Cytotherapy, 2018; ■■: ■■–■■

International Society for Cellular Therapy ISCT®

Effect of irradiation-induced intercellular adhesion molecule-1 expression on natural killer cell-mediated cytotoxicity toward human cancer cells

JAE-UK JEONG[1,*], TUNG NGUYEN THANH UONG[2,3,*], WOONG-KI CHUNG[1], TAEK-KEUN NAM[1], SUNG-JA AHN[1], JU-YOUNG SONG[1], SANG-KI KIM[4], DONG-JUN SHIN[2,4], EUGENE CHO[1,2], KYOUNG WON KIM[2,3], DUCK CHO[5] & MEE SUN YOON[1,2,3]

[1]Department of Radiation Oncology, Chonnam National University Hwasun Hospital, Chonnam National University Medical School, Gwangju, Republic of Korea, [2]Research Center for Cancer Immunotherapy, Chonnam National University Hwasun Hospital, Jeollanam-do, Republic of Korea, [3]Department of Biomedical Science, Chonnam National University Graduate School, Gwangju, Republic of Korea, [4]Department of Companion & Laboratory Animal Science, Kongju National University, Yesan, Republic of Korea, and [5]Department of Laboratory Medicine & Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

Abstract

Background aims. Irradiation enhances the adhesion between natural killer (NK) cells and target cells by up-regulating intercellular adhesion molecule-1 (ICAM-1) on target cells. Therefore, we investigated the effect of irradiation-induced ICAM-1 expression on human cancer cells on NK cell–mediated cytotoxicity. *Methods.* Expression levels of ICAM-1 on the target cell surface before and after irradiation of six human cancer cell lines (HL60, SKBR-3, T47D, HCT-116, U937 and U251) were analyzed by flow cytometry. *Ex vivo* expansion of NK cells from human peripheral blood mononuclear cells was performed by co-culture with irradiated K562 cells. The related adhesion molecule lymphocyte function–associated antigen 1 (LFA-1) on NK cells was analyzed by flow cytometry. An enzyme-linked immunosorbent assay was used to detect interferon-γ (IFN-γ), and WST-8 assays were performed to check NK cell cytotoxicity. Finally, blocking assays were performed using monoclonal antibodies against ICAM-1 or LFA-1. *Results.* LFA-1 expression increased on NK cells after expansion ($P < 0.001$). The expression of ICAM-1 was significantly upregulated by irradiation after 24 h in various cell lines, including HL60 ($P < 0.001$), SKBR-3 ($P < 0.001$), T47D ($P < 0.001$) and U937 ($P < 0.001$), although the level of expression depended on the cell line. ICAM-1 expression was extremely low before and after irradiation in U251 cells. NK cell–mediated cytotoxicity increased after irradiation of HL60 ($P < 0.001$), SKBR-3 ($P < 0.001$), T47D ($P = 0.003$), and U937 ($P = 0.004$) cells, in which ICAM-1 expression was significantly increased after irradiation. IFN-γ production by NK cells in response to HL60 ($P < 0.001$) and T47D ($P = 0.011$) cells significantly increased after irradiation. NK cell–mediated cytotoxicity against irradiated SKBR-3 ($P < 0.001$) and irradiated T47D cells ($P = 0.035$) significantly decreased after blocking of ICAM-1. Blocking of LFA-1 on NK cells resulted in reduced cytotoxicity against irradiated HL60 ($P < 0.001$) and irradiated SKBR-3 ($P < 0.001$). *Conclusions.* Irradiation upregulates ICAM-1 expression on the surface of human cancer cells and enhances activated NK cell–mediated cytotoxicity. Therefore, irradiation combined with NK cell therapy may improve the antitumor effects of NK cells.

Key Words: *immunotherapy, intercellular adhesion molecule-1, irradiation, natural killer cell*

Introduction

Immunotherapy using natural killer (NK) cells is effective because NK cells do not require specific tumor cell antigens to induce cytotoxicity and do not cause graft-versus-host disease, in contrast to cytotoxic T lymphocytes [1–4]. Enhanced cytotoxicity was observed in *ex vivo*–expanded NK cells against various tumor cells [5,6]. Furthermore, chimeric antigen receptor (CAR)-engineered NK cells intended to overcome tumor cell resistance demonstrated antitumor activity to B-cell malignancies in *in vivo* studies [7,8].

*These authors contributed equally to this work.
Correspondence: **Mee Sun Yoon**, MD, PhD, Department of Radiation Oncology, Chonnam National University Hwasun Hospital, Chonnam National University Medical School, 322 Seoyong-ro, Hwasun-eup, Hwasun-gun, Jeonnam-do 58128, Republic of Korea. E-mail: meesunyoon@jnu.ac.kr

(Received 10 August 2017; accepted 28 January 2018)

ISSN 1465-3249
https://doi.org/10.1016/j.jcyt.2018.01.010

Clinical trials using CAR-engineered NK cells are ongoing in patients with B-cell leukemia and lymphoma (clinicaltrials.gov identifiers NCT-00995137, NCT-01974479 and NCT-03056339).

Binding of NK cells to tumor cells is essential to achieve NK cell–mediated cytotoxic effects, which results from the direct release of cytotoxic granules into tumor cells [9]. This adhesion of NK cells to tumor cells is mediated by lymphocyte function–associated antigen 1 (LFA-1). LFA-1 is a member of a family of leukocyte integrins composed of β-chains (β2, CD18) and α-chains (αL, CD11a) [10]. It is expressed on the surface of NK cells and adheres to intercellular adhesion molecule-1 (ICAM-1) of tumor cells [11,12]. Adhesion of NK cells to target cells affects the cytotoxicity of NK cells, with a lack of LFA-1 on NK cells or ICAM-1 on target cells resulting in decreased cytotoxicity of NK cells [13,14].

Irradiation can induce increased expression of adhesion molecules in tumor cells. In fact, several studies have shown that irradiation increases ICAM-1 expression in human cancer cells [15–19]. However, no studies have examined the relationship between the irradiation-induced increase in ICAM-1 expression on tumor cells and cytotoxicity of NK cells.

In this study, we evaluated whether increased ICAM-1 expression by irradiation is directly related to the cytotoxicity of NK cells against human cancer cells. This is the first study to investigate the relationship between radiation-induced ICAM-1 expression and NK cell cytotoxicity and whether resistance to NK cells can be overcome by enhancing the expression of adhesion molecules in human cancer cells.

Methods

Reagents

The following anti-human monoclonal antibodies (mAbs) were used in this study: fluorescein isothiocyanate-conjugated CD3, allophycocyanin-conjugated CD56, phycoerythrin (PE)-conjugated CD54, IgG1 isotype control (BD Biosciences) and PE-conjugated CD11a/CD18, IgG1 isotype control (BioLegend). Mouse anti-human ICAM-1 blocking antibody (BBIG-l1) (R&D Systems) and mouse anti-human CD18 blocking antibody (Abcam) were used for the blocking assay. The recombinant human interleukins rhIL-2, rhIL-15, rhIL-21 (PeproTech) were used to expand NK cells. Vita-orange Cell Viability reagent (WST-8; Biotool) was used in the cytotoxicity assay and blocking assay.

Cell culture

Different human cancer cell lines, including human breast cancer (SKBR-3 and T47D), human colon cancer (HCT-116), human promyelocytic leukemia cells (HL60), human glioblastoma (U251) and human lymphoma (U937) cells, were obtained from the American Type Culture Collection. HL60, SKBR-3, T47D, HCT-116 and U937 were cultured in RPMI1640 media and an U251 cell line in Dulbecco's Modified Eagle's Medium. All medium contained 10% inactivated fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin (all from Invitrogen). All cell lines were incubated at 37°C in a humidified 5% CO_2 incubator.

NK cell expansion

NK cell expansion was performed as described previously [20]. Peripheral blood mononuclear cells (PBMCs) were isolated using Lymphoprep (Axis-Shield) from healthy donors. PBMCs were co-cultured with 100-Gy gamma-irradiated conventional K562 cells (ATCC) in a 24-well plate with RPMI 1640 medium (10% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin and 4 mmol/L L-glutamine) containing 5 ng/mL rhIL-21 and 10 U/mL rhIL-2. The medium was exchanged on days 3 and 5 with fresh medium containing 10 U/mL rhIL-2. From day 7, the medium was exchanged every 2 days with fresh medium containing 100 U/mL rhIL-2 and 5 ng/mL rhIL-15. Expanded NK cells were continuously cultured until days 14–17.

Irradiation of human cancer cell lines

Human cancer cell lines including HL60, SKBR-3, T47D, HCT-116, U937 and U251 were exposed to 20-Gy ionizing radiation. The expression level of ICAM-1 was measured at 24 h after irradiation. To determine whether radiation-induced upregulation of ICAM-1 occurred in a time-dependent manner, HL60 and U251 cell lines were harvested at different time points (6, 24 and 48 h) after irradiation.

After each time point, the irradiated human cancer cells were harvested into a new 15-mL tube and centrifuged at 1300 rpm for 3 min. Human cancer cells were placed in new media and immediately using for fluorescence-activated cell-sorting analysis, cytotoxicity assay, blocking assay, and interferon-γ (IFN-γ) enzyme-linked immunosorbent assay (ELISA).

Flow cytometry

The expression of adhesion molecules on cancer cell lines and expanded NK cells was analyzed by flow cytometry. Expanded NK cells (2×10^5) were stained with mAbs specific for LFA-1, and cancer cell lines (2×10^5) with or without irradiation were stained with mAbs specific for ICAM-1 after washing twice with phosphate-buffered saline (PBS) and 5% bovine serum albumin (BSA). All cells were stained on ice for 15 min.