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Enhancement effect of dihydroartemisinin on human γδ T cell proliferation and killing pancreatic cancer cells

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ABSTRACT

γδ T cells play important roles in innate immunity against tumors and infections. Inhibitory effect of dihydroartemisinin on growth of cancer cells has been found in recent years. In this study, we investigated the effect of dihydroartemisinin on human γδ T cell proliferation by MTT assay and killing activity against pancreatic cancer cells SW1990, BxPC-3 and PANC-1 by LDH release assay in vitro. Intracellular molecule alterations were verified by flow cytometry. The results suggested that appropriate concentration of dihydroartemisinin favored the expansion of γδ T cells and enhanced γδ T cell mediated killing activity against pancreatic cancer cells. Up-regulation of intracellular perforin, granzyme B expression and IFN-γ production may be the important mechanism of dihydroartemisinin on increased antitumor activity of γδ T cells.

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1. Introduction

Human peripheral blood γδ T lymphocytes constitute approximately 1–5% of the total T cell population. Most of the circulating γδ T cells belong to the Vγ9δ2 subset, which recognizes non-peptide phosphoantigen such as the mevalonate pathway-derived isopentenyl pyrophosphate (IPP) and its structural analog aminobisphosphonates [1]. As part of innate immune system, activated γδ T cells produce proinflammatory cytokines and chemokines and kill infected cells and most tumor cells including leukemia/lymphoma cells [2,3]. Therefore, antitumor reactivity and the ability to intentionally increase this activity have raised great interest in exploring the immunotherapeutic potential of γδ T cells [4,5].

Dihydroartemisinin, a semi-synthetic derivative of Artemisinin, is isolated from *Artemisia annua*. Artemisinin and its derivatives are well known as a treatment for malaria [6], but more recent studies have revealed that it also has a preferentially cytotoxic effect on cancer cells, including leukemia cells, breast cancer cells and cervical cancer cells [2–4]. Moreover, some studies have indicated that Artemisinin derivatives have anti-cancer activities in vitro and in vivo [7,8]. The fact that dihydroartemisinin induces cytotoxicity and apoptosis in cancer cells has been reported by several groups. However, immunomodulatory activity and the mechanism of dihydroartemisinin on human immune cells such as γδ T cells are incompletely understood. In this study we attempt to investigate antitumor immune response of γδ T cells treated with dihydroartemisinin. Our findings indicate that dihydroartemisinin enhances antitumor cytotoxic effects mediated by γδ T cells. The killing of tumor cells is associated with increased expression of perforin, granzyme B and IFN-γ. Our results suggest that dihydroartemisinin may be a useful complementary agent in the immunotherapy of pancreatic cancer and other malignancies.

2. Materials and methods

2.1. Reagents and antibodies

Dihydroartemisinin was purchased from Sigma-Aldrich. Recombinant human IL-2 was purchased from Xiamen Amoytop Biotech Co., Ltd. Isopentenyl pyrophosphate (IPP) was purchased from Sigma-Aldrich Inc. The following Abs used for extracellular and intracellular stainings: PE-conjugated anti-human granzyme B, PE-conjugated anti-human Perforin and isotype matched controls were obtained from eBioscience Inc. FITC-conjugated anti-human TCRγδ, APC-conjugated anti-human CD107a, APC-conjugated anti-human IFN-γ and isotype matched controls were purchased from BD Biosciences.

2.2. Pancreatic cancer cells culture

Human pancreatic cancer cells (SW1990, BxPC-3, PANC-1) obtained from the Shanghai Cell Institute, Chinese Academy of Sciences were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum

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(FCS), 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin in a humidified 5% CO_2 incubator at 37 °C.

2.3. Expansion of human γδ T cells

PBMCs of 10 cases of human healthy donors (average age 28 ± 10) were isolated from blood samples after Ficoll-Hypaque centrifugation. For γδ T cell expansions, fresh PBMCs (5×10^5 cells/ml) were specifically activated by IPP (7.5 μmol/l) in RPMI 1640 medium supplemented with recombinant human IL-2 (100 U/ml), 10% FCS and 5% human AB serum in 5% CO_2 at 37 °C. Fresh medium containing same component was added every 3 days. After 8 days, cells were observed under an inverted phase contrast microscope and oil immersion lens (Wright–Giemsa staining). Specific expansion of γδ T cells from PBMC was measured by flow cytometry (CD3/TCRγδ, Vδ1/Vδ2 staining) at day 8 following activation, mouse anti-human IgG1, κ-FITC was served as isotype control. Cell viability ratio was observed under microscope using trypan blue exclusion test.

2.4. Magnetic cell sorting

γδ T cells cultured for 8 days were resuspended in phosphate-buffered saline (PBS) supplemented with 0.5% bovine serum albumin and 2 mM EDTA and incubated with anti-TCRγδ hapten antibody for 10 min at 4 °C, and then added MACS anti-hapten microbead-FITC and incubated for 15 min at 4 °C. The cells were purified by positive magnetic immunoselection by using automated magnetic cell sorting (autoMACS; Miltenyi Biotec, Germany) according to the manufacturer's instructions.

2.5. Proliferation assays

After preliminary inoculation (5×10^5 cells/ml) in 96-well plates for 24 h, γδ T cells were incubated with various concentrations of dihydroartemisinin containing 200 μl of 10% FCS/RPMI 1640 medium in 5% CO_2 at 37 °C for another 48 h. Final concentrations of dihydroartemisinin were 0 μmol/ml, 12.5 μmol/ml, 25 μmol/ml, 50 μmol/ml, 100 μmol/ml, and 200 μmol/ml respectively. After incubation,

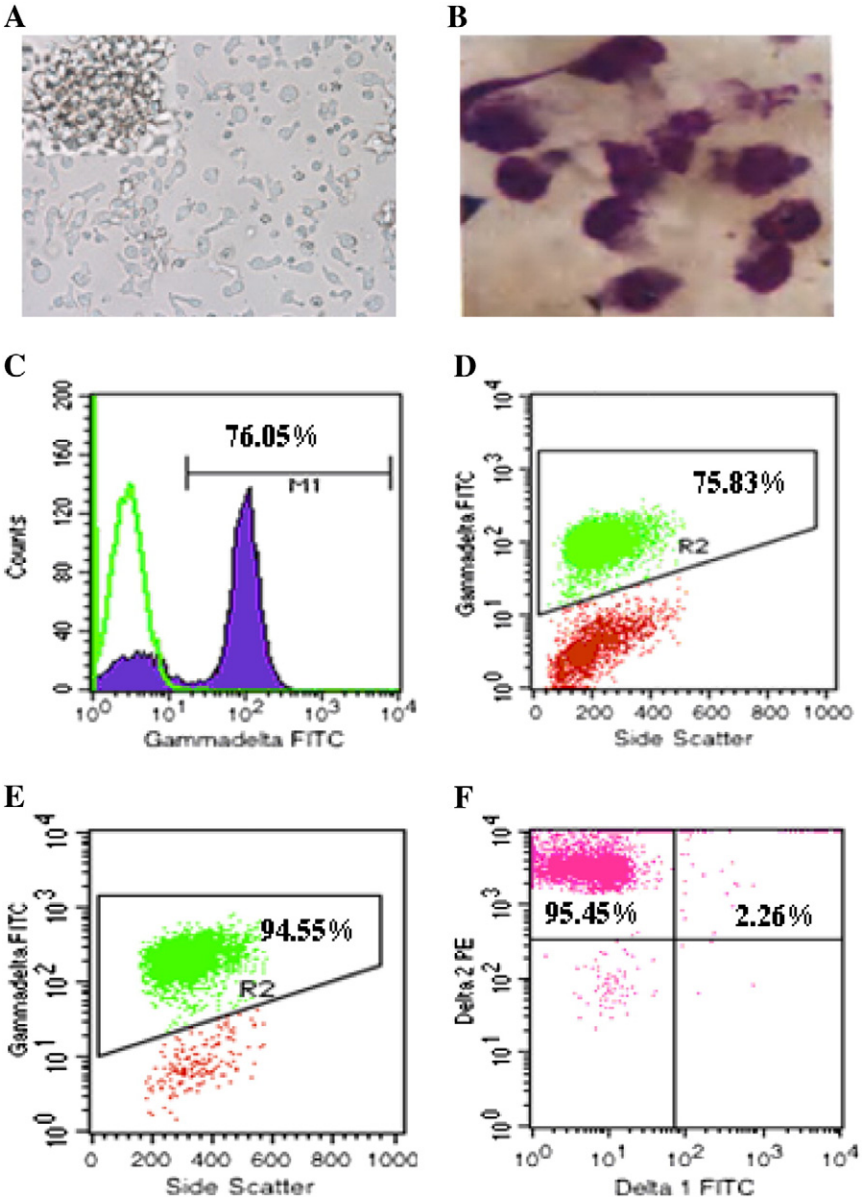

Fig. 1. Expansion of peripheral blood γδ T cells with IL-2 plus IPP. A: Cellular morphous of γδ T cells under an inverted microscope (400×). B: Cellular morphous of γδ T cells with Wright–Giemsa staining under oil immersion lens (1000×). C: Representative histogram plots of γδ T cell percentage after culture for 8 days. The open histogram is the isotype control. The filled histogram is the specific staining. D: Representative dot plot of γδ T cell percentage after culture for 8 days. E: Representative dot plot of γδ T cell percentage after magnetic cell sorting. F: Percentage of δ1 and δ2 cells in γδ T cells cultured with IPP plus IL-2 for 8 days.

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