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STAT3 as a target for sensitizing prostate cancer cells to irradiation

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ABSTRACT

Radioresistance of prostate cancer (PCa) is a major factor leading to local failure of radiotherapy. STAT3 is an oncogenic protein that was recently found to be activated in PCa tumors. This study aimed to investigate the radiosensitization effect of targeting STAT3 in PCa tumors. Here, the radiosensitization effect of STAT3 blockade was investigated by clonogenic assay, flow cytometry and western blot analysis in human PCa cells *in vitro* and *in vivo*. We demonstrated that STAT3 blockade with a STAT3 inhibitor or siRNA increased the radiosensitivity of PCa cells and that radiation together with STAT3 blockade induced more apoptosis and double-strand breaks (DSBs) than radiation alone in LNCaP cells. In addition, radiation induced STAT3 activation and survivin expression in PCa cells, which was inhibited by STAT3 blockade. Transfection with survivin cDNA attenuated the radiosensitization effect of STAT3 blockade. These effects were further confirmed by *in vivo* studies, which showed that the STAT3 inhibitor enhanced the treatment efficacy of radiation on LNCaP xenografts with decreased STAT3 activation and survivin expression. These findings suggest that STAT3 blockade radiosensitizes PCa cells through regulation of survivin. Thus, our study has revealed STAT3 as a potential sensitizer for irradiation in PCa cells. Its clinical application as an adjuvant in radiotherapy of PCa should be explored in the future.

Keywords: STAT3; radiosensitivity; prostate cancer (PCa); survivin; apoptosis

INTRODUCTION

Prostate cancer (PCa) is the second most frequent cancer in men and the fifth leading cause of death worldwide [1]. Radiation therapy (RT) is a standard treatment for patients with intermediate- to high-risk PCa [2]. Despite the use of radiation dose-escalation with modern techniques such as Intensity-modulated radiotherapy (IMRT) and Stereotactic body radiation therapy (SBRT), which improves local disease control, intermediate- and high-risk PCa patients continue to develop recurrence after definitive radiotherapy [3]. Treatment failure after radiotherapy may be due to the low intrinsic radiosensitivity of PCa cells within the primary tumor. PCa radiosensitivity remains the most investigated issue in PCa radiation treatment.

Signal transducer and activator of transcription 3 (STAT3) is an oncogenic protein and STAT3 signaling pathway plays a critical role in the occurrence and development of tumor cells [4]. After activation by Janus kinases (JAK) via phosphorylation, STAT3 translocates into the nucleus and acts on the transcription of several genes involved in anti-apoptosis, proliferation and metastasis [5]. STAT3 was recently found to be expressed and constitutively activated in PCa tumors and cell lines [6–8]. In recent years, increasing studies have focused on its potential therapeutic value in PCa. Activated STAT3 regulates gene transcription to promote antiapoptosis, proliferation and metastasis [5], and emerging evidence suggests that STAT3 promotes tumor radioresistance. Activation of STAT3 was found to increase radioresistance in breast cancer [9], and the JAK2/STAT3 axis promotes colorectal cancer stem cell persistence and radioresistance [10]. Additionally, an *in vitro* study showed that blockade of STAT3 elicited radioresistant tumor cells [11].

The purpose of the current study was to investigate whether STAT3 can be a target to sensitize PCa cells to radiation and to determine

whether survivin is involved in the regulation of cell radiosensitivity induced by STAT3 blockade.

MATERIALS AND METHODS

Cell lines and reagents

The human PCa cell lines LNCaP and DU-145 were purchased from National collection of Authenticated cell Cultures, Shanghai, China. Cell lines were authenticated at source by STR profiling, morphology (ATCC) and DNA profiling (ECACC). The cells were cultured in RPMI-1640 medium (Gibco, CA, USA) supplemented with 10% calf serum (Gibco, CA, USA), 100 units/ml penicillin and 100 mg/ml streptomycin (Sigma-Aldrich, St. Louis, USA). Stattic hydrochloride (> 95%) which is a potent STAT3 inhibitor and inhibits STAT3 phosphorylation (at Y705 and S727) was purchased from Sigma-Aldrich. Antibodies against STAT3 (Mouse IgG, Cat. # 9139, Cell Signaling), phospho-STAT3 (pSTAT3) (Tyr705) (Rabbit IgG, Cat. # 9145, Cell Signaling) and β-actin (Mouse IgG, Cat. # 3700, Cell Signaling) were obtained from Cell Signaling Technology Inc., CA, USA. Antibodies against survivin (Rabbit IgG, Cat. # ab76424, Abcam) were obtained from Abcam Inc., Cambridge, UK.

Irradiation protocols

Irradiation (IR) was performed with 6 MV X-rays at a dose rate of 5.66 Gy/min using an X-ray irradiator (Elekta, Sweden) at room temperature. Animals received 8 Gy (4.48 cGy/min) for tumor radiotherapy (RS-200 Pro Biological Irradiator).

Quantitative real-time PCR

Paraffin embedded prostate carcinomas samples of patients treated at the Hubei Cancer Hospital have been retrospectively collected and analyzed for this study. Informed consent was obtained from each patient. This study was inspected and approved by Ethics Committee of Hubei Cancer Hospital; the approval number is LLHBCHLW-15. Macrodissection of tumor tissue from several paraffin sections was used to minimize the influence of surrounding normal tissues. RNA was isolated using TRIzol reagent (RNAiso; TaKaRa). RNA was extracted with phenol-chloroform, precipitated with ethanol, and resuspended in diethyl pyrocarbonate-treated H2O. cDNA was prepared with a QuantiTect Reverse Transcription kit (Qiagen) and subjected to quantitative real-time PCR (Applied Biosystems ABI 7500). Primer pairs for STAT3 and survivin were purchased from Qiagen. STAT3: 5'-CATATGCGGCCAGCAAAGAA-3′ (forward), 5'-ATACCTGCTCTGAAGAAACT-3′ (reverse). β-actin: 5'-AGAGCTACGAGCTGCCTGAC-3′ (forward), 5'-AGCACTGTGTTGGCGTACAG-3′ (reverse). Survivin: 5'-CCCTGCCTGGCAGCCCTTTC-3′(forward), 5'-CTGGCTCCCAGCCTTCCA-3′(reverse). Quantitative real-time PCR was carried out using EvaGreen (ABM) according to the manufacturer's protocol.

Cell transfection

Cells were transfected using the liposome delivery system (Cat. # 11668027, Thermo Fisher Scientific). Briefly, cells were grown on petri dishes overnight and incubated with a plasmid containing the cDNA of wild-type survivin (Biovector, China)/liposome complex in serum-free medium for 5 to 6 h followed by replacement of serum-containing medium and incubation at 37°C for 16 to 24 h. For transfection of small interfering RNA (siRNA), siRNA oligonucleotides targeting STAT3 and nonspecific siRNA oligonucleotides were purchased from Sigma-Aldrich: 5'-AACAUCUGCCUAGAUCGGCUAdTdT-3′; 3'-dTdTGUAGACGGAUCUAGCCGAU-5′, along with a universal control set of siRNAs (Sigma Aldrich, MO, USA). The siRNA oligonucleotides were transfected into PCa cells using liposomes according to the standard protocol provided by the manufacturer. Forty-eight hours after transfection, cells were harvested and analyzed by western blotting or used in further experiments.

Cell proliferation assay

PCa cells in early log phase were trypsinized and plated in 96-well plates at a density of 4000 cells/well. After 24 h, the medium was removed and replaced with fresh medium containing different concentrations of stattic (0, 2.5, 5, 10, 20 μM). Cell viability was measured for 24 h by using a methylthiazolyldiphenyl-tetrazolium bromide (MTT) kit (Sigma-Aldrich) following the manufacturer's instructions. Briefly, 10 μl of MTT was added to each well at a final concentration of 0.5 mg/ml. Then, the cells were incubated for 4 h at 37°C. Subsequently, the media/MTT mixture was removed, and 150 μl of dimethyl sulfoxide (DMSO) was added to dissolve the MTT crystals (formazan). The absorbance of the sample at 490 nm was read using a Bio-Rad microplate reader (model 630; Hercules, CA, USA).

Clonogenic survival assay

Cells were seeded into 6-well plates. Then, the cells were treated with or without stattic for 24 h or 48 h after transfection with STAT3 siRNA. Next, the cells were subjected to 0, 2, 4, 6 and 8 Gy X-rays at room temperature, cultured at 37°C for 12 days, fixed with methanol and stained with Giemsa. Finally, the numbers of colonies containing at least 50 cells were counted by microscopy. The surviving fraction (SF) was calculated with respect to the survival of unirradiated controls.

Annexin V-PI staining

Cells in early log phase were trypsinized and plated in 6-well plates at a specific density. The cells were treated with or without stattic for 24 h or 48 h after transfection with STAT3 siRNA and then exposed to X-rays (6 Gy). After 24 h, the samples were harvested. Cells were collected and labeled with Annexin V reagent (Sigma Aldrich, MO, USA) according to the manufacturer's instructions. Cell death was analyzed by staining with propidium iodide (Sigma Aldrich, MO, USA) for 15 min, and apoptotic cells were detected by BD FACScan flow cytometer (BD Bioscience, Oxford, UK).

Western blotting assay

Total cell lysates were prepared by harvesting cells in protein extraction buffer, and the protein concentration was analyzed using the BCA protein assay kit (Beyotime, Jiangsu, CN). Equal amounts of protein were separated by 10% SDS-polyacrylamide gel electrophoresis, transferred to Protran nitrocellulose membranes (Schleicher and Schuell

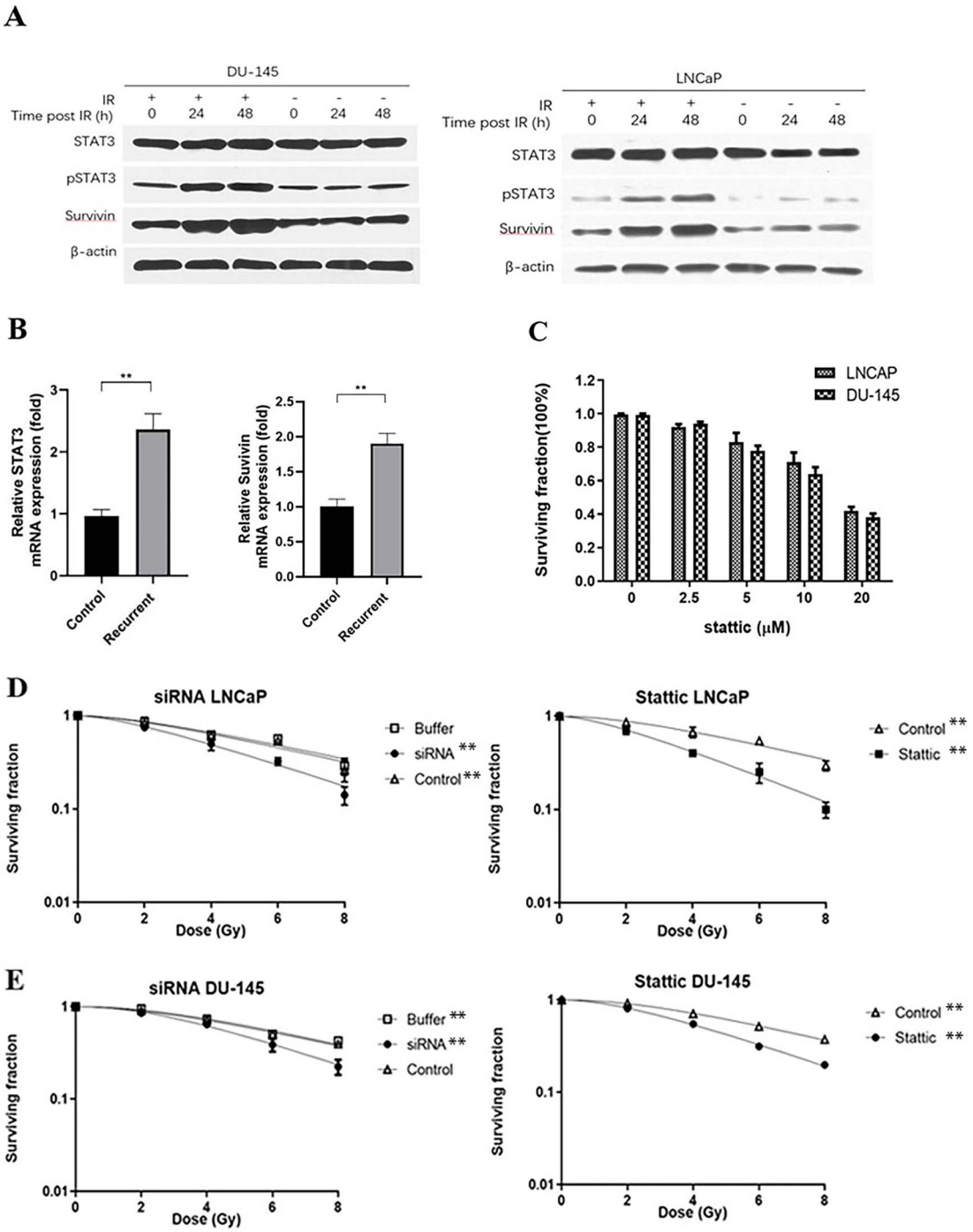

Fig. 1. Irradiation increases the activation of STAT3 in PCa cells, and STAT3 blockade increases radiosensitivity. A Western blot analysis was used to detect the levels of pSTAT3 and survivin in LNCaP and DU-145 cells 0 h, 24 h and 48 h after irradiation (5 Gy). B Stat3 and survivin expression in PCa tissue was detected by real-time PCR in patients who developed recurrence (recurrent group, n = 6) after radiotherapy and in patients who had no recurrence (control group) after radiotherapy, $^{**}P < 0.01$. The data are shown as the mean ± SD (n = 6). C LNCaP and DU-145 cells were treated with stattic at the indicated concentration for 24 h, and cell viability was detected by MTT assay. Stattic inhibited the viability of PCa cells in a dose-dependent manner. The inhibitory effect of stattic and siRNA silencing on STAT3 was confirmed by western blotting. D Clonogenic survival assay of LNCaP cells pretreated with stattic (5 μM) or STAT3 silencing and exposed to 0–8 Gy irradiation, $^{**}P < 0.01$. E Clonogenic survival assay of DU-145 cells exposed to 0–8 Gy irradiation and pretreated with stattic (5 μM) or STAT3 silencing, $^{**}P < 0.01$. The data are shown as the mean ± SD (n = 3).

Bioscience), blocked with 5% nonfat milk in TBST (Tris-buffered saline, pH 7.4 and 0.05% Tween 20) and incubated with antibodies against STAT3 (1:500), pSTAT3 (1:500), survivin (1:500) and β-actin (1:250) overnight at 4°C. Next, the membranes were incubated with alkaline phosphatase-conjugated goat anti-mouse IgG or goat anti-rabbit IgG (1:2000) for 1 h at room temperature. Finally, the signals were detected by using an enhanced chemiluminescence detection kit (Thermo Fisher Scientific, IL, USA).

Immunofluorescence

Cells were exposed to X-ray radiation (1 Gy). Then, the cells were fixed with 4% paraformaldehyde at 0.5, 4 and 24 h, permeabilized with 0.1% Triton X-100, stained with γ-H2AX antibody (Rabbit IgG, Cat. # ab11174, Abcam) (diluted 1:200) at 4°C overnight and incubated with fluorescein isothiocyanate (FITC)-conjugated secondary antibody (Goat anti-Rabbit IgG, Cat. # 65–6111, Thermo Fisher Scientific) (diluted 1:100) for 1 h at room temperature. Finally, the slides were observed under a laser scanning confocal microscope (Zeiss LSM510).

Tumor xenograft mouse models

Four- to five-week-old male BALB/c nude mice were provided by Tongji Medical College Animal Center. All procedures were in accordance with the guidelines of the laboratory animal ethics committee of Hubei Cancer Hospital. Tumors were induced by subcutaneous (s.c.) injection of LNCaP cells (5×10^6 cells in 0.1 ml of PBS) at one site of the right armpit. When the average volume of the tumor mass reached approximately 150 mm^3, the mice were randomly divided into four groups: (i) vehicle (control), (ii) 25 mg/kg stattic alone, (iii) single dose of 8 Gy IR, and (iv) 25 mg/kg stattic plus 8 Gy IR. The mice in the control group were treated with vehicle control only, whereas groups 3 and 4 were given daily intraperitoneal injections of stattic. Tumors were irradiated by an RS-2000 biological irradiator at a dose of 8 Gy with X-rays. IR was delivered 2 h after injection on day 5 for groups 2 and 4. Tumor growth was measured every three days. On day 34, the mice were sacrificed. The tumor volume, tumor growth inhibition (TGI) and tumor doubling time (DT) were calculated. Tumor volume was calculated with the formula: tumor volume (mm^3) = length diameter (mm) × width diameter $(mm)^2/2$.

Statistical analysis

Statistical comparisons between two groups were performed with Student's t-test. Data are represented as the means ± standard deviations (SDs). A two-tailed one-way analysis of variance (ANOVA) with a post hoc Dunnett's multiple comparison test was used for comparing statistical differences in data from the cell survival assay. Differences between mean values were considered statistically significant when the associated p-value was less than 0.05.

RESULTS

Irradiation increases the activation of STAT3 in PCa cells

To investigate IR-induced activation of STAT3 in PCa cells, pSTAT3 expression was evaluated by western blotting. The accumulation of pSTAT3 increased in a time-dependent manner in irradiated PCa cells (Fig. 1A). Survivin plays important roles in cell proliferation and survival [12], and we found that survivin expression was also increased in irradiated PCa cells. To further investigate the correlation of STAT3 with the radiosensitivity of PCa, we compared STAT3 expression in PCa tissue in patients who developed recurrence (recurrent group, n = 6) after radiotherapy and in patients who had no recurrence (control group) by using real-time PCR (patient characteristics are shown in Supplementary Table 1). The recurrent group showed higher STAT3 expression than the control group (Fig. 1B).

STAT3 blockade increases the radiosensitivity of PCa cells

To evaluate the toxicity of the STAT3 inhibitor stattic and siRNA silencing on PCa cells, an MTT assay was performed. The cells were treated with increasing doses of stattic for 24 h. As shown in Fig. 1C, stattic inhibited the viability of LNCaP and DU-145 cells in a dose-dependent manner. The IC50 values for LNCaP and DU-145 cells were calculated, and a dose of 5 μM stattic was selected in further experiments to avoid toxicity. Transfection of STAT3 siRNA (10 nM) showed no toxicity on PCa cells by the MTT assay at 48 h and 72 h after transfection ($P > 0.05$). The inhibitory effect of stattic and siRNA silencing on STAT3 was confirmed by western blotting (Fig. 3).

To investigate the effect of STAT3 blockade on the radiosensitivity of PCa cells, the cells were pretreated with stattic (5 μM) for 24 h or transfected with STAT3 siRNA (10 nM) and then subjected to a clonogenic assay. The clonogenic assay generated radiation dose–response survival curves for LNCaP and DU-145 cells with or without stattic treatment (Fig. 1D and E), which demonstrated that stattic significantly suppressed the clonogenic formation of LNCaP and DU-145 cells ($P < 0.01$). To further confirm that STAT3 blockade could increase the radiosensitivity of PCa cells, STAT3 siRNA was used. The results showed that STAT3 silencing also demonstrated radiosensitization effects in LNCaP and DU-145 cells ($P < 0.01$) (Fig. 1D and E). The radiosensitization effects of stattic and STAT3 silencing in PCa cells are summarized in Supplementary Tables 2-5.

STAT3 blockade increases radiation-induced apoptosis of LNCaP cells

To investigate whether STAT3 blockade-induced radiosensitization is due to increased apoptosis in PCa cells, flow cytometry was used to detect apoptosis, and treatment with stattic (5 μM) alone for 24 h induced apoptosis ($P < 0.01$) in LNCaP cells. Furthermore, stattic combined with IR (6 Gy) induced more apoptotic cell death than treatment with IR (6 Gy) alone in LNCaP cells ($P < 0.01$) (Fig. 2A and B). Flow cytometry analysis showed that STAT3 silencing combined with IR (6 Gy) similarly induced more apoptotic cell death than IR (6 Gy) alone in LNCaP cells ($P < 0.01$) (Fig. 2A and B).

IR induces apoptosis via the generation of DNA double-strand breaks (DSBs). Therefore, we investigated the effect of STAT3 blockade on the generation of IR-induced DSBs. DSBs were detected by

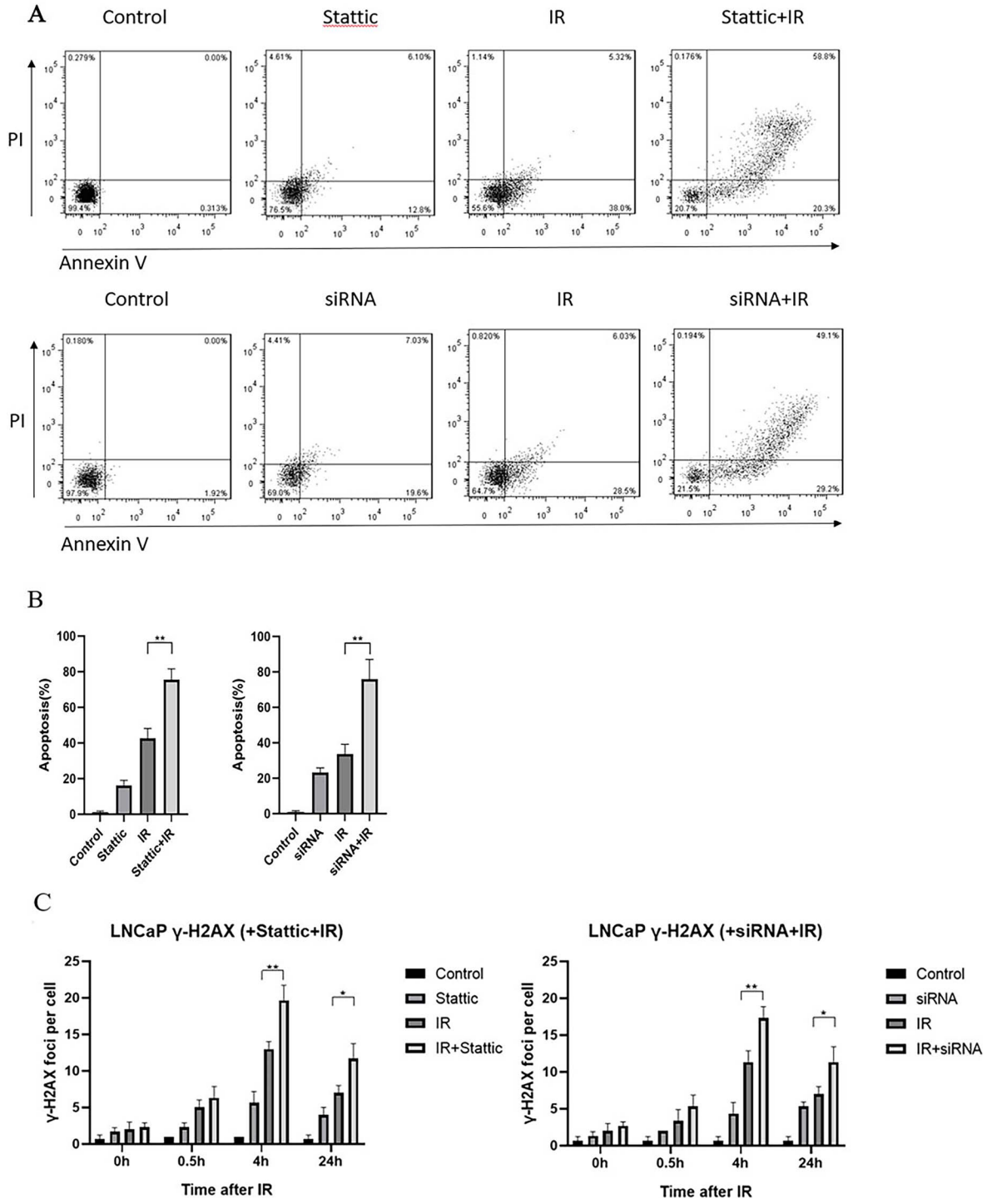

Fig. 2. STAT3 inhibition significantly enhance irradiation-induced apoptosis. A, B Flow cytometry was used to detect the apoptosis rate (annexin V+ cells) of LNCaP cells treated with irradiation (5 Gy) in combination with the STAT3 inhibitor stattic (5 μM) or STAT3-specific siRNA, $^{*}P < 0.05$, $^{**}P < 0.01$. C Determination of γ-H2AX foci in LNCaP cells 0 h, 4 h, and 24 h after irradiation with 1 Gy combined with 5 μM stattic or STAT3 siRNA (mean $\pm$ SD, n = 3), $^{*}P < 0.05$, $^{**}P < 0.01$.

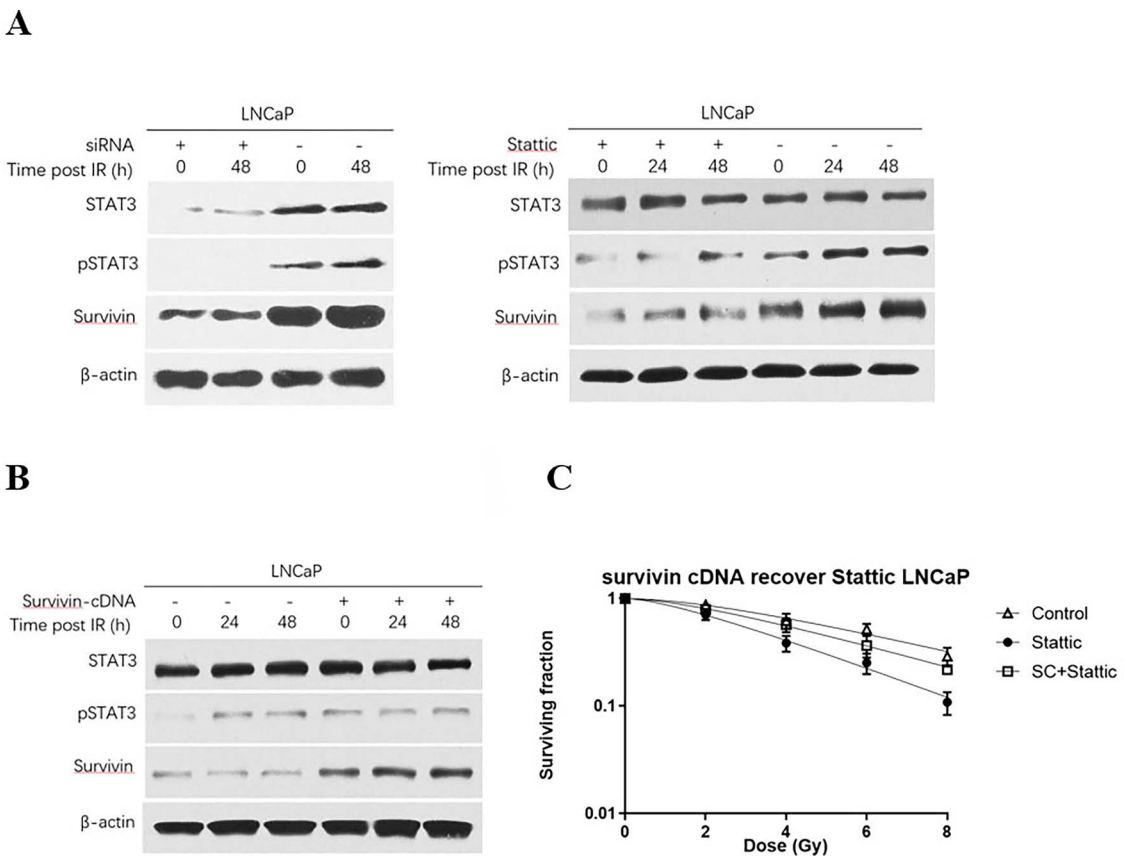

Fig. 3. STAT3 inhibition decreases radiation-induced survivin expression. Survivin overexpression attenuates the radiosensitization effect of STAT3 inhibition. A LNCaP cells pretreated with STAT3-specific siRNA (left panel) or the STAT3 inhibitor stattic (right panel) were irradiated (5 Gy) and then subjected to western blotting to detect irradiation-induced pSTAT3 activation and survivin expression at different time points. B LNCaP cells pretreated with stattic were transiently transfected with either the plasmid containing wild-type survivin cDNA (SC) or empty plasmid vector. Clonogenic survival assays were performed to analyze the SF of LNCaP cells after irradiation. C Western blotting was used to detect the expression of survivin and pSTAT3.

immunofluorescence staining of γ-H2AX foci in LNCaP cells at different time points after IR. We observed increased DSB formation with a maximum at 4 h after IR ($P < 0.01$) (Fig. 2C). The average number of γ-H2AX foci per cell was significantly greater in cells treated with both IR and stattic or STAT3 silencing than in cells treated with IR alone at 0.5, 4 and 24 h ($P < 0.01$). STAT3 silencing inhibited the repair of IR-induced DSBs to a similar extent as stattic. These data suggest that STAT3 blockade enhances IR-induced generation of DSBs to promote PCa cell apoptosis.

STAT3 inhibition decreases radiation-induced survivin expression

In further investigation of the potential mechanism of STAT3 blockade on radiosensitization, western blotting results showed that stattic (5 μM) could reduce the IR-stimulated increase in survivin levels at 24 h and 48 h after IR in LNCaP cells. STAT3 silencing also decreased the IR-stimulated survivin accumulation at 48 h after IR in LNCaP cells (Fig. 3A). To further confirm whether STAT3 inhibition enhances radiosensitivity by decreasing survivin expression, LNCaP cells pretreated with stattic were transiently transfected with either the plasmid containing wild-type survivin cDNA (SC) or empty plasmid vector. Western blotting showed that survivin expression decreased in cells pretreated with stattic (Fig. 3B). The clonogenic survival assay showed a lower SF in cells pretreated with stattic, and transfection with survivin cDNA partially recovered the SF and survivin expression in cells pretreated with stattic (Fig. 3C). This finding indicates that survivin overexpression attenuates the radiosensitization effect of STAT3 inhibition.

STAT3 inhibitor enhances the radiosensitivity of LNCaP xenografts *in vivo*

To investigate the radiosensitization effect of STAT3 inhibition *in vivo*, LNCaP cells were implanted subcutaneously into mice to establish tumors. Then, the mice were exposed to 8 Gy IR and received intraperitoneal injection of stattic every 3 days, IR alone or IR combined with stattic (Supplementary Fig. S1). Compared to the control, both IR alone and the combined treatment significantly delayed tumor growth ($P < 0.001$) (Fig. 4C) and reduced tumor

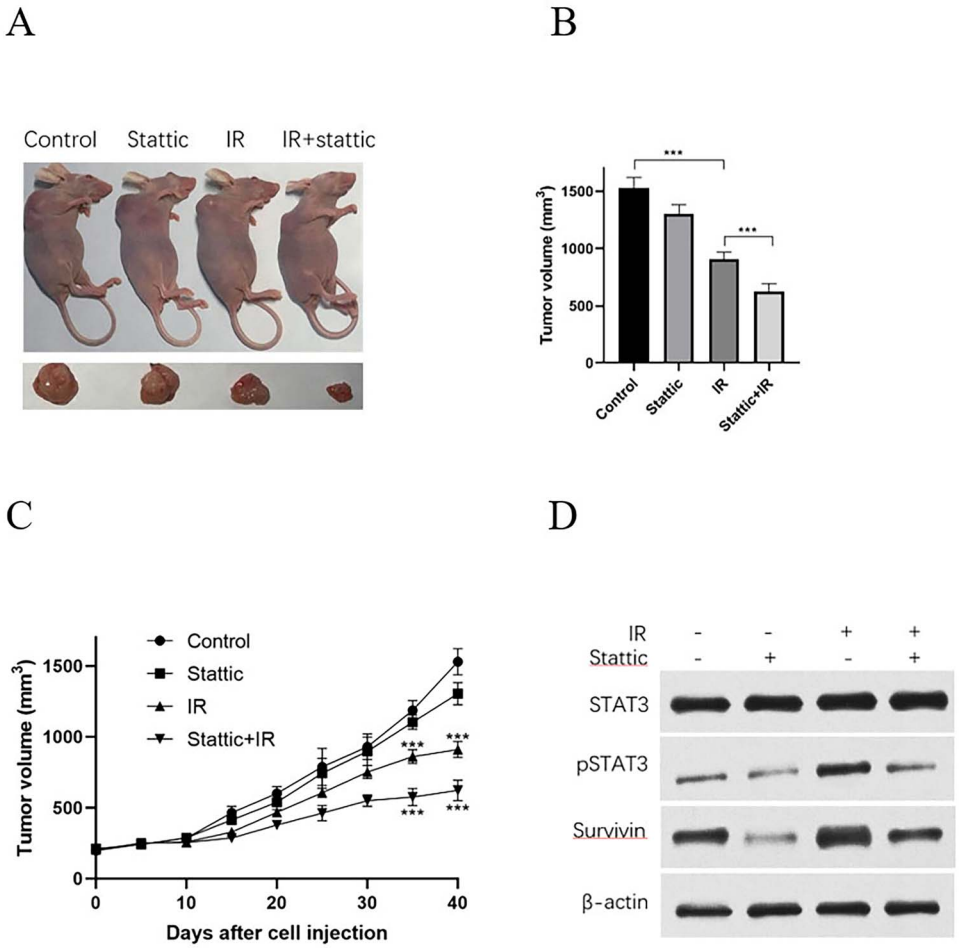

Fig. 4. STAT3 inhibitor enhances the radiosensitivity of LNCaP xenografts in nude mice. LNCaP cells were introduced subcutaneously into mice to establish tumors. Tumor-bearing mice received an intratumoral injection of either PBS or stattic, irradiation (8 Gy) or irradiation together with stattic. A Visual examination of representative mice from each group. B Tumor volume on the last day in each group. The data are shown as the mean ± SD from six mice in each group (n = 6). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. C Tumor growth curves of each group. D Western blots showing the expression of pSTAT3 and survivin in LNCaP mouse xenograft tissues.

volume (Fig. 4A). The tumor volume on the last day in the IR group was significantly lower than that in the control group. The tumor volume in the IR combined with stattic group was lower than that in the IR alone group ($P < 0.001$) (Fig. 4B). After treatment with stattic and/or IR, tumor xenografts were sectioned for western blotting. As shown in Fig. 4D, pSTAT3 and survivin expression were further decreased in the stattic and IR combined treatment group compared with the stattic or IR treatment alone group.

DISCUSSION

Increased expression and persistent activation of STAT3 is a characteristic of many malignancies [13]. Recent studies have shown that STAT3 is expressed and activated in primary prostate tumors and metastatic lesions [4–6]. Activated STAT3 signaling in malignant cells promotes cancer cell proliferation and tumor survival [14, 15]; thus, STAT3 may be a potential molecular target for PCa treatment [16].

IR can promote STAT3 activation in cancer cells [17], and prior studies have shown that activated STAT3 plays important roles in the radioresistance of tumor cells. Activation of STAT3 contributes to the development of radioresistance in breast cancer, and inhibition of STAT3 could radiosensitize breast cancer cells to radiation [8]. JAK2/STAT3 activation inhibits cell apoptosis and enhances the clonogenic potential, which promotes tumor initiation and radioresistance [9]. Our previous study also showed that STAT3 inhibitors enhance radiosensitivity in esophageal squamous cell carcinoma [18].

To demonstrate the effect of STAT3 blockade on the radiosensitivity of PCa cells, we conducted clonogenic survival assays, cell proliferation assays, flow cytometry analysis and *in vivo* experiments. We observed that STAT3 blockade increased the radiosensitivity of PCa cells. In addition, STAT3 blockade with siRNA or inhibitors suppressed the clonogenic formation of irradiated LNCaP and DU-145 cells. We further tested IR-induced apoptosis and determined that

compared with treatment of LNCaP cells with IR (6 Gy) alone, IR combined with STAT3 inhibition induced more apoptotic cell death. Furthermore, IR with STAT3 silencing showed a similar effect. These results indicate that STAT3 blockade increases radiosensitivity by promoting apoptosis of irradiated cancer cells. In line with other studies, we found that STAT3 inhibition alone induced apoptosis [19]. However, STAT3 inhibition alone resulted in a lower apoptosis rate than STAT3 inhibition combined with IR, which suggests a synergistic effect between STAT3 inhibition and IR. DSBs are a common cause of IR-induced apoptosis [20]. We further evaluated IR-induced DSBs and observed increased DSB formation after IR in PCa cells. The average number of DSBs increased more dramatically in irradiated cells treated with STAT3 inhibitor or siRNA. This finding suggests that STAT3 blockade may inhibit IR-induced DNA repair and increase DSB formation, which trigger cell apoptosis. These results may partially explain why STAT3 blockade increases apoptosis and radiosensitivity. Therefore, combining STAT3-targeted drugs with radiotherapy potentially produces a synergistic effect.

Survivin is known as an inhibitor of the apoptosis protein family that promotes the survival of tumor cells, leading to resistance to radiotherapy [11]. A previous study showed that STAT3 activation could promote survivin expression to decrease apoptosis, which led to radiotherapy resistance in tumor cells [21]. We also tested the level of survivin expression, and increased survivin was observed in irradiated PCa cells, while STAT3 blockade decreased radiation-induced survivin expression. These results indicate that STAT3 blockade may increase PCa cell radiosensitivity through the downregulation of survivin. To further confirm these results, we transfected stattic-pretreated PCa cells with wild-type survivin cDNA. Although stattic decreased survivin expression in pretreated cancer cells and clonogenic formation after IR, transfection with survivin cDNA partially restored survivin expression and clonogenic formation in cells. These results indicate that survivin overexpression may attenuate the radiosensitization effect of STAT3 inhibition in PCa cells. This finding supports the hypothesis that STAT3 blockade increases radiosensitivity through downregulation of survivin.

In vivo, we used LNCaP cell xenografts to confirm that IR alone or combined with a STAT3 inhibitor effectively reduced prostate tumors in animal models. The tumor volume in the IR group was significantly lower than that in the control group. The tumor volume in the IR combined with stattic group was even more strongly reduced than that in the IR alone group. This demonstrates that stattic enhanced the inhibitory effect of IR on xenograft growth. Consistent with these findings, western blotting showed that the STAT3 inhibitor stattic decreased IR-induced STAT3 activation and survivin expression, leading to increased apoptosis in tumors. Our results show that animals tolerated the treatment well and that increased efficacy was achieved with a combined therapy of stattic and IR.

In summary, this study demonstrates that STAT3 blockade increases the radiosensitivity of PCa cells *in vitro* and *in vivo*. STAT3 blockade increased the apoptosis rate of irradiated PCa cells, leading to increased radiosensitivity. Further results provide evidence for STAT3 as a promoter of radioresistance through regulation of DSB repair and survivin expression. Our study proposes a novel approach of targeting STAT3 to enhance the efficacy of radiotherapy in PCa patients with STAT3 expression and activation.

SUPPLEMENTARY DATA

Supplementary data is available at *RADRES Journal* online.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

ETHICS STATEMENT

This study was inspected and approved by Ethics Committee of Hubei Cancer Hospital; the approval number is LLHBCHLW-15.

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