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Original Article

Increased concentration of sialidases by HeLa cells might influence the cytotoxic ability of NK cells

Wen-Chi Lee [a,b,1], Wen-Ling Lee [c,d,1], Wen-Yuann Shyong [a,b], Ling-Wei Yang [a,b,e], Min-Chun Ko [a,b,f], Bor-Ching Sheu [g], Shie-Liang Edmond Hsieh [e,f], Peng-Hui Wang [a,b,e,f,*]

[a] *Department of Obstetrics and Gynecology, National Yang-Ming University School of Medicine, Taipei, Taiwan*
[b] *Department of Obstetrics and Gynecology, Taipei Veterans General Hospital, Taipei, Taiwan*
[c] *Department of Medicine, Cheng-Hsin General Hospital, Taipei, Taiwan*
[d] *Department of Nursing, Oriental Institute of Technology, New Taipei City, Taiwan*
[e] *Immunology Research Center and Institute of Clinical Medicine, National Yang-Ming University School of Medicine, Taipei, Taiwan*
[f] *Immunology Research Center, Taipei Veterans General Hospital, Taipei, Taiwan*
[g] *Department of Obstetrics and Gynecology, National Taiwan University Hospital, College of Medicine, National Taiwan University, Taipei, Taiwan*

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Abstract

Objective: Cancer cells reportedly have the ability to escape from the immune system, mainly from natural killer (NK) cells. Although the real mechanisms are complicated, some inhibitors that are secreted from the cancer cells might play an important role. This study's aim was to investigate the potential mediator released by cancer cells (HeLa) that contributes to the decreased cytotoxicity of NK cells.
Methods and Materials: An NK-HeLa coculture system was used to test the hypothesis that the presence of the potential mediator from cancer cells contributes to the decreased cytotoxicity of NK cells.
Results: After coculturing with HeLa cancer cells, the cytotoxicity of NK cells was decreased. When the coculture medium and culture medium containing commercialized sialidase were used to culture NK cells, the cytotoxicity of the NK cells was also inhibited. However, cytotoxicity was partially restored by a sialidase inhibitor (DANA). Western blot analysis of the HeLa cells after coculturing with NK cells demonstrated increased Neu2 and Neu3 expression in HeLa cells.
Conclusions: The finding that Neu2 and Neu3 expression in cancer cells might be involved in the impaired function of NK cells, which could be restored by a sialidase inhibitor, provides a new concept that could be applied to the management of cancer.
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Keywords: cervical cancer cell line; cytotoxicity; natural killer cell; neuraminidase; neuraminidase inhibitor; sialidase

Introduction

Natural killer (NK) cells were initially identified as a lymphoid population that represents 10−20% of peripheral blood mononuclear cells (PBMC), and they are important players in the first line of defense against diseases because of their ability to lyse major histocompatibility complex class I (MHC-I)-negative tumors and virus-infected cells and orchestrate the innate immunity of the organism. The rapid cytotoxic actions and broad target range suggest that NK cells may be promising candidates for use in cancer cell therapy, with the potential to target a wide range of malignancies [1]. However, cancer cells often escape from NK cells, which results in therapeutic failure. Although the tumor escape mechanisms developed by cancers are complicated and uncertain, some of them could be explained by inhibitors that are secreted from cancer cells [2]. The restoration of immune

* Corresponding author. Department of Obstetrics and Gynecology, National Yang-Ming University and Taipei Veterans General Hospital, 201, Section 2, Shih-Pai Road, Taipei 112, Taiwan.
E-mail addresses: phwang@vghtpe.gov.tw, phwang@ym.edu.tw (P.-H. Wang).
[1] Both authors contributed equally to this article.

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functions is of great importance to tumor eradication, most particularly in regard to the residual disease [3]. Therefore, restoration of the cytotoxicity of the NK cells by blockage or neutralization of these cancer cell-secreting inhibitors might provide be valuable for use in cancer treatment.

In this report, we used a coculture system that mixed NK cells and cancer cells (a cervical cancer HeLa cell line) to investigate the possible mechanisms of tumor-induced immunosuppression.

Materials and methods

Cell lines

A human cervical cancer cell line, HeLa, was obtained from the Culture Collection and Research Center, Food Industry and Development Institute, Taiwan. HeLa cells were grown in DMEM (GibcoBRL, Grand Island, NY, USA) that was supplemented with 10% fetal bovine serum (FBS). The NK-sensitive cell line, K562, was cultured in RPMI 1640 (GIBCO BRL) that was supplemented with 10% FBS. All cell types were cultured in 10-cm dishes at 37°C in a humidified atmosphere with 5% CO_2. Cell number and viability were determined by staining with trypan blue, and the cell count was determined using a hemocytometer (MARIENFELD/MATSUNAMI, Germany).

NK cells

PBMCs were separated from leukapheresed adult peripheral blood by Ficoll-Hypaque density gradient centrifugation (Amersham Biosciences, GE Healthcare Life Sciences. Piscataway, NJ, USA) [4]. PBMCs were resuspended at a concentration of 10^6 cells/mL and cultured for 18 days. Then, the NK cells were isolated from the PBMCs by autoMACS (NK Isolation Kit; Miltenyi Biotec, Germany) and cultured for 1 day. The PBMCs and NK cells were maintained in RPMI 1640 medium that was supplemented with 10% FBS and 1000 U/mL recombinant human interleukin (IL)-2 (PeproTech Asia, Israel) at 37°C in a humidified atmosphere with 5% CO_2.

Interactions between NK and HeLa cells

A constant number of NK cells were directly cultured with the HeLa cells (at a 1:1 ratio) for 1.5 hours or 3.5 hours at 37°C in the NK-HeLa coculture system. Meanwhile, the cells were also treated with or without DANA (sodium 5-Acetamido-2,6-anhydro-2,3-dehydro-3,5-dideoxy-D-glycero-D-galactononanoate; DANA, CALBIOCHEM, La Jolla, CA, USA).

Cytotoxicity assay

K562 cells were incubated with 100 μCi of ^{51}Cr for 60 minutes at 37°C and then extensively washed with medium to remove any free ^{51}Cr. These radiolabeled target cells were seeded onto round-bottom 96-well microplates, then the NK cells or NK-HeLa cells were cultured with radiolabeled K562 cells (E:T ratio of 1:1) and incubated for 3 hours at 37°C. Supernatants containing the ^{51}Cr that was released from the lysed targets were collected and the radioactivity was counted using a Packard gamma counter (PerkinElmer Inc., United States). All experiments were repeated at least three times. Percent-specific cytotoxicity was calculated according to the formula: (sample ^{51}Cr released − spontaneously released)/(maximum amount released − amount spontaneously released) × 100 [5].

Sialidase and DANA treatment

NK cells were suspended at a concentration of 10^6 cells/mL in the RPMI 1640 medium and incubated in the presence or absence of 0.2 U/mL sialidase (N-6514; Sigma, St. Louis, MO, USA) and different doses of 1mM or 2mM DANA for 2 hours at 37°C followed by extensive washing with RPMI 1640 medium and 0.25% FBS.

Detection of sialidase activity and expression of HeLa cells in the coculture system

HeLa cells were seeded in 96well plate with 4×104 cells/well and fixed with cold methanol (100μL/well) for 5 minutes. Then remove cold methanol and incubated with the substrate 100μL per well contained 1 mM of X-Neu5Ac and 0.1 M sodium acetate (pH 4.8) and Fast Red Violet LB (0.1 mg/mL) for 1 hour at 37°C. The cells were then washed twice with PBS and detected using a fluorescence reader (Molecular Devices Corporation, SpectraMax M2, Molecular Devices, Sunyvale, CA, USA) with excitation wavelengths ranging from 520−550 nm and an emission wavelength of 580 nm [6,7]. In addition, the sialidase activity of the cocultured medium was detected using an Amplex Red Neuraminidase (Sialidase) Assay Kit (Invitrogen, San Diego, CA, USA), according to the manufacturer's recommendations.

Immunoblotting

Cells were homogenized and solubilized in SoluLyse-M Protein Extraction Reagent (Genlantis, San Diego, USA). The protein content was determined using the Bradford assay (Bio-Rad Laboratories, Inc., California, USA). The cell lysates were separated using SDS-PAGE and analyzed by immunoblotting with the respective antibodies using Western Lightning Chemiluminescence Reagent Plus (PerkinElmer, California, USA) [8]. The blots were probed using monoclonal anti-Neu2 antibody (Abnova, Taipei City, Taiwan) and anti-Neu-3 mAb (MBL Japan, Nagoya, Japan), respectively. Capture and analysis of the images of the protein bands was performed by using an imaging system equipped with a CCD camera (AlphaImager 2200; Alpha Innotech Corporation) and quantified by software AlphaEaseTM (Alpha Innotech Corporation, San Diego, CA, USA).

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