Cancer Letters 335 (2013) 278–288

Contents lists available at SciVerse ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Enhanced anti-cancer activity of human dendritic cells sensitized with gamma-irradiation-induced apoptotic colon cancer cells

Sun Kyung Kim [a], Cheol-Heui Yun [b], Seung Hyun Han [a,*]

[a] Department of Oral Microbiology and Immunology, DRI, and BK21 Program, School of Dentistry, Seoul National University, Seoul, Republic of Korea
[b] Animal Science and Biotechnology Major and World Class University Biomodulation Major, Department of Agricultural Biotechnology and Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea

A R T I C L E I N F O

Article history:
Received 15 January 2013
Received in revised form 14 February 2013
Accepted 14 February 2013

Keywords:
Dendritic cells
Cancer cell-killing
γ-Irradiation
Cytotoxic molecules
HT-29

A B S T R A C T

Properly sensitized dendritic cells (DCs) can be an effective immunotherapeutic against cancers. We investigated the phenotypic and functional changes in human DCs sensitized with γ-irradiated colon cancer cell-line HT-29 (GIH). GIH induced maturation and activation of DCs. GIH-sensitized DCs showed increased cytotoxic activity against HT-29 through higher expression of perforin and granzyme B. They further induced expression of effector cytokines, cytotoxic molecules, and mucosal-homing receptor in autologous T-cells. Conclusively, these results suggest that effective anti-cancer activity is induced when DCs are sensitized with γ-irradiated cancer cells via both direct augmentation of the cytotoxicity and indirect activation of T cells.

© 2013 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Dendritic cells (DCs) are the professional antigen-presenting cells that link innate and adaptive immunities and play an important role in the elimination of cancer cells. DCs sense, phagocytose, and digest malignant cells, and at the same time, migrate into draining lymph nodes to initiate the induction of adaptive immune responses [1]. In the lymph nodes, DCs present the epitopes derived from the cancer cells to naïve helper T (T_H) cells, which in turn activate cytotoxic T lymphocytes (CTLs) to kill the cancer cells, mainly by releasing death-related molecules such as perforin and granzymes [2]. DCs also express cytokines and co-stimulatory molecules that affect the type, strength, and duration of adaptive immune responses [3] required for effective anti-cancer immune responses [1]. In addition to the role of DCs as antigen-presenting cells, DCs can also eliminate cancer cells directly. DCs provide cancer cells with death signals such as tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) or Fas ligand (FasL), resulting in apoptotic death [4]. They also utilize soluble cytotoxic effectors like perforin and granzyme B to eliminate cancer cells [5].

Pre-clinical and clinical studies show convincing evidence on the activation of cellular immunity and cancer regression following treatment of cancer with DCs. For example, vaccination with DCs carrying cancer antigens demonstrated improvement in the antigen-specific cytotoxic activity of CTLs against melanoma and reduction of the metastases [6]. In addition, patients with melanoma who received DCs pulsed with lysates of three allogenic melanoma cell lines elicited anti-melanoma immune responses, reduction of regulatory T cells (Treg), and prolonged survival [7]. For DC therapy, preparation of the cancer antigens to sensitize DCs is substantially important in maximizing efficacy [8]. Antigen-sensitizing methods commonly used in pre-clinical and clinical trials include: (i) transduction of viral vectors encoding tumor-associated antigen (TAA) peptides in DCs, resulting in a specific immune response limited to the transduced TAA [9]; (ii) fusion of DCs with cancer cells possessing multiple cancer antigens, but low fusion efficacy and immunosuppressive properties of the hybridoma obscure the efficacy of the therapy [10]; and (iii) sensitizing DCs with killed cancer cells that contain a number of various cancer-specific antigens [11]. Various methods have been introduced for the inactivation of cancer cells such as chemotherapeutic treatment, freeze-thawing, and ionizing radiation, which exhibit distinctive phenotypes and immunostimulating capacities in the activation of DCs [12].

Ionizing radiation, including γ-rays, has been used as the conventional anti-cancer therapy for years because the local irradiation to the area of the tumor in the patient directly induces cancer cell death and limits progression of tumor [13]. Recently, γ-irradiation was reported to induce immunogenic tumor cell death [12] and immunotherapy together with γ-irradiation has

* Corresponding author. Address: Department of Oral Microbiology and Immunology, DRI, and BK21 Program, School of Dentistry, Seoul National University, 28 Yongon-Dong, Chongno-Gu, Seoul 110-749, Republic of Korea. Tel.: +82 2 740 8641; fax: +82 2 743 0311.
E-mail address: shhan-mi@snu.ac.kr (S.H. Han).

0304-3835/$ - see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.canlet.2013.02.038

focused on the development of cancer vaccines using DCs [14,15], calling for further studies into the mechanisms by which irradiated cancer cells modulate DCs and subsequent immune responses. In the present study, we investigated whether γ-irradiated cancer cells could sensitize and enhance the anti-cancer immune properties of DCs targeting a human colon cancer cell-line, HT-29.

2. Materials and methods

2.1. Reagents and chemicals

Ficoll–Paque plus was obtained from GE Healthcare (Uppsala, Sweden). Anti-human CD14 magnetic particles were purchased from BD Biosciences (San Diego, CA, USA). Fetal bovine serum (FBS), penicillin–streptomycin solution, RPMI 1640 and DMEM were purchased from HyClone (Logan, UT, USA) and Trypsin–EDTA solution was purchased from Invitrogen (Carlsbad, CA, USA). Recombinant human GM-CSF and interleukin (IL)-4 were purchased from Peprotech (Rocky Hill, NJ, USA) and R&D Systems (Minneapolis, MN, USA), respectively. Annexin V-FITC apoptosis detection kit I for analyzing cancer cell death and 5-(and-6)-carboxyfluorescein diacetate succinimidyl ester (CFDA-SE/CFSE) for fluorochrome-labeling cancer cells were purchased from BD Biosciences and Molecular Probes (Eugene, OR, USA), respectively. Monoclonal antibodies (mAbs) used for flow cytometric analyses were PE-labeled anti-human CD80 antibody (Ab), APC-labeled anti-human CD86 Ab, FITC-labeled anti-human HLA-DR, DP, DQ Ab for MHC class II, Alexa Fluor 647-labeled anti-human CD205 Ab, and PE-labeled anti-human IL-12p70 Ab were obtained from BD Biosciences. FITC-labeled anti-human TNF-α Ab, APC-labeled anti-human IL-6 Ab, APC-labeled anti-human IL-10 Ab, Alexa Fluor 647-labeled anti-human DC-SIGN Ab, APC-labeled anti-human PD-L1 Ab, PE-labeled anti-human PD-L2 Ab, PE-labeled or APC-labeled anti-human perforin Ab, Alexa Fluor 647-labeled anti-human granzyme B Ab, FITC-labeled or APC-labeled anti-human CD25 Ab, APC-labeled anti-human CD69 Ab, PE-labeled or APC-labeled anti-human CD3 Ab, PE-labeled anti-human CD4 Ab, PE-labeled anti-human CD8 Ab, Alexa Fluor 647-labeled anti-human FoxP3 Ab, APC-labeled anti-human α4β7, Alexa Fluor 647-labeled CCR6 Ab and Alexa Fluor 647-labeled anti-human CCR9 Ab were purchased from BioLegend (San Diego, CA, USA). All isotype-matched control (IC) Abs were obtained from BD Bioscience or BioLegend. Concanamycin A and staurosporine were purchased from Sigma–Aldrich (St. Louis, MO, USA). Z-AAD-CMK was obtained from Calbiochem (Darmstadt, Germany).

2.2. Generation of human monocyte-derived DC

All experiments using human blood were conducted under the approval of the Institutional Review Board of the Seoul National University (IRB No. S-D20060001). Peripheral blood mononuclear cells (PBMCs) were obtained from heparinized adult peripheral blood by density gradient centrifugation using Ficoll–Paque plus. Monocytes were isolated using anti-human CD14 magnetic beads (BD Biosciences). Then, 2×10^6 cells/ml of $CD14^+$ cells were plated on 100-mm cell culture dishes and cultured in complete RPMI 1640 supplemented with 800 U/ml of GM-CSF and 500 U/ml of IL-4 for 5 days. The cytokines were changed after 3 days. Expression of phenotypic markers of monocytes and iDCs were analyzed by flow cytometry (FACSCalibur, BD Biosciences).

2.3. Preparation of inactivated cancer cells and DC sensitization

HT-29 cells were purchased from the American Type Culture Collection (Manassas, VA, USA). The cells were cultured on 100-mm cell culture dishes until 70–80% confluent in complete DMEM containing 10% FBS and 1% penicillin–streptomycin, and then detached by trypsinization using 0.25% trypsin–EDTA solution. Inactivated HT-29 cells were prepared and mixed with iDCs as follows. HT-29 cells were detached and re-suspended in complete DMEM at 1×10^6 cells/ml. To prepare γ-irradiated HT-29 cells (GIH), the cells were irradiated with γ-rays at 10, 50, or 100 Gy. Freeze-thawed HT-29 (FTH) cells were snap-frozen in liquid nitrogen, and immediately thawed in a 37 °C water bath, and this procedure was repeated three times. To prepare chemotherapeutic-induced apoptotic HT-29 (SH) cells, the cells were treated with 5 nM of staurosporine for 48 h. Gamma-irradiation/staurosporine-co-treated HT-29 (ISH) cells were also prepared to examine the combined effect of radiation and chemotherapy. The condition of the cells was analyzed by propidium iodide (PI) and annexin V-FITC staining according to the manufacturer's instructions followed by flow cytometric analyses. Live HT-29 cells (LH) were used as a negative control. For sensitization, the iDCs were incubated with inactivated HT-29 cells for 24–48 h.

2.4. Characterization of DCs

iDCs (1×10^6 cells/ml) were stimulated with either live or inactivated HT-29 cells (1×10^6 cells/ml) in the presence of GM-CSF and IL-4 for 48 h. DCs were harvested and stained with fluorochrome-conjugated mAbs specific to typical DC markers including CD80, CD86, MHC class II, CD205, PD-L1 and PD-L2 on ice for 30 min. Then, the cells were washed three times with cold PBS containing 2% FBS and analyzed by flow cytometry (FACSCalibur, BD Biosciences). At least 1×10^4 -cells were acquired for each sample and dead cells and cell debris were gated out. All flow cytometric data were analyzed using FlowJo software (Tree Star, San Carlos, CA, USA).

2.5. Intracellular staining of cytokines

iDCs (1×10^6 cells/ml) were stimulated with either live or killed HT-29 cells (1×10^6 cells/ml) for 24 h in the presence of GM-CSF and IL-4. Then, brefeldin A (10 μg/ml) was added to the culture at 8 h before the harvest. The cells were stained with mAbs specific to MHC class II or DC-SIGN, which HT-29 cells do not express. The cells were fixed with 4% paraformaldehyde for 15 min, and then permeabilized with PBS containing 0.1% saponin for another 15 min on ice. The permeabilized cells were stained for human IL-6, TNF-α, IL-12p70, or IL-10 on ice for 30 min, and then washed three times with cold PBS containing 0.1% saponin. The cells were acquired and analyzed as described above.

2.6. Phagocytosis assay

To label HT-29 cells, the cells (5×10^6 cells/ml) were suspended in PBS containing 10 μM of CFSE for 15 min at 37 °C. The cells were washed with PBS three times, re-suspended in complete DMEM at 1×10^6 cells/ml, and then subsequently irradiated (50 Gy). The irradiated CFSE-HT-29 cells were incubated for 48 h, and mixed with iDCs (1×10^6 cells/ml) at various culture ratios (DC:HT-29 = 1:0.1, 1:0.2, 1:0.5, 1:1, and 1:2) for 12 h at 37 °C or 4 °C to measure specific uptake and non-specific uptake, respectively. Then, DC uptake was analyzed by flow cytometry.

2.7. Activation of autologous T cells by DCs sensitized with GIH

DCs sensitized with GIH (1×10^5 cells) and autologous $CD14^+$ cells-depleted PBMCs (1×10^6 cells) were plated in flat bottom 96-well cell culture plates and incubated for 4 days. Anti-human CD3 Ab was used with either anti-human CD4 Ab or CD8 Ab to analyze the $CD4^+$ or $CD8^+$ T cell population. To analyze Treg, the cells were stained with anti-human CD4 Ab and anti-human CD25 Ab, and then intracellular expression of FoxP3 was analyzed using anti-human FoxP3 Ab. To examine T-cell activation, staining with anti-human CD25 Ab and CD69 Ab followed by flow cytometric examination was performed. To determine the expression of gut mucosal-homing receptor on T cell subpopulations, a mAb specific to human α4β7, CCR6, or CCR9 was used. To analyze expression of cytokines in $CD4^+$ cells and $CD8^+$ T cells, phorbol 12-myristate 13-acetate (PMA, 0.5 μg/ml) and ionomycin (0.5 μg/ml) were added to the DC-PBMC cultures 6 h before harvest. Brefeldin A (10 μg/ml) was also added for 6 h to block the release of cytokines. After surface staining with either anti-human CD4 Ab or CD8 Ab, the cells were fixed, permeabilized, and stained with anti-human mAb specific to IFN-γ, IL-17A, IL-4, granzyme B, or perforin. The cells were analyzed by flow cytometry.

2.8. Measurement of cancer cell-killing activity of DCs (JAM test)

Live HT-29 cells (1×10^5 cells/ml) were labeled with 1 μCi/ml of [^{3}H]-thymidine for 12 h at 37 °C. The cells were re-suspended in RPMI 1640 and seeded in 96-well flat bottom plates at 1×10^4 cells/well. DCs sensitized with GIH were mixed with the target cells at an effector to target ratio of 20:1 as described previously [5] and incubated for 48 h. The cells were harvested and radioactivity was measured. The cytotoxicity (cancer cell-killing activity) of the DCs was calculated with the following formula: cpm [1-{(target + killer)/target alone}] × 100.

3. Results

3.1. GIH efficiently induces maturation of DCs

First, we characterized the cell death in GIH. Annexin V- and PI-positive apoptotic cell populations increased as the irradiation intensity increased (Fig. 1A) and as the incubation time increased (Fig. 1B). Next, iDCs were treated with the irradiated HT-29 cells and DC maturation was analyzed. HT-29 cells undergoing apoptosis induced expression of co-stimulatory molecules (CD80 and CD86) and MHC class II on iDCs in dose- and time-dependent manner (Fig. 1C and D). In addition, iDCs phagocytosed the irradiated HT-29 cells (Fig. 1E). These results suggest that irradiated, apoptotic HT-29 cells induce the activation and maturation of DCs.

Download English Version:

https://daneshyari.com/en/article/10899926

Download Persian Version:

https://daneshyari.com/article/10899926

Daneshyari.com