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CD44+/CD24+ cervical cancer cells resist radiotherapy and exhibit properties of cancer stem cells

H. LIU[1], Y.-J. WANG[2], L. BIAN[2], Z.-H. FANG[1], Q.-Y. ZHANG[1], J.-X. CHENG[2]

[1]Department of Gynecologic Oncology, the Fourth Hospital of Hebei Medical University, Shijiazhuang, China
[2]Department of Gynecology and Obstetrics, Fourth Hospital of Hebei Medical University, Shijiazhuang, China

Abstract. – OBJECTIVE: The aim of the study is to investigate the radiosensitivity of CD44+/CD24+ cervical cancer cells and to explore its mechanism of radiotherapy resistance. Moreover, we further to test whether the CD44+/CD24+ cervical cancer cells had the characteristics of stem cells.

MATERIALS AND METHODS: The human squamous cell carcinoma SiHa cells were cultured *in vitro*, and CD44+/CD24+ SiHa cells were sorted by FACS analysis. CD44+/CD24+ SiHa cells and the parental SiHa cells were given several fractionated irradiation at a cumulative dose of 8 Gy, 16 Gy, 30 Gy, respectively. Survival curves were obtained and fitted using clonogenic assays, and the radiosensitivity of tumor cells was compared according to the radiobiological parameters, including Do, Dq, N and SF 2. Morphological changes of cell apoptosis were determined using Hoechst 33258 fluorescence staining. The ultrastructural changes in cells with apoptosis were observed by transmission electron microscopy. Cell apoptosis rate was determined by FCAS analysis. DNA "ladder" in apoptotic cells was detected by gel electrophoresis. The mRNA levels of cell apoptosis-related genes were detected by RT-PCR assay. Balling capacities of CD44+/CD24+ SiHa cells and parental SiHa cells were detected by suspension culture without FBS. The *in vivo* tumorigenicity was detected by inoculating CD44+/CD24+ SiHa and parental SiHa cells into nude mice.

RESULTS: The FACS analysis results demonstrated that there was a concomitant increase in the percentage of CD44+/CD24+ cells as the increasing irradiation doses. Colony formation assay results showed that the colony formation rate of CD44+/CD24+ SiHa cells was significantly higher than that of parental SiHa cells ($p < 0.05$). Moreover, the data from Hoechst 33258 staining, DNA fragment gel electrophoresis, transmission electron microscopy and FACS analysis showed that CD44+/CD24+ SiHa cells had no cell apoptosis after irradiation treatment. RT-PCR results showed that the mRNA levels of bcl-2, surviving and OCT4 were significantly higher in CD44+/CD24+ SiHa cells than that of parental SiHa cells ($p < 0.01$). CD44+/CD24+ SiHa cells could form more compact cell spheres with a larger volume than that of parental SiHa cells ($p < 0.05$). CD44+/CD24+ cervical cancer cells had more potent tumorigenicity than that of parental cervical cancer cells.

CONCLUSIONS: CD44+/CD24+ cervical cancer resist cell apoptosis induced by irradiation therapy and possessed the characteristics of stem cells.

Key Words:
Cervical cancer stem cells, Cell apoptosis, Irradiation resistance.

Introduction

Cervical cancer is the second most frequent gynecological malignancy worldwide[1,2]. The incidence of cervical cancer has been increasing and the onset of cervical cancer tends to be younger[3]. More and more attention is being paid to improve the prognosis and treatment outcomes for cervical cancers[4,5]. Cancer stem cells (CSC) theory reveals the tumor cell heterogeneity within the tumors[6], suggesting that CSC is the root cause for tumor immortalization, recurrence and metastasis[7-9]. Bcl-2 and survivin genetically control the apoptotic process and are the inhibitor of apoptosis proteins (IAP)[10]. Both of them have strong anti-apoptotic effects and become the focus of cancer research in recent years. Oct4 is a non-specific marker for adult stem cells, which has been shown to play a crucial role in the anti-apoptotic activity of stem cells[11-13]. Accumulating evidence demonstrates that stem cells exhibit the ability to resist apoptosis through a number of

Corresponding Author: Jianxin Cheng, MD; e-mail: chengcjianxinjx@sina.com

complex mechanisms. When cell apoptosis is induced and triggered, ordinary tumor cells will eventually undergo apoptosis, while the percentage of cancer stem cells will concomitantly increase[14,15].

The main reason for radiotherapy failure of cervical cancer is local relapse or distant metastasis. Radiotherapy is the first-line treatment for all stages of human cervical cancer; however, the efficacy of re-irradiation therapy is very poor for the patients with cervical cancers who relapse locally after primary radical radiotherapy. It is mainly related to radiation resistance. In recent years, studies have shown that cancer stem cells may contribute to radiation resistance after tumor relapse. Cancer stem cells possessed a marked capacity for self-renewal, proliferation and differentiation. Singh et al[16] had identified and purified a cancer stem cell from human brain tumors of different phenotypes, and they also found that the $CD133^+$ cells were the brain tumor stem cells and could differentiate into tumor cells. Lou et al[17] had found that two polymorphisms of CD44 3'UTR weakened the binding of miRNAs and associated with naso-pharyngeal carcinoma in a Chinese population. Bao et al[18] found the fraction of CD133 positive cells, a marker for both neural stem cells and brain cancer stem cells, was enriched after radiation in glioblastoma. Moreover, no matter it is *in vitro* cell culture or *in vivo* tumor cell xenografts, the higher percentage of $CD133^+$ cell was correlated with increased survival rate of the glioblastoma cells after radiotherapy. Thus, the radiation tolerance of brain tumor was caused by the brain tumor stem cells. Hajj et al[19] distinguished the tumorigenic (tumor initiating) from the nontumorigenic cancer cells based on cell surface marker expression, and the breast cancer stem cells were identified and purified from breast cancer cells by specific cell surface marker. So far, however, the research on cancer stem cells of cervical cancer is rare, as well as the study of the resistance to radiotherapy.

In the present work, we found that the cervical cancer cells, which were resistant to radiotherapy, exhibited a higher percentage of surface markers CD44+ and CD24+. Then, the $CD44^+/CD24^+$ SiHa cells were isolated by flow cytometry and further identified whether they possessed the characteristics of cancer stem cells. The study would provide some experimental evidence to improve radiotherapy for human cervical cancers.

Materials and Methods

Materials and Reagents

Cervical squamous cell carcinoma cell line SiHa was provided by Research Center of the Fourth Hospital of Hebei Medical University. Bovine serum albumin (BSA, fatty acid-free) and RPMI-1640 (powder) were obtained from Sigma-Aldrich (St. Louis, MO, USA). FBS was purchased from Evergreen Biotechnology Co. (Hangzhou, China). DNA-ladder extraction kit was purchased from Promega Co. (Madison, WI, USA). Monoclonal Antibodies against CD24 and CD44 were obtained from eBioscience Co. (San Diego, CA, USA). TRIzol agent used for RNA extraction was purchased from Gibco Co. (CA, USA). FACS-420 flow cytometer was purchased from BD Biosciences (Franklin Lake, NJ, USA). Linear accelerator (Varian UNIQUE_2229) was purchased from Varian Co. (Palo Alto, CA, USA).

Cell Culture and Radiation Treatment

SiHa cells were cultured in RPMI-1640 medium and supplemented with 10% fetal bovine serum (FBS) in a 5% CO_2 incubator at 37°C. The cells were weekly passed on 1 to 2 times and the SiHa cells in the logarithmic growth phase were used for subsequent experiments.

Radiation Treatment

Briefly, 6 MeV X-rays were used as radioactive sources generated by a linear accelerator (Varian 2100 C; Varian, Palo Alto, CA, USA). The SiHa cells in logarithmic growth phase were irradiated at room temperature, and a 1.0 cm-thick compensating film was added. The source to detector distance was set to 100 cm and the dose rate was 300 mu/min. After treatment, the irradiated cells were put into an incubator for further culture.

When the cells covered 70%-80% of the flask (Corning, New York, NY, USA) sidewall, they were digested with Trypsin (Solarbio, Beijing, China) and passaged. Then, the cells proliferated into the logarithmic growth phase, and the cells were irradiated again. So repeatedly, the highest cumulative radiation dose got to 30Gy.

FCM was Used to Detect $CD24^+$, $CD44^+$ Cell Content

The cells were treated with different doses of radiation, and the monolayers were digested with 0.25% trypsin and accutase (eBioscience, San Diego, CA, USA). The cell suspension was pre-

pared and the cells were adjusted into 1×10^6 cells/group. The antibodies of anti-CD44-PE (0.625 μl/test) and CD24-FITC (0.25 μl/test) were added and mixed evenly. The cells were incubated at 4°C for 30 min in dark and washed twice. Detection can be analyzed by flow cytometry.

Colony Formation Assay

The cells in logarithmic phase were digested, counted and diluted with a gradient. The cells (10^2-10^5 cells/plate) were plated into a 6-well plate. After adherence for 8 hours, the cells were irradiated at the dose of 2, 4, 6, 8 and 10 Gy. Then, the irradiated cells were cultured in a normal medium. The medium was changed every twice day. The cells were cultured for seven to ten days. The 40 g/L of paraformaldehyde was used to fix the cells for 15 min, and 1 g/L crystal violet was used for staining for 20 min. The clones with more than 50 cells were counted under a microscope. The surviving fraction (SF) = colony forming efficiency in experimental group / colony forming efficiency in control group × 100%. The single-hit, multi-target model was used to fit the survival curves and the radiobiological parameters, such as SF2, D0, Dq and N was calculated by GraphPad Prism software. Among them, Log N = Dq / D0. The plates were set up in triplicate and each study repeated three times.

Hoechst 33258 Staining is Used to Detect Cell Apoptosis

In experimental groups, the culture medium was exhausted and 0.5 ml of fixative solution was added to fix for 10 min. Fixative solution was discarded, and the cells were washed with PBS buffer for 3 min, twice times. Then, 0.5 ml of Hoechst 33258 (Sigma-Aldrich, St. Louis, MO, USA) staining solution was added for staining 5 min. Slices were observed under fluorescent microscope (Nikon Fluophot TV Lens C-06X, Tokyo, Japan).

Cell Apoptosis is Determined by Transmission Electron Microscopy (TEM)

The cells in each experimental group were collected and fixed for 30 min in 2.5% glutaraldehyde solution. The slices were prepared for TEM by conventional methods[20], including fixation, cleaning, fixation, dehydration, impregnation, embedding, polymerization and trimming to prepare thin sections. The samples were observed under a transmission electron microscope.

Electrophoretic DNA Analysis for the Detection of Apoptosis

The cells in each group were treated with different doses of irradiation and the DNA in the nucleus was extracted by DNA extraction kit (Promega, USA) according to the protocols. The DNA sample with the volume of 5 μl was added the equivalent amount of 1 × DNA sample buffer for 1.5% agarose gel electrophoresis. The electrophoresis was performed at 80 V for 30 min, and the photograph was captured by Gel imaging system (Syngene Ltd., Model: GBox EF2, Cambridge, UK).

Cell Apoptosis is Detected by Flow Cytometry Analysis

The cells were digested and collected in each experimental group. Then, single-cell suspensions were prepared and collected in 5 mL tubes. Then, the cells were centrifuged at 840 g for 15 min and washed three times with 1×PBS buffer. The cells were centrifuged again and fixed with 70% ethanol at 4°C for 30 min. Then, the cells suspended in RNase A solution were maintained in a water bath at 31°C for 30 min. Adding 5-10 □l of PI staining solution to each sample just prior to cytometry analysis. Data were processed by Modfit 1.0 software and the proportion of apoptotic cells was calculated.

The mRNA levels of Bcl-2, Surviving and OCT4 are Detected by RT-qPCR Assay

The cells were collected and total RNA was extracted by TRIzol kit (Gibco, Carlsbad, CA, USA) according to the protocols. The total RNA (450 ng) was reverse transcribed into cDNA and the cDNA was used as template for PCR amplification. The volume of the reaction system was 20 μl, including 10 μl of 2 × Ultra SYBR Mixture, 2 μl of template, 0.4 μl of upstream primer, 0.4 μl of downstream primer, and 7.2 μl sterile distilled water. The sequences were shown in Table I. The PCR reaction conditions were as follows: denaturation program (95°C for 15 s), program repeated 45 times (60°C for 20 s). Experimental data obtained were analyzed for relative quantification by using $2^{-\Delta\Delta ct}$ calculation formula.

Cell Suspension Culture

The CD44+/CD24+ SiHa cells and parental SiHa cells were sorted by fluorescence-activated cell sorting (FACS). The cells with the density of 1000 cells/ml were plated into the fresh-

ly prepared serum-free medium and were given a conventional culture at the culture flask in a low adhesion culture flask. Growth state of the cells was observed everyday and ball-forming rate was calculated (Ball forming rate = the number of the forming balls / cell number × 100%).

Tumorigenicity Testing: in vivo Models

Sixteen eight-week-old male NOD/SCID mice were purchased from the Experimental Animal Center of Hebei Medical University. The mice were housed in a specific pathogen-free (SPF) environment. The feed, water, beddings and cages must be sterilized by high temperature and pressure. They were randomly divided into 2 groups. $CD44^+/CD24^+$ SiHa and parental SiHa cells were collected and single cell suspensions were prepared. After centrifugation, the cells were resuspended in RPMI-1640 containing Matrigel. The cells were counted and the concentration was adjusted into 1×10^3 cells/ml. Then, the cell suspension with the volume of 1 ml was injected subcutaneously into the right forelimb of nude mice. The tumor growth was observed every 6 days.

Statistical Analysis

All the data were analyzed by SPSS 11.5 software (SPSS Inc., Chicago, IL, USA). The experiments were repeated three times and each experiment was triplicated. The experimental data were expressed as mean values ± standard deviation. The metering data were performed by *t*-test. $p < 0.05$ was considered statistically significant.

Results

The Expression of CD44 and CD24 is Detected by FACS

In order to detect whether CD44 /CD24 expression was related to irradiation resistance, SiHa cells were exposed to various doses (8, 16 and 30 Gy) of radiation. Compared with the non-irradiated group, the percentage of CD44/CD24 double positive SiHa cell was not changed in 8 Gy irradiation group ($p > 0.05$), and the data was not shown here. As shown in Figure 1, when the SiHa cells were treated with a boost radiation dose of 16 Gy, the percentage of $CD44^+/CD24^+$ subpopulation of SiHa cells was up to 2.81%, compared with the percentage of 0.17% in non-irradiated group ($p < 0.05$). Moreover, the percentage of $CD44^+/CD24^+$ cells was up to 4.61% at the irradiation dose of 30 Gy. There was statistically difference with the percentage of 0.21% in non-irradiated group ($p < 0.05$).

The $CD44^+/CD24^+$ SiHa Cells Have Decreased Radiosensitivity

Resistance to radiotherapy is a major cause of treatment failure in patients with cervical cancer. Here, we detected the variation of radiosensitivity in CD44/CD24 dual positive SiHa cells by testing the surviving fraction (SF) of irradiated cells. The $CD44^+/CD24^+$ SiHa cells and control SiHa cells were treated with increasing doses of irradiation. The cell survival curve was shown in Figure 2, and the results demonstrated that SF of $CD44^+/CD24^+$ SiHa cells was significantly higher than that of control SiHa cells. In the single-hit, multitarget model, its associated parameters (Table II), D0 value was 4.018 ± 0.08 in $CD44^+/CD24^+$ SiHa cells, which was significantly higher than that of 3.112 ± 0.11 in SiHa cells ($p < 0.05$). The Dq values were 1.908 ± 0.12 in $CD44^+/CD24^+$ SiHa cells *vs*. 0.3389 ± 0.21 in SiHa cells, and there was a statistical difference between the groups ($p < 0.01$).

Fluorescent Staining with Hoechst 33258 for Labeling DNA in Fluorescence Microscopy

The $CD44^+/CD24^+$ cells and SiHa cells were exposed to the cumulative radiation dose of 30 Gy and cultured for 48 h. As shown in Figure 3A, most $CD44^+/CD24^+$ SiHa cells maintained normal morphology, with uniform and week fluorescence throughout the cytoplasm in living cells. However, SiHa cells showed morphologic and apoptotic changes (Figure 3B), which had condensed and smaller nuclei with visible compact and strong fluorescence, chromatin marginalization or Chunky dense staining. Typical apoptotic bodies were seen in irradiated SiHa cells.

Changes in Ultrastructure After Radiotherapy

Electron microscopy is still considered the "gold standard" for the identification of apoptotic cells. In the present study, the $CD44^+/CD24^+$ cells and the parental SiHa cells were irradiated with a cumulative radiation dose of 30 Gy. The cell morphology was observed with a scanning electron microscope. As shown in Figure 4, the parental SiHa cells showed characteristic mor-

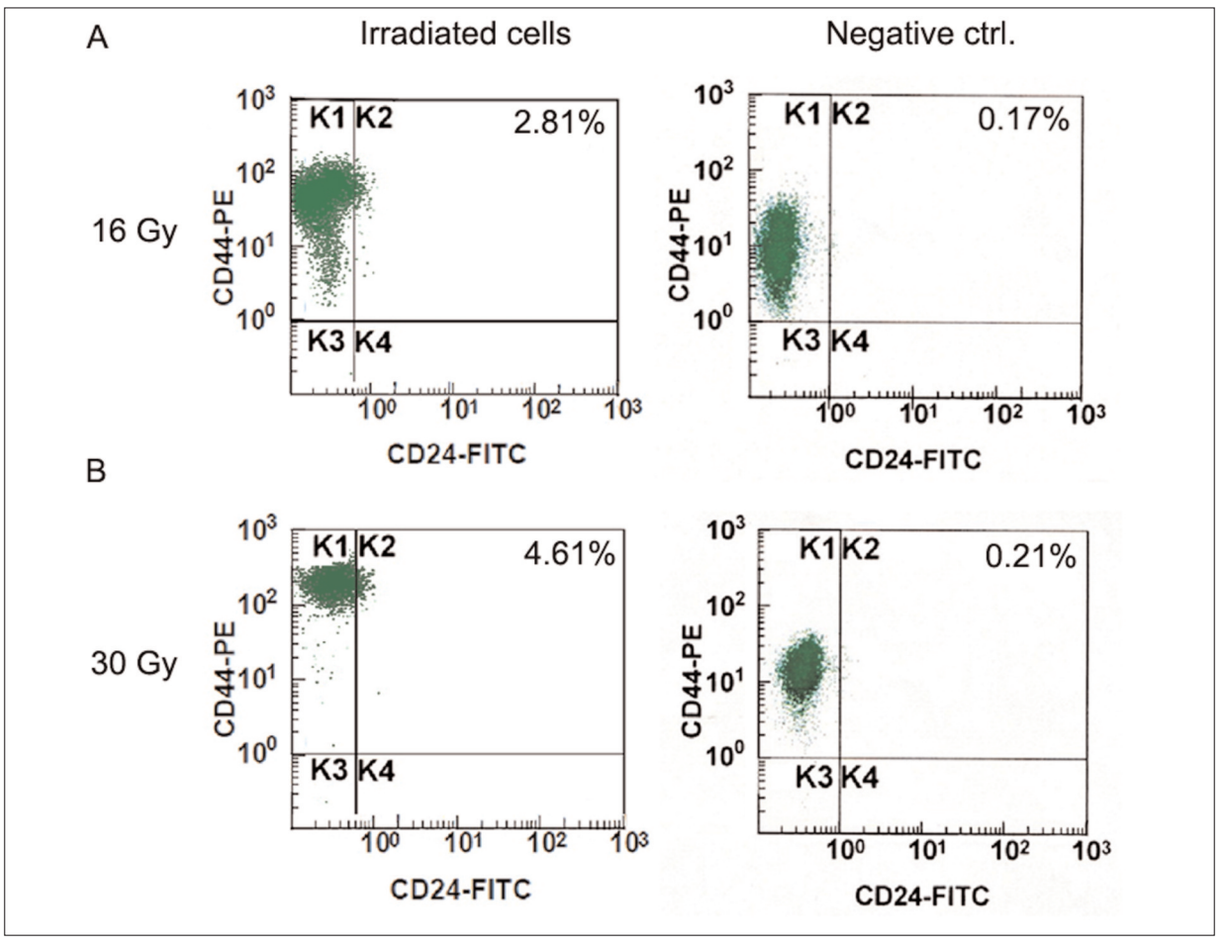

Figure 1. The expression of CD44 and CD24 is detected by FACS. SiHa cells were plated into the 6-well plate and exposed to increasing doses (8, 16 and 30 Gy) of radiation. ***A,*** The cells were irradiated with the accumulative dose of 16 Gy. The percentage of $CD44^+/CD24^+$ SiHa cells was determined by FACS analysis. ***B,*** The cells were irradiated with the accumulative dose of 30 Gy. The percentage of $CD44^+/CD24^+$ SiHa cells was determined by FACS analysis. Here, the non-irradiated cells were used as negative control cells.

phological changes of apoptosis, including nuclear chromatin condensation, margination, uneven thickness of the nuclear membrane, a part of membrane bulging and decreased cell surface microvilli (Figure 4A). However, $CD44^+/CD24^+$ SiHa cells had not shown morphological changes of apoptosis (Figure 4 B).

DNA Ladders Are Detected by Agarose Gel Electrophoresis

The characteristic DNA ladders were used to identify the apoptotic cells by conventional constant field agarose gel electrophoresis of DNA. As shown in Figure 5, the cells were treated with total radiation dose of 30 Gy, characteristic apoptotic DNA ladder with molecular weight of 180-200 bp was shown in the lane of SiHa cells; how-

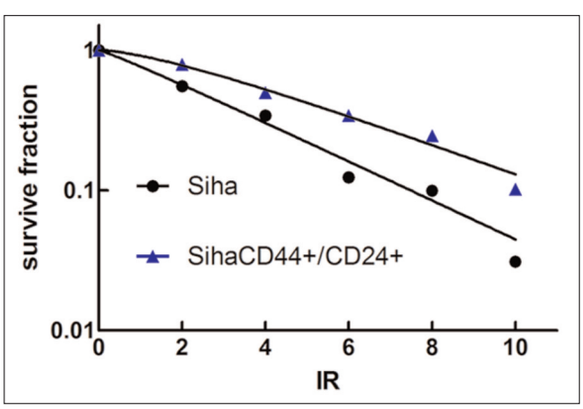

Figure 2. Radiosensitivity test of $CD44^+/CD24^+$ SiHa cells. The survival rates were fitted by single-hit, multitarget model. $CD44^+/CD24^+$ SiHa cells and parental SiHa cells were irradiated with the accumulative irradiation doses of 0, 2, 4, 6, 8 and 10 Gy. The survival fraction was calculated with GraphPad software.

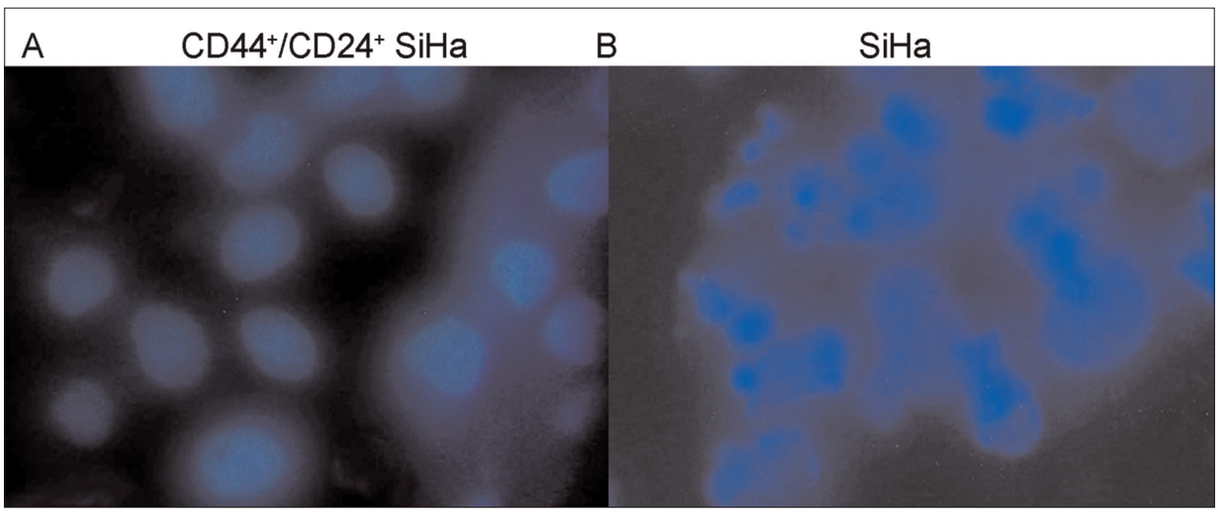

Figure 3. ***A-B,*** Fluorescent staining with Hoechst 33258 for labeling DNA in fluorescence microscopy. CD44+/CD24+ SiHa cells and parental SiHa cells were irradiated with an accumulative dose of 30 Gy and cultured for another 48 hours. The apoptotic morphology was observed by fluorescence microscopy (400×).

ever, mere macromolecules in the genome were observed near the sample holes and there was no apoptotic DNA fragmentation in the lane of CD44+/CD24+ SiHa cells.

Apoptotic Rate is Determined by Flow Cytometry Analysis

In order to detect the cell apoptosis rates in irradiated CD44+/CD24+ SiHa cells and parental SiHa cells, FACS analysis was used in the experiment. As shown in Figure 6, the cells were treated with a total radiation dose of 30 Gy, the apoptotic rate in CD44+/CD24+ SiHa cells was significantly lower than that in the parental SiHa cells. There was a statistical difference between them (0.37 ± 0.05 *vs.* 35.59 ± 0.31, $p < 0.01$). A subdiploid apoptosis peak appeared before the G1 phase of cell cycle in irradiated parental SiHa cells; however, there was no apoptosis peak shown in irradiated CD44+/ CD24+ SiHa cells.

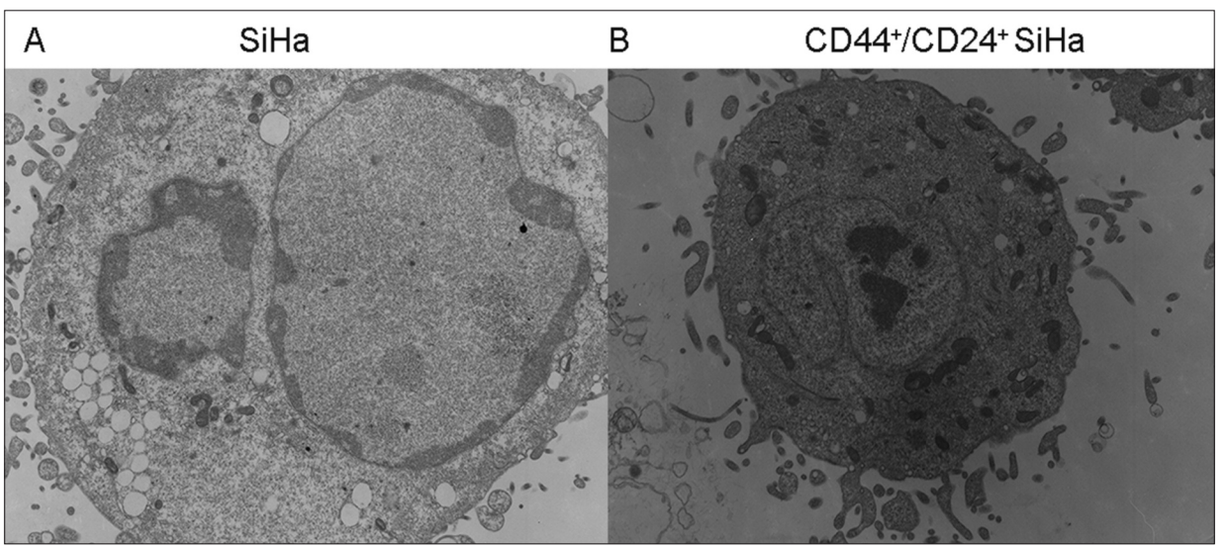

Figure 4. ***A-B,*** Cell apoptotic changes in ultrastructure after radiotherapy. The CD44+/CD24+ SiHa cells and parental SiHa cells were irradiated with an accumulative dose of 30 Gy and cultured for another 48 hours. The cell morphology was observed by a scanning electron microscope (×5000).

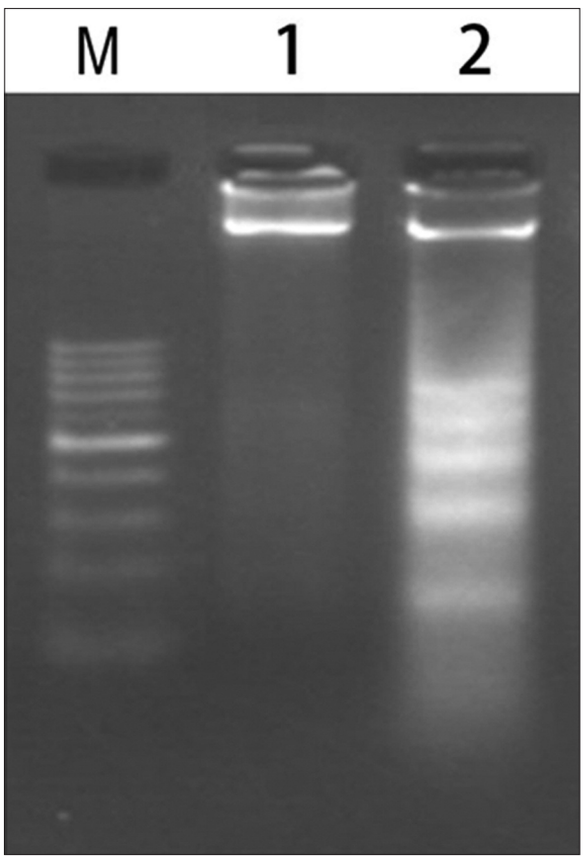

Figure 5. DNA ladders are detected by agarose gel electrophoresis. The CD44+/CD24+ SiHa cells and parental SiHa cells were irradiated with an accumulative dose of 30 Gy and cultured for another 48 hours. Total DNA in each group was extracted and agarose gel electrophoresis was used to detect the DNA ladder in irradiated cells. M: marker; 1: CD44+/CD24+ SiHa cells; 2. SiHa cells.

Higher mRNA Levels of Apoptosis Inhibitor Genes are Detected in CD44+/CD24+ SiHa Cells

Next, we also detected the levels of apoptosis inhibitor genes in irradiated CD44+/CD24+ SiHa cells and parental SiHa cells. As shown in Figure 7, the cells were treated with a total radiation dose of 30 Gy, the mRNA levels of Bcl-2, surviving and OCT4 were significantly increased in CD44+/CD24+ SiHa cells, compared with that in parental SiHa cells ($p < 0.01$).

CD44+/CD24+ SiHa Cells Have High in vitro Balling Capacity by Suspension Culture

Suspension culture assay was used to test the in vitro proliferative capacity of CD44+/CD24+ SiHa and SiHa cells. As shown in Figure 8, CD44+/CD24+ SiHa cells developed into a ball of cells earlier than that of SiHa cells. Moreover, CD44+/CD24+ SiHa cells had more compact and larger volume of cell sphere than that of SiHa cells. The rate of sphere forming was significantly higher than that of SiHa cells [$(36.0 \pm 4.52)\%$ *vs.* $(6.1 \pm 2.85)\%$, $p < 0.01$]. All of the data demonstrated that CD44+/CD24+ SiHa cells had higher sphere forming capability than that of SiHa cells.

The CD44+/CD24+ Cervical Cancer SiHa Cells Have Potent Tumorigenicity Capacity in Nude Mice

As described in Materials and Methods, the tumorigenicity potential of CD24+/CD44+ cells was

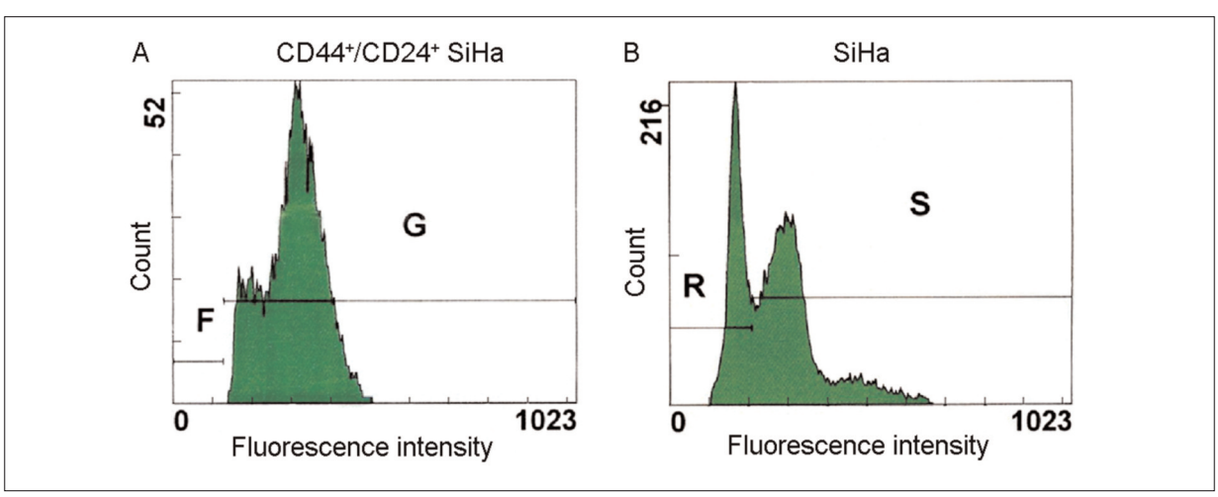

Figure 6. ***A-B,*** The apoptotic rate is determined by flow cytometry analysis. The CD44+/CD24+ SiHa cells and parental SiHa cells were irradiated with an accumulative dose of 30 Gy and cultured for another 48 hours. The apoptotic rate was determined by FACS analysis.

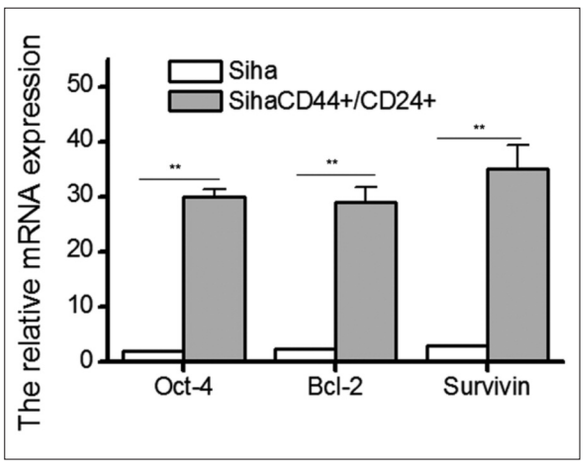

Figure 7. Higher mRNA levels of apoptosis inhibitor genes are detected in $CD44^+/CD24^+$ SiHa cells. The $CD44^+/CD24^+$ SiHa cells and parental SiHa cells were irradiated with an accumulative dose of 30 Gy. The cells (5×10^5 cells/well) were plated into the 6-well plate and cultured for 48 hours. Total RNA was extracted and transcribed with reverse transcriptase. The mRNA levels of Oct-4, Bcl-2 and survivin were determined by real-time PCR. $^{**}p < 0.01$, compared with that in SiHa cells.

tested in nude mice. When NOD/SCID mice were inoculated with $CD44^+/CD24^+$ SiHa cells for 4.5 days, the grain-like nodules were formed and the tumor-forming rate was 100%. While the NOD/SCID mice were inoculated with parental SiHa cells for 13.0 days, the grain-like nodules were touched and the final tumor-forming rate was 87.5%. Thus, $CD44^+/CD24^+$ SiHa cells had higher tumorigenic activity than the parental SiHa cells ($p < 0.01$). The tumor volumes were calculated every six-day after injection, as shown in Table III and Table IV, $CD44^+/CD24^+$ SiHa cells were faster growing transplanted tumors than that of parental SiHa cells.

Discussion

As we know, metabolic abnormalities in apoptotic pathways contributed to the development and progression of malignant tumors. Moreover, it was also an important factor to produce resistance to chemotherapy and radiation therapy.

Radiation therapy could induce cell death or cell apoptosis by damaging DNA, affecting cell proliferation and changing cell cycles, etc. In the present study, our results demonstrated that the proportion of cells gradually increased as the irradiation dose increased. The cells were separated and its biological characteristics were examined. Colony-forming assay results showed the values of SF2, Do, Dq and N in $CD44^+/CD24^+$ SiHa cells were higher than that of the parental cells, suggesting that $CD44^+/CD24^+$ SiHa cells were more resistant to radiotherapy than the parental cells.

Morphological analysis revealed that there were no typical apoptosis morphology changes in $CD44^+/CD24^+$ SiHa cells. Electrophoretic DNA

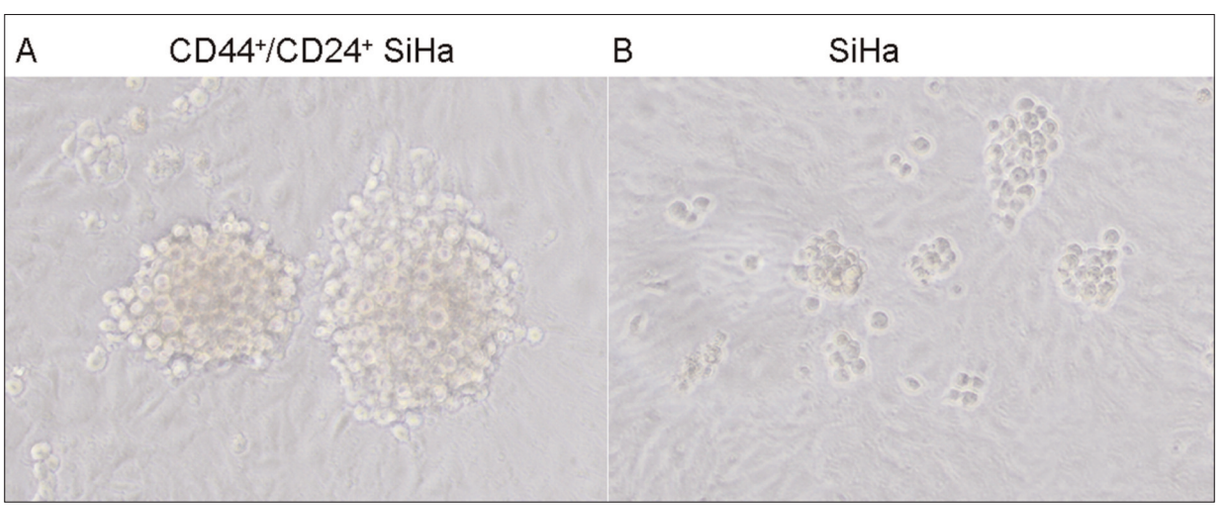

Figure 8. The mammosphere forming capability in two groups at 10 days (×200). ***A,*** $CD44^+/CD24^+$ Siha cell group. ***B,*** Parent SiHa cell group.

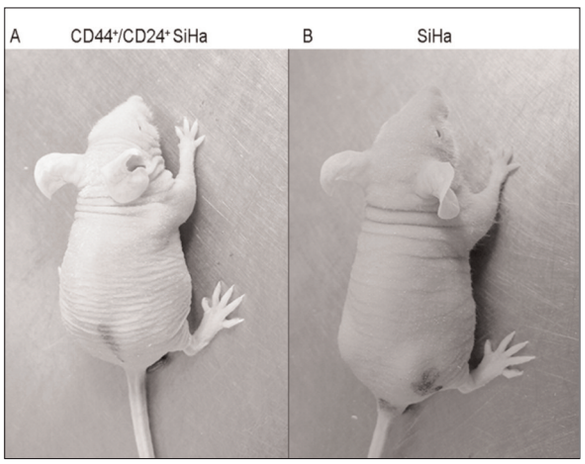

Figure 9. The CD44+/CD24+ cervical cancer SiHa cells have potent tumorigenicity capacity in nude mice. The CD44+/CD24+ cervical cancer SiHa cells and parental SiHa cells were inoculated into nude mice as described in Materials and Methods. After 18 days, the nude mice in two groups were shown here. ***A,*** CD44+/CD24+ SiHa cell group; ***B,*** parent SiHa cell group.

analysis showed that characteristic DNA ladders appeared only in parental cells. Moreover, FACS analysis showed that cell apoptosis rate in CD44+/CD24+ SiHa cells was significantly lower than that in parental cells after radiotherapy. All of the data demonstrated that CD44+/CD24+ SiHa cells were resistant to radiation therapy, resulting in enhanced resistance to cell apoptosis.

Bcl-2 family proteins play key roles in the regulation of apoptosis, which are believed to be membrane bound proteins. Anti-apoptotic Bcl-2 protein is mainly reside on nuclear membrane, endoplasmic reticulum and the outer mitochondrial membrane. Martin and Green have shown[21] that bcl-2 gene is a key factor to regulate cell apoptosis in many pathological and physiological processes, which is an apoptosis suppressor gene and also plays an important role in the X-ray-induced cell apoptosis. Survivin is a member of the inhibitor of apoptosis (IAP) gene family. It plays an important role in both the regulation of cell cycle and the inhibition of apoptosis. It is highly expressed in most cancers and associated with chemotherapy resistance, and associated with the progression, prognosis and recurrence in many tumors. Studies[22-24] have found that survivin acted directly on the downstream "effector" caspases (caspase 3 and 7) in apoptotic pathway, which had a strong anti-apoptotic effects, suggesting that high expression of survivin in tumors was likely to affect the sensitivity of the tumor cells to radiation therapy. Oct4 is a stem cell-related gene in embryonic and germ cells. As an important transcription factor, it is not only involved in regulation of embryonic stem cells, and also plays an important anti-apoptotic role in stem cells[25]. In the present study, we detected the expression levels of Bcl-2, surviving and Oct4 in CD44+/CD24+ SiHa cells and parental SiHa cells by real-time PCR. The results demonstrated that in radiation-resistant cells (CD44+/CD24+ SiHa cells), the levels of Bcl-2, surviving and Oct4 were significantly higher than that of the parental SiHa cells. Moreover, the cultivation in serum-free cell culture medium and xenografts *in vitro* experiments confirmed that CD44+/CD24+ SiHa cells had higher balling forming capacity and tumorigenic ability than that of parental SiHa cells.

Despite the continuous improvement of radiotherapy techniques, the targeted radiotherapy of cancer stem cells still has no substantial progress. Currently, the bottleneck for the treatment of recurrent and metastatic cancer is the radiotherapy toxicity and lack of tumor-specific treatment. In the present report, we demonstrated that CD44+/CD24+ cervical cancer cells had resistance for irradiation-induced cell apoptosis and showed the characteristics of stem cells. However, it needs to be further studied whether the CD44+/CD24+ could be like the surface maker of cancer stem cells in cervical cancers.

Conclusions

CD44+/CD24+ cervical cancer resist cell apoptosis induced by irradiation therapy and possessed the characteristics of stem cells. The research will give a new clue for the clinical therapy of human cervical cancers.

Acknowledgements

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Informed Consent

The samples from cancer patients were not involved in the experiments.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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