Association between B7-H1 and cervical cancer: B7-H1 impairs the immune response in human cervical cancer cells

JIANYING TAO*, JIANRONG DAI* and SHUNYU HOU

Department of Gynecology and Obstetrics, The Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou, Jiangsu 215006, P.R. China

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Abstract. The aim of the present study was to determine the preliminary mechanism of action of B7 homolog 1 (B7-H1) and investigate the association between B7-H1 and cervical cancer. The expression of B7 family proteins was measured in cervical cancer cells. Cervical cancer cells were co-cultured with T lymphocytes. An ELISA assay was subsequently conducted to analyze cytokine concentrations in the supernatants of the cultured T cells in cervical cancer cells and B7-H1 downregulated cells. Levels of interleukin (IL)-1β, IL-6 and tumor necrosis factor-α mRNA in mice injected with cervical cancer cells or B7-H1 downregulated cells were measured by reverse transcription-quantitative polymerase chain reaction. It was determined that cervical cancer cells express high levels of B7-H1, whereas the normal cervical epithelium does not express B7-H1. When co-cultured with T lymphocytes, cervical cancer cells were involved in the inhibition of lymphocyte activation. When B7-H1 was downregulated using a lentivirus, the proliferation ability did not change compared with cervical cancer cells, whereas the soluble factors secreted by T cells differed between cervical cancer cells and B7-H1 downregulated cells. In an animal model, injected B7-H1 downregulated cervical cancer cells elicited a more intense immune response, whereas cervical cancer cells had the wild immune response. Therefore, the results of the present study demonstrate that B7-H1 mediates the low immunogenicity of cervical cancer and is not attacked by the immune system.

Introduction

The cervix is often referred to as the uterus gate, due to the fact that it functions as a defense and guard. Due to the special status of the uterine cervix, it is vulnerable to attack from external bacteria and viruses. Unique morphological changes occurring in the cervical epithelial tissue that comprises the cervical epithelium may result in malignant transformation or inflammation (1). Cervical cancer is the fourth most common type of cancer and the fourth most common cause of cancer-associated mortality in women worldwide (2,3). There are different types. of cervical cancer: About 90% of all cervical cancer cases are squamous cell carcinomas, ~10% are adenocarcinomas and a small proportion of cases are other types, including small cell carcinoma and clear cell carcinoma (4). Recently, the number of cervical cancer cases detected in younger women (women aged 45 years or younger is defined as here) has increased from 18.02/100,000 in 2012 to 19.71/100,000 in 2013 (5). Tumors occur as the result of a number of factors, including a weakening of the body's immune system, exposure to chemical carcinogenic factors, physical carcinogenic factors or biological carcinogenic factors and a variety of chronic stimulation (6).

Current treatment for cervical cancer consists of a combination of surgery, radiotherapy and chemotherapy (7) and the five-year survival rate for patients with cervical cancer in the United States is 68%, which is a high 5-year survival rate compared with pancreatic cancer or lung cancer (8). Outcomes, however, depend heavily on how early the cancer is detected. It has been reported that the cervical cancer microenvironment often has low levels of T cell immunity, which is important as T cell immunity serves a vital role in recognizing and inhibiting the growth and proliferation of tumor cells (9).

B7 homolog 1 (B7-H1) is a recently identified family of B7 co-stimulatory molecules that serve an important role in inhibiting T cell activation and promoting T cell apoptosis (10). It has been reported that B7-H1 is exploited by tumors to evade immune responses (11). In many different types of human cancer, B7-H1 is expressed on the cell surface and a correlation between its expression and poor clinical prognosis has been identified in gastric, renal, breast, ovarian and esophageal carcinoma (12-16). A number of studies have investigated the association between B7-H4 and cervical cancer (17-19). However, few studies have focused on the role of the B7-H1 molecule in cervical cancer. The present study attempted to identify the association between B7-H1 and cervical cancer, and the role B7-H1 serves in the immune regulation of cervical cancer.

Correspondence to: Dr Shunyu Hou, Department of Gynecology and Obstetrics, The Affiliated Suzhou Hospital of Nanjing Medical University, 26 Daoqian Road, Suzhou, Jiangsu 215006, P.R. China
E-mail: hou_shunyu@sina.com

*Contributed equally

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A total of 12 specimens of cervical cancer tissue were investigated in the present study and using immunohistochemistry, it was determined that they all expressed B7-H1. However, immunohistochemistry indicated that normal cervical epithelium taken from healthy controls was negative for B7-H1. It has been demonstrated that cervical cancer cells can inhibit the activation of T lymphocytes (17). However, following the downregulation of B7-H1 expression using specific blocking antibodies, suppression of T lymphocytes was inhibited. When B7-H1 was downregulated using a lentivirus carrying a small interfering RNA (siRNA) specific for B7-H1, the ratio of human cervical cancer cells (the green fluorescent protein ratio in mice injected with green fluorescent protein cancer cells) was decreased, whereas the immune response was increased. Therefore, the results of the present study suggest that B7-H1 may be developed as a novel target for the gene therapy of cervical cancer.

Materials and methods

Ethics statement. All methods were performed in accordance with the approved guidelines. In the present study, samples were collected with the written consent of subjects and the written approval of the Ethical Review Board of the Suzhou Hospital, Affiliated with Nanjing Medical University (Suzhou, China). Mice used in the present study were handled in strict accordance with the appropriate animal practices. All experimental procedures using mice in the present study were reviewed and approved by the Ethical Review Board of Nanjing Medical University.

Isolation and culture of normal cervical epithelium and cervical cancer cells. A total of 27 patients were studied in the present study. From these 27 patients, 15 normal cervical epithelial samples (from 15 female patinents with hysteromyoma aged 45 years or younger who had undergone routine womb excision) and 12 cervical cancer samples were obtained (from 12 female patients with cervical cancer aged 45 or younger who had undergone colposcopy and biopsy of the suspicious cancerous area and pathological diagnosis to confirm cervical cancer) at Suzhou Hospital, which is Affiliated with Nanjing Medical University (Suzhou, China), between September 2015 to September 2016. Normal cervical epithelial and cervical cancer cell samples were isolated by a two-step combined dissociation method using dispase and trypsin prior to being plated into a 10-cm culture dish (Corning Inc., Corning, NY, USA) and expanded (20). The cells were cultured in keratinocyte serum-free medium (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) in a humidified incubator in 5% CO_2 at 37˚C and were passaged by trypsinization (0.25% trypsin, 0.1% EDTA) and expanded serially with a split ratio of 1:3 at 80% confluence after 10 days. Cells were maintained in a humidified incubator in 5% CO_2 at 37˚C for ~1 week. In the present study, all procedures were performed following the guidelines established by Suzhou Hospital-Affiliated Nanjing Medical University Ethics Boards and written approval was granted from the Ethical Review Board of Suzhou Hospital. Written consent was obtained from all women, after they were informed that the samples would be used for study purposes.

RNA isolation and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was isolated using the RNeasy Mini extraction kit (Qiagen, Inc., Valencia, CA, USA) according to the manufacturer's instructions. A total of 1 μg RNA was used for reverse transcription and cDNA was synthesized from total RNA following the manufacturer's instructions (Qiagen, Inc.). For qPCR, primer mixes were loaded in duplicate wells in 96-well plates and PCR was performed following the addition of SYBR Green PCR Master mix (Thermo Fisher Scientific, Inc.) and 1 μg (final) cDNA. The following thermocycling conditions were applied: Pre-denaturation at 95˚C for 5 min; followed by 40 cycles of denaturation for 15 sec at 95˚C; and annealing and elongation at 65˚C for 35 sec. (Bio-Rad Laboratories, Inc., Hercules, CA, USA). As an internal control, GAPDH levels were quantified in parallel with target genes. Relative quantification of the target was determined by the $2^{-\Delta\Delta Cq}$ method (21). The primers used were as follows: B7-H1 forward, 5'-TGGCATTTGCTGAACGCATTT-3' and reverse, 5'-TGCAGCCAGGTCTAATTGTTTT-3'; B7-H2 forward, 5'-TGGCATTTGCTGAACGCATTT-3' and reverse, 5'-AAAGTTGCATTCCAGGGTCAC-3'; B7-H3 forward, 5'-CCCACAGGTTGCTTTGCTTAA-3' and reverse, 5'-GCAGACCCCTGGAGAACCA-3'; B7-H4 forward, 5'-TCTGGGCATCCCAAGTTGAC-3' and reverse, 5'-TCCGCCTTTTGATCTCCGATT-3'; interleukin (IL)-1β forward, 5'-TTCAGGCAGGCAGTATCACTC-3' and reverse, 5'-GAAGGTCCACGGGAAAGACAC-3'; IL-6 forward, 5'-CTGCAAGAGACTTCCATCCAG-3' and reverse, 5'-AGTGGTATAGACAGGTCTGTTGG-3'; tumor necrosis factor (TNF)-α forward, 5'-CAGGCGGTGCCTATGTCTC-3' and reverse, 5'-CGATCACCCCGAAGTTCAGTAG-3'; GAPDH forward, 5'-AGAAGGCTGGGGCTCATTTG-3' and reverse, 5'-AGGGGCCATCCACAGTCTTC-3'.

Flow cytometry analysis. The expression of cell surface antigens on cervical cancer cells was analyzed by flow cytometry. A total of $2x10^5$ cells were detached by trypsin and re-suspended in phosphate-buffered saline (PBS) containing 1% bovine serum albumin. They were then counted using a hemocytometer and $2x10^5$ cells were incubated at 37˚C for 30 min with diluted pre-immune immunoglobulin (Ig) G to block nonspecific binding of the antibodies (22). Following incubation at 4˚C for 1 h with a primary antibody specific for B7-H1 (cat. no. ab210931, dilution 1:100; Abcam, Cambridge, UK), the cells were washed three times in PBS. Cells were then incubated with phycoerythrin (PE)-labeled goat anti-mouse IgG secondary antibody (cat. no. ab5881, dilution 1:100; Abcam) at 37˚C for 1 h. Negative controls were conducted by performing incubation without primary antibodies. The results were analyzed using a FACSCalibur™ flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA). All data were analyzed using FlowJo 7.2.2 software (Tree Star Inc, Ashland, OR, USA), following recently updated guidelines (23).

Histological and immunochemical analysis. Cervical cancer tissue from human patients and mice, respectively were immediately fixed at room temperature in 4% paraformaldehyde for 24 h and were subsequently embedded in paraffin. Consequently, 5-μm thick paraffin sections were

evaluated using, immunohistochemistry and the light microscope (magnification, x20) was used to evaluate staining. Mouse anti-human B7-H1 monoclonal antibody (cat. no. ab210931, dilution 1:100; Abcam) was used for immunohistochemistry. Immunohistochemistry was performed according to the indirect streptavidin-biotin-peroxidase method (24). For immunostaining, the samples were incubated with a PE-conjugated secondary antibody (cat. no. ab5881, dilution 1:100; Abcam) and then counterstained with DAPI (Southern Biotech, Birmingham, AL, USA) and observed under a fluorescence microscope (magnification, x10; Leica DM 2500; Leica Microsystems, Inc., Buffalo Grove, IL, USA).

Preparation of peripheral blood mononuclear cells (PBMCs) to obtain T lymphocytes. Whole blood collected from healthy adult donors was provided by the Suzhou Red Cross Blood Center (Suzhou, China). PBMCs were isolated from blood using Ficoll-Biocoll Separation Solution (Jingyang, Tianjin, China), as previously described (25). PBMCs were added to RPMI-1640 medium (Hyclone, Logan, UT, USA) and centrifuged at ~600 x g for 5 min at room temperature. The supernatant was discarded, cells were resuspended in RPMI-1640 medium and counted using a light microscope. PBMCs were seeded in triplicate at $2x10^5$ cells/well in 96-well plates and incubated at 37˚C for 3 days in a humidified 5% CO_2 environment.

T lymphocyte proliferation assay. To assess the various effects of cervical cancer cells and the normal cervical epithelium on T cell proliferation, both types of cells were treated with 10 μg/ml mitomycin C (Sigma-Aldrich; Merck kGaA, Darmstadt, Germany) for 2 h at 37˚C. For the proliferation assay, $2x10^4$ cervical cancer cells/well and normal cervical epithelium were plated in triplicate into 96-well plates, respectively. T lymphocytes were stimulated using 0.4 μg/ml anti-human cluster of differentiation CD3 (cat. no. 10977-H001, dilution 1:100; BD Pharmingen; BD Biosciences) and CD28 antibodies (cat. no. 560684, dilution 1:100; BD Pharmingen; BD Biosciences). The ratios of T cells to cervical cancer cell or normal cervical epithelium were both 5:1. Cell proliferation was measured at 48 and 72 h independently following incubation using the Cell Counting Kit-8 (CCK-8) (Takara Biotechnology Co., Ltd., Dalian, China) assay.

Infection. Green fluorescent protein (GFP)-positive cervical cancer cells and B7H1-downregulated GFP positive cervical cancer cells were constructed. A lentivirus containing the GFP gene (LV-EGFP) was obtained from Stemcell Technologies, Inc. (Shanghai, China). Lentivirus containing the GFP gene and interfering RNA for B7H1 downregulation were constructed using the same vector. A total of $2x10^5$ cells were seeded and then added $1.3x10^6$ IU/ml of lentivirus with 10 mg/ml polybrene. After 24 h at 37˚C, the medium was changed, the cells were cultured in a humidified incubator in 5% CO_2 at 37˚C. The culture medium was consisted of 90% Dulbecco's modified Eagle's medium (Hyclone) and 10% fetal bovine serum (Invitrogen; Thermo Fisher Scientific, Inc.). When the cervical cancer cells and B7H1 downregulated cervical cancer cells were ~80% confluent, GFP-positive cells were detected using a flow cytometer (BD Biosciences).

MTT assay. B7H1 downregulated cervical cancer cells and cervical cancer cells were seeded in 96-well culture plates at an optimal density of $5x10^3$ cells/well in triplicate wells (15). Following incubation at 37˚C for 0, 24, 48, 72, 96 and 120 h, the cells were stained with 20 μl MTT (5 mg/ml) at 37˚C for 4 h and subsequently solubilized with 150 ml dimethyl sulfoxide. Absorbance was measured at 490 nm using a microtitration plate spectrophotometer and calculated using GraphPad Prism 5 software (GraphPad Software, Inc., La Jolla, CA, USA). Cell growth curves were calculated as the mean values of each group.

Immunofluorescence. Slides (Nalgene Nunc International, Naperville, IL, USA) that were 5-μm thick were obtained via frozen section at -20˚C were washed twice in PBS and fixed in acetone for 5 min. Cells were blocked for 30 min in 3% BSA (Sigma-Aldrich; Merck kGaA) in PBS. Nuclei were counterstained with 4',6-diamindino-2-phenylindole-2 (Sigma-Aldrich; Merck KGaA) for 3 min at room temperature. Preparations were observed under a fluorescence microscope (magnification, x10; Leica DM 2500; Leica Microsystems, Inc.).

ELISA assay. The ELISA reagents for interferon (IFN)-γ (cat. no. RAB0223), IL-10 (cat. no. RAB0244) and transforming growth factor (TGF)-β1 (cat. no. RAB0460) were all purchased from Sigma-Aldrich (Merck kGaA). Experiments were performed according to the manufacturer's instructions. Supernatants were collected from the T cells of the cervical cancer cells and B7-H1 downregulated cells. The cells were cultured for 12, 24 and 48 h. The experiments were processed as triplicate samples and the analysis was completed using a Bio-Tek ELX800 microplate reader at 450 nm (Bio-Tek Instruments, Inc., Winnoski, VT, USA).

Animal model. A total of 20 BALB/c female mice (weigh, 25-30 g; age, 4 weeks old) were obtained from the Chinese Academy of Sciences (Beijing, China). The temperature of the housing room was 22˚C, with 60% humidity. Mice had free access to food and water every day and were observed for 5 weeks. Mice were housed individually and observed every day. Mice were randomly divided into 2 groups (n=10 per group). Briefly, 40 mg/kg nembutal (Sigma-Aldrich; Merck kGaA) was used to anesthetize mice. A total of $2x10^{10}$/l GFP-positive cervical cancer cells or $2x10^{10}$/l B7-H1 downregulated cells were hypodermically injected into 10 mice. The end-point of the experiment was 35 days, which meant that days the mice were sacrificed and the samples were obtained at days 30 and 35. Subsequently, mice were sacrificed via overdose using 80 mg/kg nembutal intraperitoneal injection. Both B7H1 downregulated cervical cancer cell and cervical cancer cell tumors in mice experienced the following treatment: Fixing, frozen section and the images were captured of GFP cells using a fluorescent microscope. Both tumor samples were digested into single cells and the GFP ratio was measured by flow cytometry. Furthermore, the expression levels of IL-1β, IL-6 and TNF-α in both tumor samples, were analyzed using mouse specific primers. The detail methods involved in animal experiment were described in the previous sections.

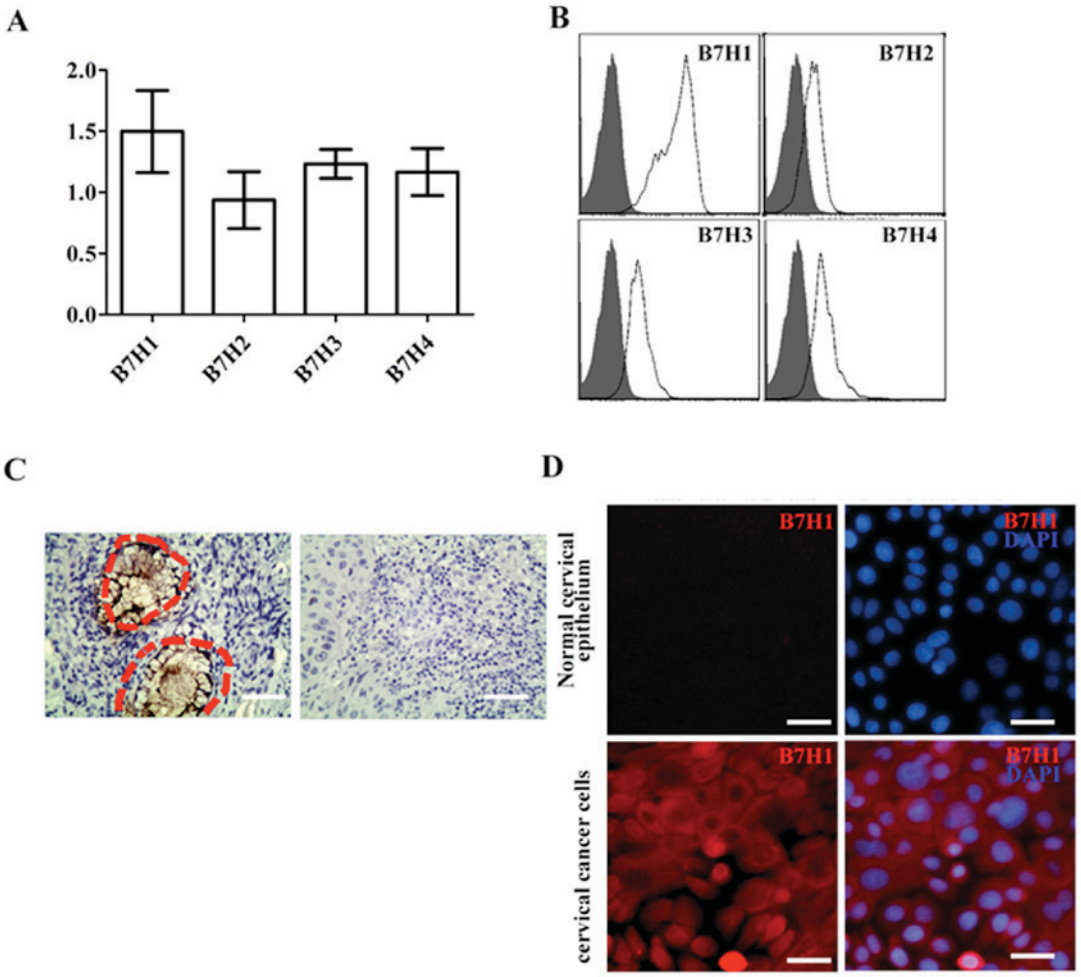

Figure 1. High expression of B7-H1 in cervical cancer cells and tissue. (A) mRNA expression levels of B7-H1, B7-H2, B7-H3 and B7-H4 in cervical cancer cells. (B) Levels of B7-H1, B7-H2, B7-H3 and B7-H4 protein in cervical cancer cells. (C) Immunohistochemistry results indicated that B7-H1 was highly expressed in cervical cancer cells (right-hand side; indicated by the red dotted-lined circles). However, no B7-H1 expression was observed in paracancerous tissue or normal cervical tissue (left-hand side; magnification, x10). (D) Immunostaining of B7-H1 (red) in normal cervical epithelium and cervical cancer cells. Nuclei (blue) were stained with 4',6-diamindino-2-phenylindole-2 (magnification, x20). Scale bar, 40 μm. B7-H, B7 homolog.

Statistical analysis. Data were presented as the mean ± standard deviation of at least three independent experiments and analyzed using Prism 5 software (Graph Pad Software, Inc., La Jolla, CA, USA). Differences between each group were analyzed using one-way analysis of variance and the Dunnett's post hoc test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Expression of B7-H1 in cervical cancer cells. To assess the expression of the B7 family of negative co-stimulatory molecules in cervical cancer, cervical cancer cells were studied. As presented in Fig. 1A, cervical cancer cells expressed the negative co-stimulatory molecules B7-H1, B7-H2, B7-H3 and B7-H4, of which, expression of B7-H1 was the highest. Results from flow cytometry indicated that in cervical cancer cells, B7-H1 protein was expressed at higher levels compared with B7H2, B7H3 or B7H4 (Fig. 1B). Thus, the present study focused on B7-H1. The expressions of B7-H1 in the 12 cervical cancer samples were measured using immunohistochemistry. The results demonstrated that B7-H1 was highly expressed in patients with cervical cancer (inside the red cycle; Fig. 1C), whereas B7-H1 was not expressed in paracancerous tissue and normal cervical tissue. In addition to the cancer tissue, immunostaining demonstrated that the cervical cancer cells expressed B7-H1. Conversely, the normal cervical epithelium was negative for B7-H1 expression (Fig. 1D).

The role of B7-H1 in the inhibitory effect of cervical cancer cells on human lymphocytes. To investigate the role of B7-H1 in the inhibitory effect of the immune response to T cells, cervical cancer cells were co-cultured with human T lymphocytes. CD3 was also used to stimulate the activation of T lymphocytes. Mitomycin-treated cervical cancer cells inhibited the activity of T lymphocytes, whereas normal cervical epithelial tissue did not have any marked inhibitory effects (Fig. 2). Following the use of the specific blocking antibodies of B7-H1 and siRNA to downregulate the expression of B7-H1, and specific blocking antibodies of B7-H3 to downregulate the expression of B7-H3, it was demonstrated that the B7-H1 blocking antibody and siRNA are able to inhibit the suppression of T lymphocytes by cervical cancer cells to a greater extent than the B7-H3 blocking antibody.

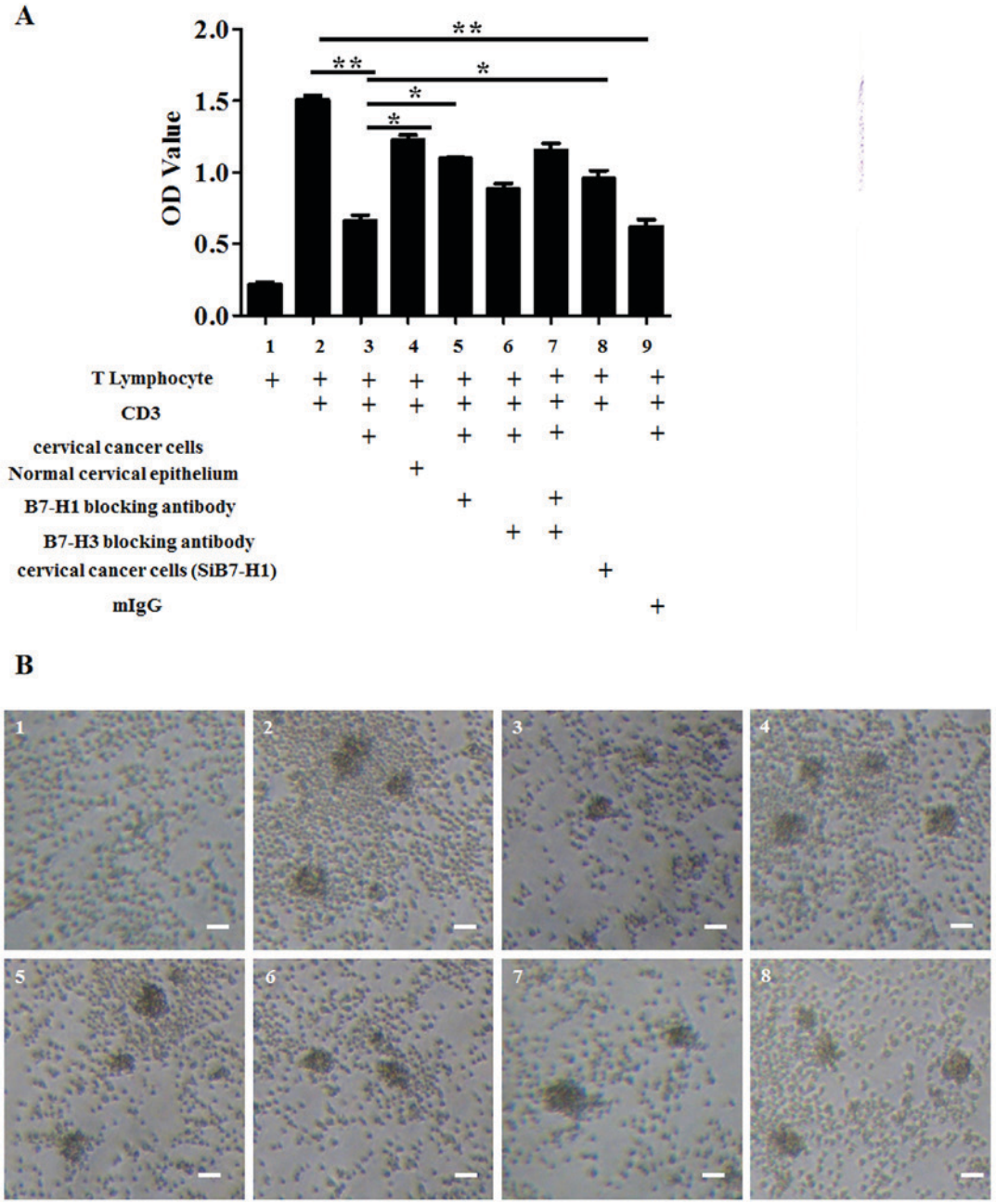

Figure 2. Downregulation of B7-H1 in cervical cancer suppresses the inhibitory ability of cervical cancer cells on human T lymphocytes. (A) Analysis of the inhibitory effect of cervical cancer cells on human lymphocytes and the effect of the B7 family blocking antibody on the inhibitory ability of cervical cancer cells. Cell Counting Kit-8 data are presented as the mean ± standard deviation of 3 independent experiments. Analysis was performed with GraphPad Prism; *P<0.05; **P<0.01. (B) The corresponding cell images: 1, T lymphocyte; 2, T lymphocyte + CD3; 3, T lymphocyte + CD3 + cervical cancer cells; 4, T lymphocyte + CD3 + normal cervical epithelium; 5, T lymphocyte + CD3 + cervical cancer cells + B7-H1 blocking antibody; 6, T lymphocyte + CD3 + cervical cancer cells + B7-H3 blocking antibody; 7, T lymphocyte + CD3 + cervical cancer cells + B7-H1 blocking antibody + B7-H3 blocking antibody. 8, T lymphocyte + CD3 + B7-H1 downregulated (SiB7-H1) cervical cancer cells (magnification, x10). Scale bar, 40 μm. CD, cluster of differentiation; B7-H, B7 homolog; OD, optical density; Ig, immunoglobulin.

Furthermore, B7-H1 and B7-H3 combined did not exert a significantly greater effect than B7-H1 or B7-H3 alone (Fig. 2; cells represented by each bar are shown in figure legend). It was therefore concluded that B7-H1 is an important molecule in inhibiting the immune response in cervical cancer cells.

The role of B7-H1 in the proliferation and cytokine secretion by T cell of cervical cancer cells. The results from ELISA demonstrated that following co-culture with T cells, the concentration of IFN-γ in the B7-H1 downregulated group increased slightly following 12 h co-culture. At 24 and 48 h, the concentration gradually increased and at 48 h was significantly higher in the B7-H1 group compared with the control (P<0.05). However, IL-10 and TGF-β1 secretion was significantly lower than in the cervical cancer cell group at all time points measured (P<0.05; Fig. 3A). B7-H1 is associated with a number of tumor-infiltrating $CD8^{+}$ T lymphocytes and therefore increases IFN-γ production (26).

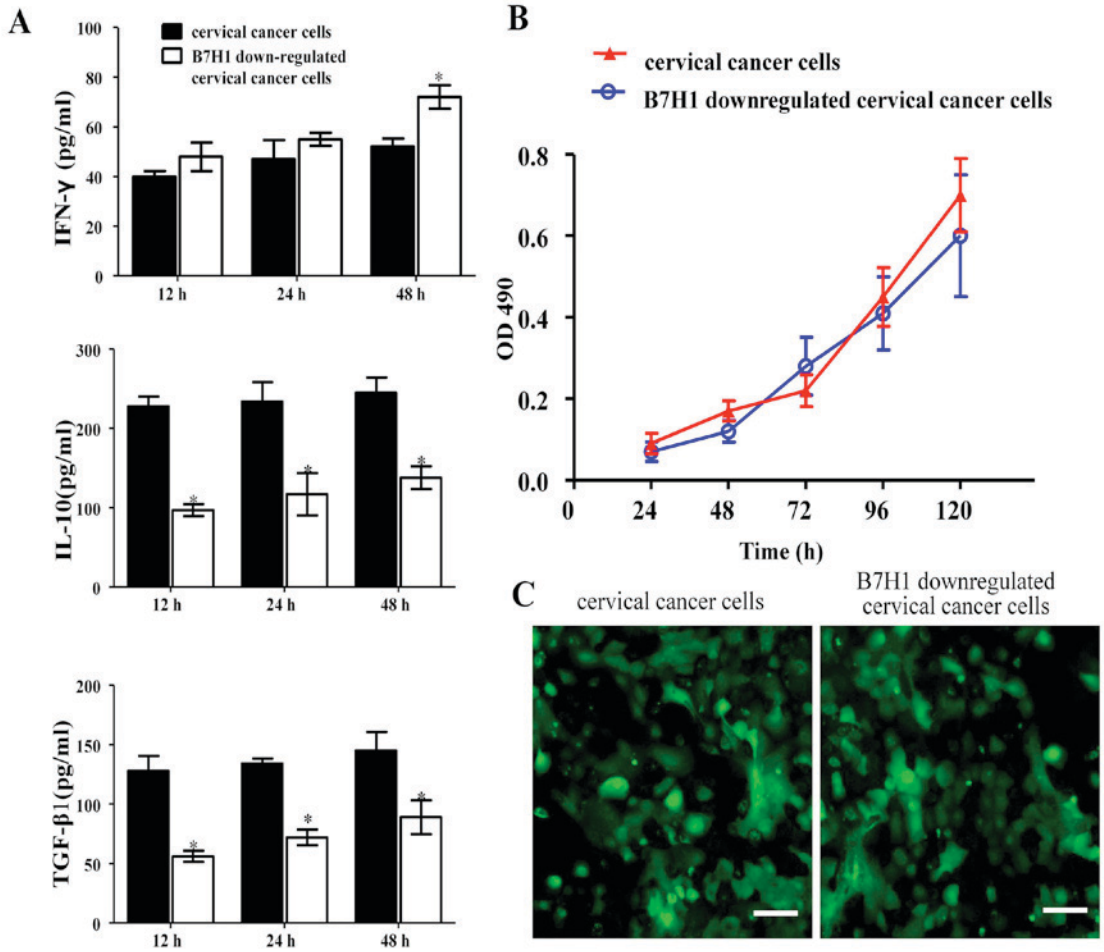

Figure 3. The role of B7-H1 in the viability and cytokine secretion by T cell of cervical cancer cells. (A) ELISA results indicating cytokine concentrations in the supernatants of the cultured T cells from cervical cancer cells and B7-H1 downregulated cervical cancer cells group after 12, 24 and 48 h. Analysis was performed with GraphPad Prism. *P<0.05. (B) MTT assay demonstrated the viability of cervical cancer cells and B7-H1 downregulated cervical cancer cells. Analysis was performed using GraphPad Prism. *P<0.05. (C) A GFP reporter gene was delivered into cervical cancer cells and B7-H1 downregulated cervical cancer cells using a lentiviral vector. Immunofluorescence showed that almost 100% of the cells were GFP positive (magnification, x10). Scale bar, 40 μm. B7-H, B7 homolog; GFP, green fluorescent protein; IL, interleukin; IFN, interferon; TGF, transforming growth factor; OD, optical density.

Results from the MTT assay demonstrated that when B7-H1 was downregulated in cervical cancer cells, cell viability was the same as in cervical cancer cells, which implies that the proliferation ability of both types of cells did not differ significantly (Fig. 3B). To further investigate the underlying mechanism, a GFP reporter gene was introduced into cervical cancer cells and B7-H1 downregulated cervical cancer cells using a lentiviral vector. These cells were then injected into BALB/c nude mice. Immunofluorescence showed that almost 100% of the cells were GFP positive, confirming the credibility of the following experiment (Fig. 3C). The aforementioned results suggested that when these two types of cells (B7H1 downregulated cervical cancer cells and cervical cancer cells) were transplanted into the subcutis of BALB/c nude mice, the difference in the ability to resist immune rejection was only influenced by the B7H1 downregulated cervical cancer cells and cervical cancer cells.

In the present study, the delitescence period of tumor formation was ~4 weeks. To observe the proportion of GFP-positive cells engrafted into the mice, the tumor and surrounding normal tissue were excised. Results from immunofluorescence staining indicated that the cervical cancer cell group had a larger number of GFP-positive cells than the B7-H1 downregulated cervical cancer cell group on days 30 and 35 (Fig. 4A). Flow cytometry analysis indicated that the proportion of GFP-positive cells in the cervical cancer cell group was ~25% on day 30, with this proportion increasing to ~36% on day 35. Conversely, the proportion of GFP-positive cells in the B7-H1 downregulated cervical cancer cell group was ~12% on day 30 and declined to ~3% by day 35 (Fig. 4B). Additionally, tissue from cervical cancer cells and B7-H1 downregulated cells with the surrounding precancerous lesions were excised and dispersed into a single-cell suspension, respectively. The expression of IL-1β, IL-6 and TNF-α was then analyzed using mouse specific primers. As presented in Fig. 4C, the expression of IL-1β in cervical cancer cells was significantly lower than in the B7-H1 downregulated cervical cancer cells on the first day (P<0.05). On day 14, the expression of IL-1β in B7-H1 downregulated cervical cancer cells reached a peak. A similar pattern was observed regarding the significant difference in expression of IL-6 on day 1 and TNF-α on day 7 (P<0.05). This indicates that B7-H1 aids the evasion of tumors from the immune system.

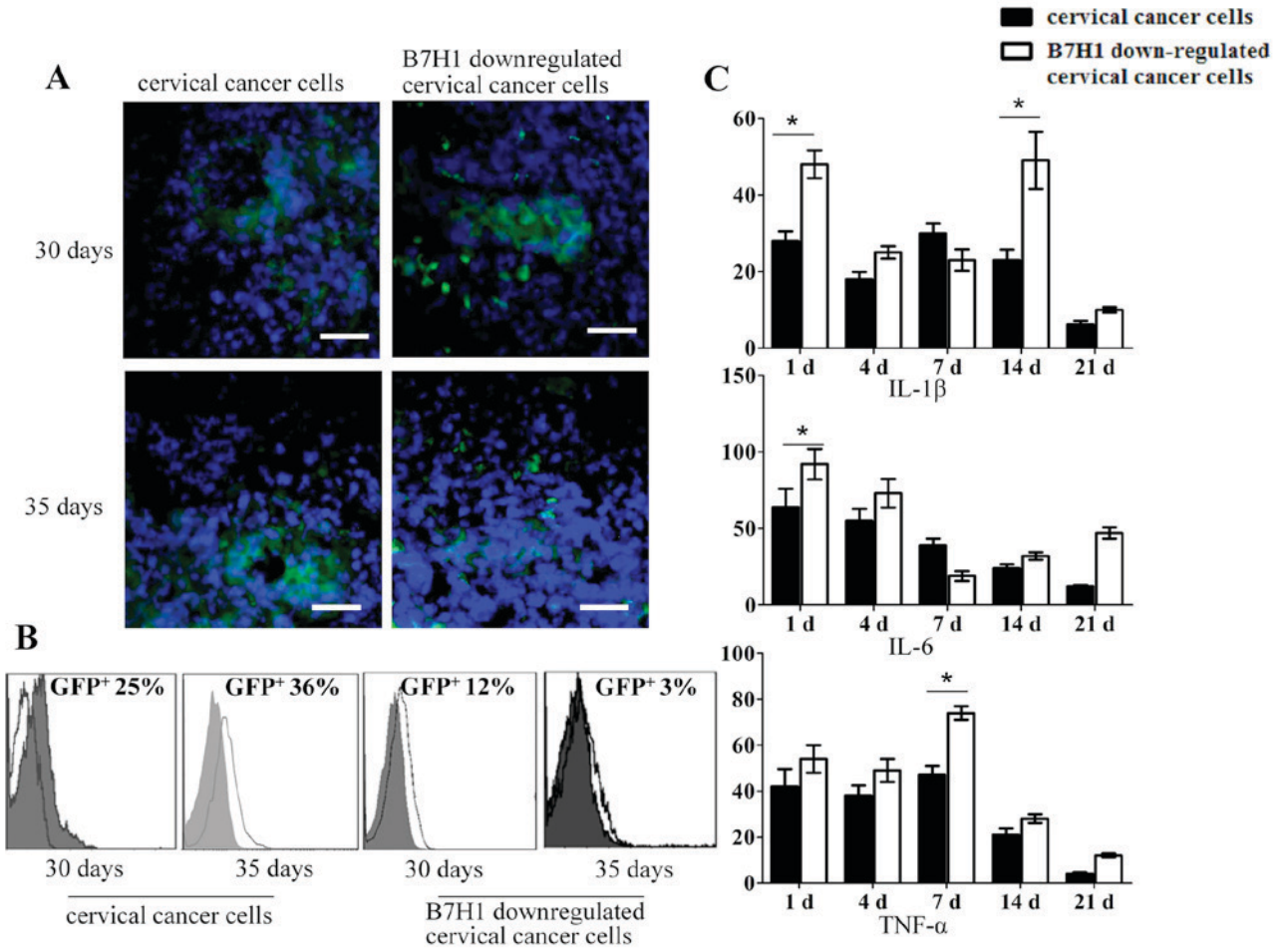

Figure 4. B7-H1 may aid the evasion of tumors from the immune system. (A) Immunofluorescence analysis of the presence of GFP-positive cells in cervical cancer cells and B7-H1 downregulated cervical cancer cells after 30 and 35 days' injection in mice. Scale bar, 40 μm. (B) Flow cytometry of GFP-positive cells from cervical cancer cell and B7-H1 downregulated cervical cancer cell-formed cervical cancer tissues after cell injection into BALB/c nude mice for 30 and 35 days, respectively (magnification, x10). (C) Reverse transcription-quantitative polymerase chain reaction analysis of IL-1β, IL-6 and TNF-α mRNA levels on days 1, 4, 7, 14 and 21 in the cervical cancer cells and B7-H1 downregulated cervical cancer cells group. The data are presented as the mean ± standard deviation of three independent experiments. Analysis was performed with GraphPad Prism; *P<0.05. B7-H, B7 homolog; IFN, interferon; IL, interleukin; TGF, transforming growth factor; GFP, green fluorescent protein; TNF, tumor necrosis factor.

Discussion

Cervical cancer is the most common malignant tumor detected in the female genital tract and its incidence rate has recently increased (1,2). T cell immunity serves a vital role in the recognition and killing of tumor cells, as well as the inhibition of tumor growth and proliferation (27). The occurrence and development of cervical cancer is associated with the low immune function of the local microenvironment of the host, particularly the immune function of T cells (28). B7-H1, also known as programmed death ligand, is a member of the B7 family. The binding of B7-H1 (programmed death-ligand 1) to its receptor, programmed cell death protein 1, can inhibit the proliferation of T cells and the secretion of some cytokines *in vitro* (29-31). B7-H1 acts as a negative co-stimulatory molecule in the process of T cell activation and may serve a major role in suppressing the immune system during certain events including pregnancy, tissue allografts (32), autoimmune disease (33) and other disease states such as hepatitis (34). A number of studies have detected elevated B7-H1 expression in numerous tumors and cancer cell lines, including non-small cell lung cancer (35), melanoma (36), colon cancer (37), renal cell carcinoma (38), ovarian cancer (39), pancreatic cancer (40), gastric cancer (41) and breast cancer (42).

The present study found that B7-H1 was highly expressed in cervical cancer cells and also in cervical cancer tissue, whereas normal cervical epithelium and the paracancerous tissue did not express B7-H1. Furthermore, it was demonstrated that when B7-H1 was downregulated via its specific blocking antibody and siRNA, it significantly inhibited the suppression of T lymphocytes. When co-cultured with B7-H1 downregulated cervical cancer cells, the levels of the cytokines IFN-γ, IL-10 and TGF-β1 secreted by T cells differed significantly compared with cervical cancer cells. Cervical cancer cells can form tumors more easily than B7-H1 downregulated cervical cancer cells *in vivo* and elicit a more moderate immune response. Therefore, B7-H1 is able to mediate the immune escape of cervical cancer cells.

It has been demonstrated that when B7-H1 is blocked effectively *in vivo*, the lethality of immune cells in cancer tissue can be strengthened (43) and this could be potentially applied to treat B7-H1-positive tumors. Thus, the immunoregulatory

function of B7-H1 holds important clinical value for treatment and for the molecular targeted therapy of gynecological malignant tumors.

In conclusion, findings of the present study indicated that B7-H1 mediated the inhibition of T lymphocyte activation of cervical cancer cells. When B7-H1 was downregulated, the cell viability of B7-H1 downregulated cervical cancer cells did not change compared with cervical cancer cells, whereas the soluble factors that are secreted by T cells changed between cervical cancer cells and B7-H1 downregulated cervical cancer cells. In an *in vivo* animal model, injected B7-H1 downregulated cervical cancer cells exhibited a potent immune response, whereas cervical cancer cells provoked the weak immune response. The findings suggest that B7-H1 mediated the low immunogenicity of cervical cancer and evaded attack from the immune system.

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