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Phenotypic characterization and anti-tumor effects of cytokine-induced killer cells derived from cord blood

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Abstract

Background aims. Cytokine-induced killer (CIK) cell therapy represents a feasible immunotherapeutic option for treating malignancies. However, the number of anti-tumor lymphocytes cannot be easily obtained from the cancer patients with poor immunity status, and older patients cannot tolerate repeated collection of blood. Cord blood–derived CIK (CB-CIK) cells have shown efficacy in treating the patients with cancer in several clinical trials. This study was conducted to evaluate the biological characteristics and anti-tumor function of CB-CIK cells. *Methods.* The immunogenicity, chemokine receptors and proliferation of CB-CIK cells were analyzed by flow cytometry. The CIK cells on day 13 were treated with cisplatin and the anti-apoptosis capacity was analyzed. The function of CB-CIK cells against the human cancer was evaluated both *in vitro* and *in vivo*. *Results.* Compared with peripheral blood–derived CIK (PB-CIK) cells, CB-CIK cells demonstrated lower immunogenicity and increased proliferation rates. CB-CIK cells also had a higher percentage of main functional fraction $CD3^+CD56^+$. The anti-apoptosis ability of CB-CIK cells after treatment with cisplatin was higher than that of PB-CIK cells. Furthermore, CB-CIK cells were effective for secreting interleukin-2 and interferon-γ and a higher percentage of chemokine receptors CCR6 and CCR7. In addition, tumor growth was greatly inhibited by CB-CIK treatment in a nude mouse xenograft model. *Conclusions.* CB-CIK cells exhibit more efficient anti-tumor activity in *in vitro* analysis and in the preclinical model and may serve as a potential therapeutic approach for the treatment of cancer.

Key Words: *cancer, cord blood, cytokine-induced killer cells, peripheral blood*

Introduction

Adoptive T-cell transfer for cancer immunotherapy involves the transfer of immune cells that have been expanded and activated *ex vivo* into patients to kill tumor cells. This approach has become an essential component in current cancer treatment [1, 2]. Various types of immune cells have been used in clinical trials, including natural killer (NK) cells, tumor-infiltrating lymphocytes (TILs) and cytokine-induced killer (CIK) cells [3–5]. NK cells are a subset of lymphocytes that provide innate effector mechanisms against viruses and tumor cells through direct cellular cytotoxicity on target cells and cytokine secretion [6]. The lack of major histocompatibility complex class I expression can render tumor cells susceptible to NK cell–mediated lysis. However, tumor cells have the ability to escape from NK cell recognition through the modulation of NK-activating receptors [7]. The *ex vivo* expansion of TIL populations to more favorable numbers followed by the transfer of these cells back into the host can eradicate the tumor. Nevertheless, the clinical application of TILs is limited by available tumor lesions [8].

CIK cells are a heterogeneous population with different cellular phenotypes: $CD3^-CD56^+$, $CD3^+CD56^-$ and $CD3^+CD56^+$ [9]. These cells can

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be readily amplified from bone marrow, peripheral blood (PB) and more recently from cord blood (CB) in the presence of IFN-γ, IL-2 and anti-CD3 monoclonal antibody (mAb), with a $CD3^+$ heterogeneous T-cell population including $CD3^+CD56^+$ cells responsible for their cytotoxicity; this subpopulation is derived from $CD3^+CD56^-$ T cells that acquire the CD56 marker [10]. The peripheral blood—derived CIK (PB-CIK) cells exhibit potent anti-tumor efficacy against various malignancies in preclinical models and have been proven as safe, feasible and effective in clinical studies [11—14]. In animal studies, CIK cells not only prevent tumor growth but also improve host immune function [15]. In clinical research, CIK cells have been evaluated as an immunotherapy for patients with advanced cancer such as hepatocellular carcinoma, lung cancer, melanoma and renal carcinoma [16—18]. However, the application of autologous CIK is limited, owing to obstacles such as short survival period *in vivo* and lower anti-tumor ability. Furthermore, it is very difficult to get enough effective CIK cells from the cancer patients with low immunity status, and older people with poor health conditions cannot tolerate repeated collection of blood. The allogeneic CIK cells from adult healthy donors might be a better source because their stronger anti-tumor effect than are autologous CIK cells derived from cancer patients [19]. However, the transfusion of allogeneic CIK cells from adult donors has a risk of acute graft-versus-host disease (GvHD) because of the human leukocyte antigen (HLA) disparity between donor and patient [20].

Studies have shown that CB-derived CIK (CB-CIK) cells can be largely expanded *in vitro* [21]. It has an overlapping phenotype with the traditional adult PB-CIK cells. The $CD3^+CD56^+$ cells contribute to their cytotoxicity [22, 23]. The cytotoxic effect of CB-CIK cells can be enhanced by interferon (IFN)-γ [24]. Nevertheless, the biological characteristics and function of CB-CIK cells are not well-defined. In this study, we investigated the immunogenicity, proliferation, anti-apoptosis capacity, chemokine receptor expression and cell surface markers of CB-CIK. Furthermore, the anti-tumor efficacy of CB-CIK cells was analyzed in the preclinical model.

Methods

Cell lines

Leukemia cell line NB4, human ovarian cancer cell line SKOV3 and human esophagus cancer cell line KYSE70 were purchased from the American Type Culture Collection. The myelogenous leukemia cell line K562 (American Type Culture Collection) was used as a positive control in cytotoxicity analysis. All cell lines were cultured in Roswell Park Memorial Institute (RPMI) 1640 (Gibco, USA) containing 10% fetal bovine serum (FBS, Sigma, USA), 100 U/mL penicillin and 100 μg/mL streptomycin.

Generation of CB-CIK and PB-CIK cells

Human PB samples were collected from subjects with histologically confirmed stage IV esophageal carcinoma and ovarian cancer. The CB samples were obtained from the First Affiliated Hospital of Zhengzhou University. Written informed consent was obtained from all participants involved in this study. The entire consent procedure was in accordance with the standard defined by Institutional Review Boards of the First Affiliated Hospital of Zhengzhou University.

Cord blood mononuclear cells from umbilical cord blood and peripheral blood mononuclear cells from patients with cancer were isolated by Ficoll density gradient (Tianjin HY, China) centrifugation, washed and resuspended at 2×10^6 cells/mL in RPMI 1640 containing 10% FBS, penicillin and streptomycin, and then were induced with recombinant IFN-γ at 1000 U/mL (Beijing SL, China). After 24 h of incubation in the atmosphere with 5% CO_2 at 37°C, 1000 U/mL interleukin (IL)-2 (Beijing SL, China) and 25 ng/mL mAb against human CD3 (Boehringer Mannheim, Germany) were added. Fresh medium with IL-2 was added as needed. After 13 days of culture, the CB-CIK and PB-CIK cells were ready for further analysis.

Fluorescence-activated cell sorting analysis

After 13 days of culture, CB-CIK and PB-CIK cells were harvested by means of centrifugation and washed twice with phosphate-buffered saline (PBS). These CIK cells were then stained with fluorescence-conjugated Abs against CD3, CD4, CD8, CD56, CD27, CD28, PD-1, LFA-1, intracellular adhesion molecule (ICAM)-1, CXCR3, CXCR4, CCR5, CCR6, CCR7, HLA-I and HLA-II. In brief, a total of 5×10^5 CIK cells were harvested and then incubated with appropriate mAbs for 20 min at 4°C. After incubation, CIK cells were washed before flow cytometry analysis. Nonspecific binding was determined with the use of irrelevant anti-human immunoglobulin isotypes. Data were acquired on a fluorescence-activated cell sorting anto II flow cytometer (BD, USA) and analyzed with the use of Diva software (BD, USA).

Proliferation assay

CB-CIK and PB-CIK cells were collected on day 6 and labeled with 5 μmol/L carboxy fluorescein