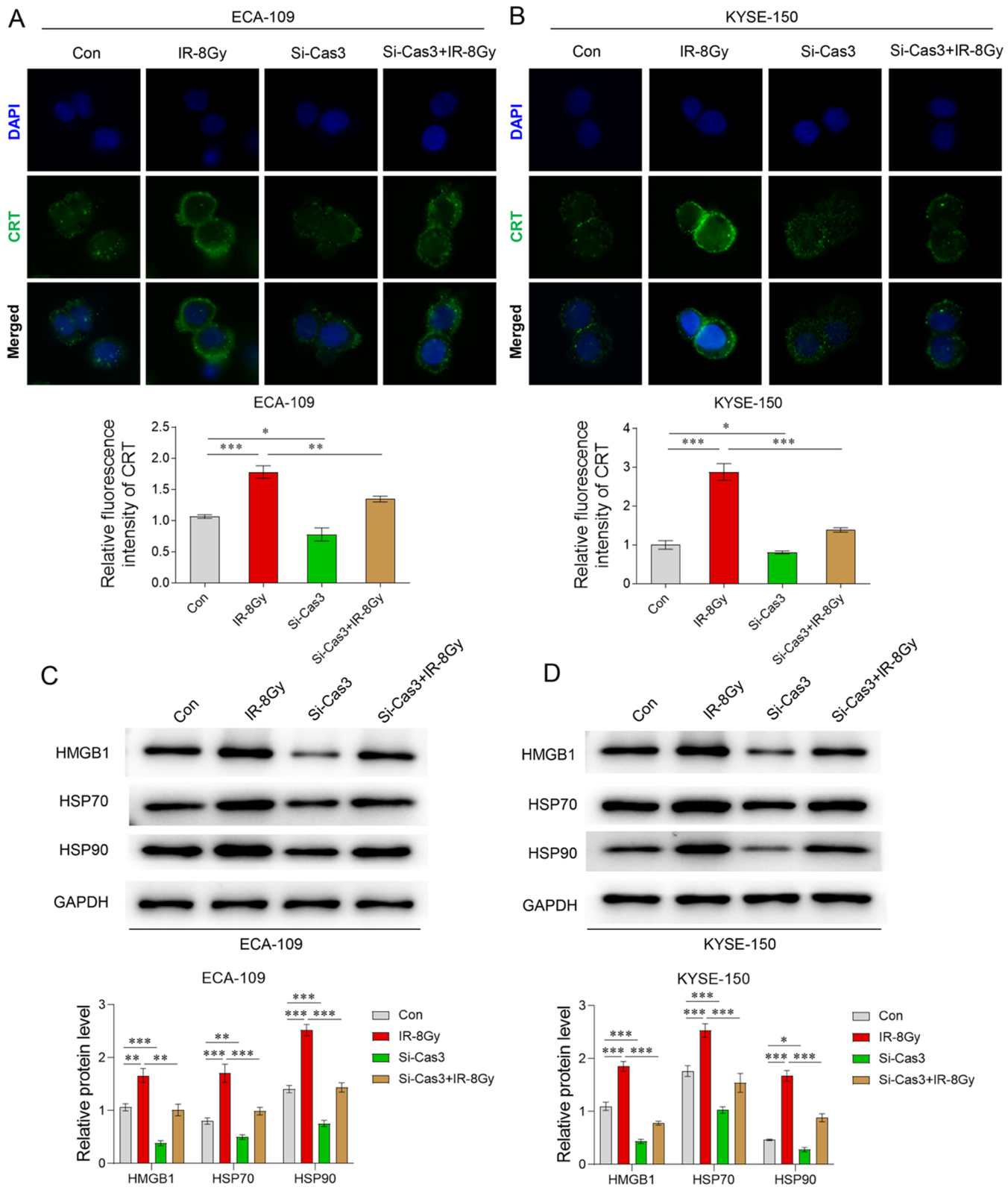

Figure 5. Silencing of caspase-3 reverses irradiation-induced ICD. (A–D) ECA-109 and KYSE-150 cells were transfected with caspase-3 specific siRNA and then exposed to 8 Gy of X-ray. CRT-positive ECA-109 (A) and KYSE-150 cells (B) were detected by using immunofluorescence staining. The expression of HMGB1, HSP70, and HSP90 in ECA-109 (C) and KYSE-150 cells (D) was measured using western immunoblotting. *$p < 0.05$; **$p < 0.01$; and ***$p < 0.001$ vs the indicated group.

cells. Additionally, irradiation induced a dose-dependent cleavage of caspase-3 and GSDME in ECA-109 and KYSE-150 cells ($p < 0.05$, Figure 2C,D). Cellular morphology was observed under a light microscope. Cells subjected to X-rays exhibited a typical pyroptosis morphology, including cell swelling and membrane blebbing (Figure 2E).

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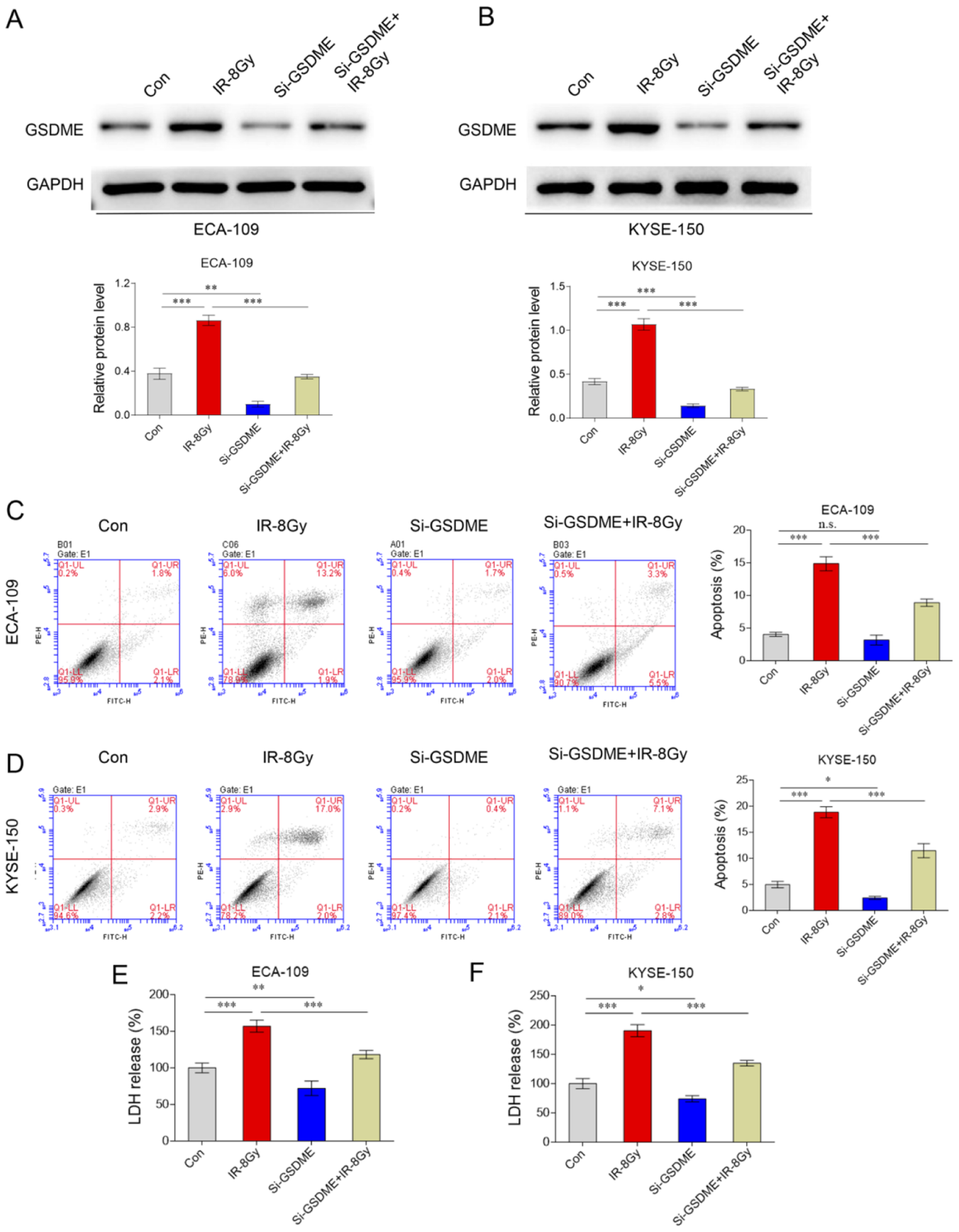

Figure 6. Silencing of GSDME reverses irradiation-induced tumor cell death. (A–F) ECA-109 and KYSE-150 cells were transfected with GSDME-specific siRNA and then exposed to 8 Gy of X-ray. The expression of GSDME in ECA-109 (A) and KYSE-150 cells (B) was measured by using western immunoblotting. The apoptosis of ECA-109 (C) and KYSE-150 cells (D) was analyzed using an annexin V-FITC/PI detection kit. The

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Figure 6. continued

LDH levels in ECA-109 (E) and KYSE-150 cells (F) were measured using an LDH detection kit. n.s., not significant; *$p < 0.05$; **$p < 0.01$; and ***$p < 0.001$ vs the indicated group.

Caspase-3 Silencing Reverses Irradiation-Induced EC Cell Death. To evaluate the effects of caspase-3 on irradiation-induced tumor cell death, expression of caspase-3 was silenced by transfection with a specific siRNA in both EC cell lines. Caspase-3 siRNA markedly suppressed 8 Gy irradiation-induced caspase-3 expression ($p < 0.05$, Figure 3A,B). Furthermore, caspase-3 siRNA significantly inhibited 8 Gy irradiation-induced apoptosis, from 13.8 to 9.4% in ECA-109 cells, and from 19.0 to 10.0% in KYSE-150 cells ($p < 0.05$, Figure 3C,D).

Caspase-3 siRNA transfection also suppressed 8 Gy irradiation-induced LDH release ($p < 0.05$, Figure 4A,B) and markedly inhibited GSDME expression and cleavage in EC cells (with or without 8 Gy irradiation) ($p < 0.05$, Figure 4C,D). These data suggest that caspase-3 silencing can inhibit irradiation-induced GSDME cleavage and tumor cell death.

Caspase-3 Silencing Reverses Irradiation-Induced ICD. CRT, an ICD hallmark, was analyzed using immunofluorescence staining. As shown in Figure 5A,B, 8 Gy irradiation increased the CRT fluorescence intensity in ECA-109 and KYSE-150 cells. The 8 Gy irradiation-induced CRT production was inhibited by transfection with caspase-3 siRNA ($p < 0.05$). Western blotting results showed that 8 Gy irradiation upregulated HMGB1, HSP70, and HSP90 protein levels, whereas caspase-3 siRNA downregulated them ($p < 0.05$, Figure 5C,D). These results indicate that irradiation-induced ICD in EC cells depends on caspase-3 expression.

GSDME Silencing Reverses Irradiation-Induced Tumor Cell Death. The effects of GSDME on irradiation-induced tumor cell death were studied by the transfection of EC cell lines with a GSDME-specific siRNA. Western blotting showed that the GSDME siRNA effectively suppressed GSDME protein levels ($p < 0.05$, Figure 6A,B). Furthermore, GSDME siRNA significantly inhibited 8 Gy irradiation-induced apoptosis, from 14.9 to 8.9% in ECA-109 cells and from 18.8 to 11.5% in KYSE-150 cells ($p < 0.05$, Figure 6C,D). Additionally, GSDME siRNA significantly reduced 8 Gy irradiation-induced LDH release in both EC cell lines ($p < 0.05$, Figure 6E,F), suggesting that irradiation-induced tumor cell death occurs in a GSDME-dependent manner.

GSDME Silencing Reverses Irradiation-Induced ICD. Immunofluorescence staining showed that GSDME siRNA markedly suppressed 8 Gy irradiation-induced CRT in ECA-109 and KYSE-150 cells ($p < 0.05$, Figure 7A,B). Furthermore, GSDME siRNA decreased HMGB1, HSP70, and HSP90 protein levels, induced by 8 Gy irradiation, in both EC cell lines ($p < 0.05$, Figure 7C,D). Altogether, these results suggest that irradiation-induced EC cell ICD occurs in a GSDME-dependent manner.

Overexpression of Caspase-3 and GSDME Aggravates Irradiation-Induced ICD. Next, the overexpression of caspase-3 and GSDME was evaluated to confirm the results. Overexpression plasmid transfection markedly increased caspase-3 and GSDME expression in ECA-109 cells ($p < 0.05$, Figure 8A,B). Apoptosis induced by 8 Gy irradiation was increased from 12.9 to 26.2% by caspase-3 overexpression and from 14.8 to 22.9% by GSDME overexpression ($p < 0.05$, Figure 8C,D). The LDH levels induced by 8 Gy irradiation were also increased in ECA-109 cells transfected with caspase-3 and GSDME overexpression plasmids ($p < 0.05$, Figure 8E,F). In addition, caspase-3 and GSDME overexpression increased the expression of HSP70 and HSP90 induced by 8 Gy irradiation in ECA-109 cells ($p < 0.05$, Figure 8G,H).

DISCUSSION

In this study, two EC cell lines, ECA-109 and KYSE-150, were exposed to a 0−8 Gy range of irradiation. As expected, we observed irradiation-induced dose-dependent viability loss and apoptosis. In addition, irradiation rapidly cleaved caspase-3 and GSDME in the EC cells. Caspase-3 silencing using a specific siRNA significantly inhibited irradiation-induced GSDME activation and EC cell death. The cell death suppressed by caspase-3 silencing was identified as ICD through specific changes in the levels of ICD markers, including CRT, HMGB1, HSP70, and HSP90. Similarly, GSDME silencing also decreased irradiation-induced EC cell ICD. Overexpression of caspase-3 and GSDME accelerated irradiation-induced EC cell ICD.

In the clinic, radiotherapy is one of the most commonly used methods for curative and palliative purposes. More than 50% of patients with cancer receive radiotherapy, and it contributes to approximately 40% of curative cases in all cancers.[19] Ionizing radiation, such as X-rays, shows high toxicity in tumor cells, as well as in the surrounding normal cells. Nonetheless, radiation is effective in killing tumor cells and controlling micrometastases.[7,19] Currently, two main mechanisms are recognized as being responsible for the antitumor effects of radiotherapy: (1) radiation directly damages tumor cell DNA, inducing cell cycle arrest and cell death, and (2) radiation indirectly produces reactive oxygen species, which damages DNA and eventually promotes tumor cell death.[20] Beyond its local action, radiation also induces systemic effects responsible for the long-term antitumorigenic effects of radiotherapy.[21] Cells dying after radiation exposure release DAMPs to initiate immune system reactions.[10,21] Recent studies have shown the immunogenic potential of radiation in tumor cells through the triggering of ICD.[22] For instance, fractionated radiation increases the production of HMGB1 and HSP70 in glioblastoma cells.[23] In prostate cancer cells, multifractionated radiation induces HMGB1 production and cytokine release to activate immune response.[24] In breast, lung, and prostate cancer cells, radiation induces production of HMGB1 to enhance T-cell ability to kill tumor cells.[25] In our current study, the overproduction of CRT, HMGB1, HSP70, and HSP90 was observed in EC-irradiated cells. This finding is consistent with other reports,[11,26] suggesting ICD as another mechanism of radiotherapy-induced antitumor effects.

Apart from ICD, radiotherapy can also induce tumor cell pyroptosis.[9] Radiation induces GSDME-triggered pyroptosis, which enhances radiosensitivity and suppresses tumor growth of colorectal cancer homografts in mice.[17] Furthermore, activation of lung cancer cell pyroptosis induced by circNEIL3 knockdown enhances the antitumorigenic effect of radiotherapy.[27] The caspase-3/GSDME signal is a major trigger in the conversion of tumor cell apoptosis to pyroptosis.[15] As a

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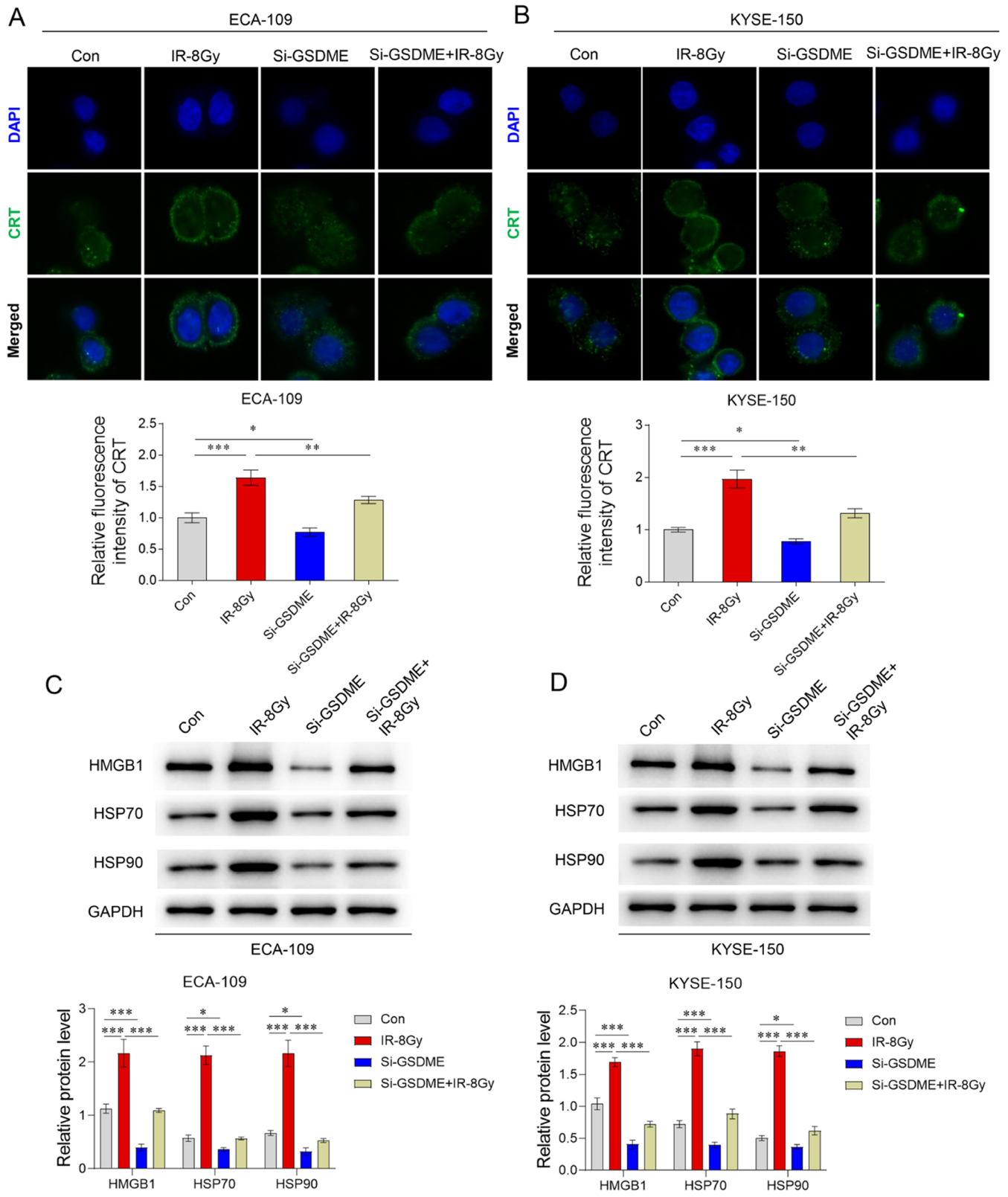

Figure 7. Silencing of GSDME reverses the irradiation-induced ICD. (A–D) ECA-109 and KYSE-150 cells were transfected with GSDME-specific siRNA and then exposed to 8 Gy of X-ray. CRT-positive ECA-109 (A) and KYSE-150 cells (B) were detected using immunofluorescence staining. The expression of HMGB1, HSP70, and HSP90 in ECA-109 (C) and KYSE-150 cells (D) was measured using western immunoblotting. *$p < 0.05$; **$p < 0.01$; and ***$p < 0.001$ vs the indicated group.

critical executor of pyroptosis, GSDME has been recognized as a tumor suppressor in various human cancers.[16] Furthermore, several therapeutic strategies, such as photodynamic therapy,[28] dihydroartemisinin,[29] cisplatin,[30] and the natural compound from *Garcinia bracteata*,[31] can induce caspase-3-mediated GSDME activation and trigger EC cell pyroptosis. In this

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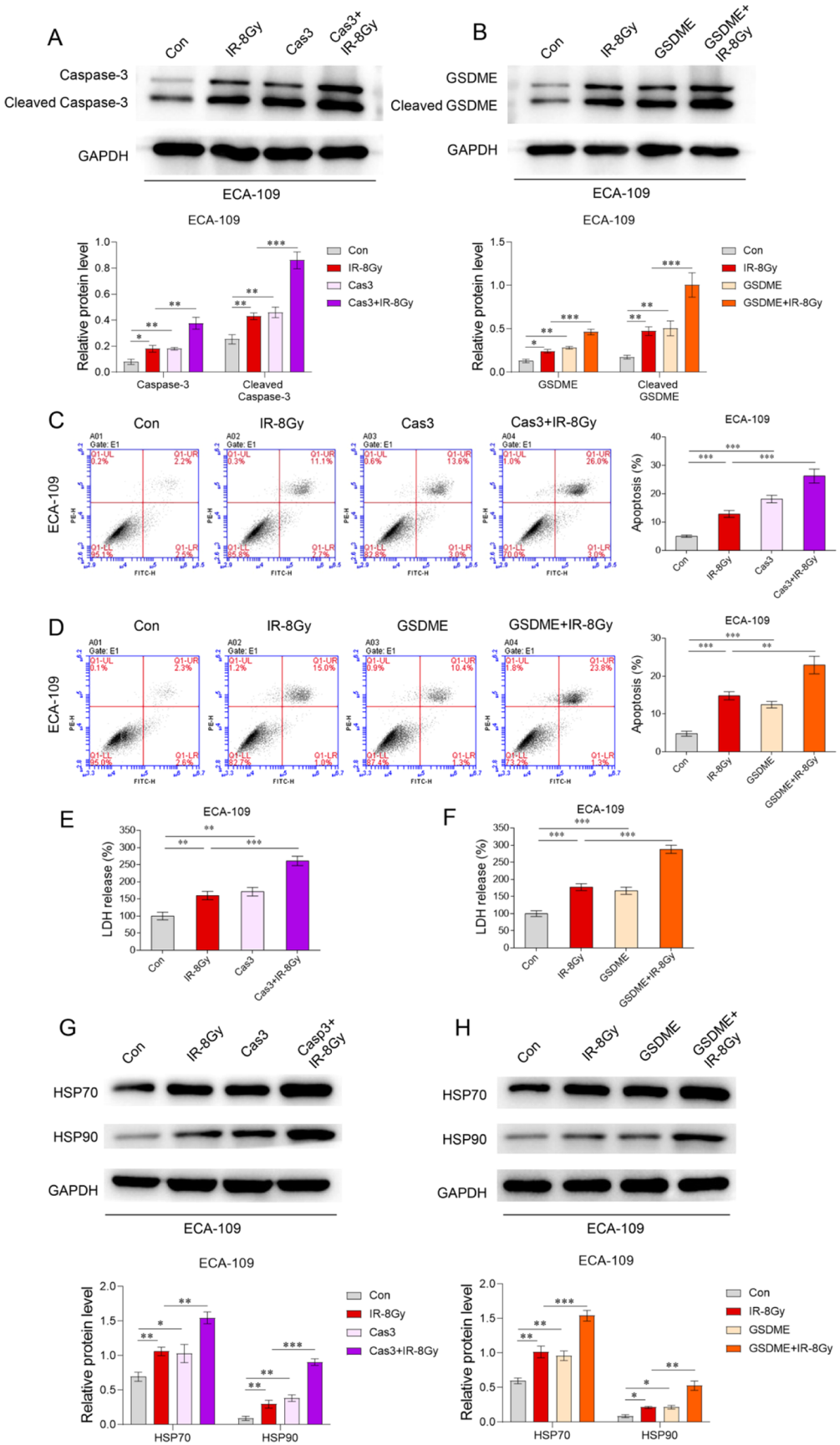

Figure 8. Overexpression of caspase-3 and GSDME aggravates irradiation-induced ICD. (A−H) ECA-109 cells were transfected with caspase-3 or GSDME overexpression plasmids and then exposed to 8 Gy of X-ray. The expression of caspase-3 (A) and GSDME (B) in ECA-109 cells was measured by using western immunoblotting. Apoptosis of ECA-109 cells following transfection with caspase-3 (C) and GSDME (D) overexpression plasmids were analyzed using an annexin V-FITC/PI detection kit. The LDH release of ECA-109 cells followed transfection with

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Figure 8. continued

caspase-3 (E) and GSDME (F) overexpression plasmids using an LDH detection kit. Expression of HSP70 and HSP90 in ECA-109 following transfection with caspase-3 (G) and GSDME (H) overexpression plasmids were measured by western immunoblotting. *$p < 0.05$; **$p < 0.01$; and ***$p < 0.001$ vs the indicated group.

study, we show for the first time the activation of caspase-3 and GSDME in irradiated EC cells, suggesting radiation-induced pyroptosis. In fact, caspase-3/GSDME signal-mediated pyroptosis has been associated with antitumor immunity.[16] Here, we demonstrate that radiation in EC cells induces GSDME-mediated pyroptosis and activates antitumor immunity, as the levels of CRT, HMGB1, HSP70, and HSP90 were downregulated by caspase-3/GSDME silencing.

Altogether, this study demonstrated that radiation inhibits EC cell survival in vitro via GSDME-mediated pyroptosis and ICD. Studying the inflammatory factors, such as caspase-3 and GSDME, which are upregulated by radiation, may help us to further understand the systemic antitumor effects of radiotherapy. Further studies should explore the link between radiation and GSDME-mediated antitumor immunity in vivo and whether targeting GSDME can overcome immune evasion.

■ ASSOCIATED CONTENT

Data Availability Statement

The data sets used and analyzed during the current study are available from the corresponding author upon reasonable request.

■ AUTHOR INFORMATION

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Notes

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