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Potent antitumor activities of recombinant human PDCD5 protein in combination with chemotherapy drugs in K562 cells

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ABSTRACT

Conventional chemotherapy is still frequently used. Programmed cell death 5 (PDCD5) enhances apoptosis of various tumor cells triggered by certain stimuli and is lowly expressed in leukemic cells from chronic myelogenous leukemia patients. Here, we describe for the first time that recombinant human PDCD5 protein (rhPDCD5) in combination with chemotherapy drugs has potent antitumor effects on chronic myelogenous leukemia K562 cells *in vitro* and *in vivo*. The antitumor efficacy of rhPDCD5 protein with chemotherapy drugs, idarubicin (IDR) or cytarabine (Ara-C), was examined in K562 cells *in vitro* and K562 xenograft tumor models *in vivo*. rhPDCD5 protein markedly increased the apoptosis rates and decreased the colony-forming capability of K562 cells after the combined treatment with IDR or Ara-C. rhPDCD5 protein by intraperitoneal administration dramatically improved the antitumor effects of IDR treatment in the K562 xenograft model. The tumor sizes and cell proliferation were significantly decreased; and TUNEL positive cells were significantly increased in the combined group with rhPDCD5 protein and IDR treatment compared with single IDR treatment groups. rhPDCD5 protein, in combination with IDR, has potent antitumor effects on chronic myelogenous leukemia K562 cells and may be a novel and promising agent for the treatment of chronic myelogenous leukemia.

1. Introduction

Chronic myelogenous leukemia (CML) is a myeloproliferative disorder characterized by increased proliferation of myeloid cells at all stages of maturation due to uncontrolled growth and resistance to apoptosis. Leukemic cells from more than 95% of CML patients have the Philadelphia (Ph) chromosome, which expresses a BCR-ABL fusion protein. The BCR-ABL protein has potent tyrosine kinase activity, which results in an increase in cellular proliferation and a decrease in apoptosis and thus leads to a leukemic phenotype [1,2].

Imatinib mesylate can target BCR-ABL and/or its downstream effector molecules. Currently, this medication is the first-line therapy for CML patients [3], but numerous clinical trials have shown that the effects of imatinib on patients with accelerated phase or blast phase (advanced phase) CML are limited, and many patients experience relapse [4,5]. Conventional chemotherapy is still frequently used in CML patients, especially in those with relapsed or blastic phase CML, or as an induction regimen before hematopoietic stem cell transplantation. However, drug toxicity and drug resistance are the primary causes of chemotherapy failure in patients with CML.

Programmed cell death 5 (*PDCD5*), formerly designated as *TF-1 cell apoptosis-related gene-19* (*TFAR19*), is upregulated in TF-1 cells that are undergoing apoptosis [6]. The overexpression of PDCD5 facilitates apoptosis triggered by growth factor withdrawal or serum withdrawal, and enhances paraptotic cell death induced by TAJ/TROY, a novel member of the tumor necrosis factor receptor family [6,7]. Furthermore, PDCD5 is expressed at low levels in multiple solid tumors and in acute and chronic myeloid leukemia [8–14]. The administration of the anti-PDCD5 antibody or short interfering RNA against PDCD5 can suppress its apoptotic effects in HeLa cells [15,16]. These observations strongly suggest that a reduction in PDCD5 expression may play an important role in the pathogenesis of leukemia and other cancers.

Recombinant human PDCD5 protein (rhPDCD5) employs clathrin-independent endocytosis, which involves lipid rafts as well as

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cell surface heparan sulfate proteoglycans, in order to enter the cells and promotes apoptotic activity [17]. However, no reports have been published concerning the roles of the rhPDCD5 protein in promoting chemotherapeutic-induced cytotoxicity and apoptosis of tumors *in vivo*.

In our study, we evaluated the effect of rhPDCD5 protein in enhancing its chemosensitivity to myelogenous leukemia K562 cells in which apoptosis has been triggered by the chemotherapy drugs idarubicin (IDR) or cytarabine (Ara-C). Most importantly, we investigated the antitumor effect of intraperitoneal (i.p.) administration of rhPDCD5 protein combined with IDR in the K562 human CML xenograft model. Our results indicated that rhPDCD5 protein can markedly sensitize K562 cells to IDR and Ara-C. The *in vivo* antitumor effect of rhPDCD5 protein and IDR combined treatment is significant.

2. Material and methods

2.1. Drugs

Imatinib mesylate was from Novartis Pharmaceuticals, Switzerland. IDR was from Sigma, USA. Ara-C was from Pharmacia Italia SpA. Cyclophosphamide was from Jiangsu Hengrui Medicine Co. Ltd. The rhPDCD5 protein was supplied by Beijing Biosea Biotechnology Co (the endotoxin activity of the rhPDCD5 protein was <10 EU mg^{-1} as measured by the limulus amebocyte lysate assay, and the purity of the rhPDCD5 protein was >95%).

2.2. Cell lines and culture conditions

Human chronic myelogenous leukemia K562 cells were maintained in RPMI 1640 (Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum (Biochrom). Cells were cultured at 37 °C in a 5% CO_2 incubator.

2.3. Clonogenic assay

The clonogenic capability of K562 cells in soft agar media was investigated. Briefly, a 2.5×10^3 single-cell suspension of K562 cells was resuspended in 1 ml growth media with an equal volume of saline (buffer control), rhPDCD5, chemotherapy drugs or a combination of rhPDCD5 and chemotherapy drugs, containing 0.5% low melting temperature agarose (Promega, Madison, WI, USA). The samples were plated on 24-well plates, and the plates were incubated for 14–15 days until colonies formed. A colony with more than 30 cells was counted as one positive colony.

2.4. Flow cytometric analysis for apoptosis

K562 cells were plated in 24-well culture dishes to a density of 2×10^5 cells/ml and treated with the chemotherapy drugs IDR or Ara-C and rhPDCD5 for 24 h. Fluorescein isothiocyanate (FITC)-conjugated Annexin V (AV) (0.5 μg/ml final concentration) was added to the suspended cells according to the manufacturer's instructions (Biosea, China). After incubation for 20 min at room temperature, 400 μl of binding buffer was added again, and samples were immediately analyzed on a FACS Calibur flow cytometer (Becton Dickinson Biosciences) with a 488-nm argon ion laser. Cell Quest Software (Becton Dickinson) was used for data acquisition and analysis.

2.5. Animals

BALB/c nu/nu female mice (4–6 weeks of age; Experimental Animal Center, Peking University Health Science Center, Beijing, China) were used in this study. Mice were pretreated by i.p. injections of cyclophosphamide once daily at a dose of 100 mg/kg for two consecutive days. Two days after the second cyclophosphamide injection, 1.5×10^7 K562 cells in a total volume of 50 μl were injected subcutaneously into the single flanks of the mice. All of the experimental procedures, care and handling of animals were performed with the approval of the Institutional Authority for Laboratory Animal Care of Peking University.

2.6. Antitumor activity of rhPDCD5 protein in vivo

Mice bearing K562 cell xenografts were divided randomly into nine groups ($n = 6$ in each group) including i.p. treated with (a) an equal volume of saline (buffer control); (b) rhPDCD5 (10 mg/kg); (c) rhPDCD5 (20 mg/kg); (d) rhPDCD5 (40 mg/kg); (e) IDR (1.25 mg/kg,); (f) IDR (1.25 mg/kg) plus rhPDCD5 (10 mg/kg); (g) IDR (1.25 mg/kg) plus rhPDCD5 (20 mg/kg); (h) IDR (1.25 mg/kg) plus rhPDCD5 (40 mg/kg) and (i) orally treated with imatinib (60 mg/kg). The i.p. treatments began on day 14, when the average tumor volume was 140 mm^3, and were given every other day, for a total of five treatments. Imatinib was given orally, at a dosage of 60 mg/kg, by gavage every day, for a total of 10 days.

In another *in vivo* experiment, treatment began on day 12, when the average tumor volume was 57.7 mm^3. Mice bearing K562 cell xenografts were divided randomly into four groups ($n = 4$ in each group) including i.p. treated with (a) an equal volume of saline (buffer control); (b) IDR (3.5 mg/kg); (c) IDR (3.5 mg/kg) plus rhPDCD5 (20 mg/kg) and (d) IDR (3.5 mg/kg) plus rhPDCD5 (40 mg/kg). Treatments were given every other day, for a total of five treatments.

K562 xenografted tumor volume was calculated using the following formula: $\pi ls^2/6$, where l = long diameter and s = short diameter. Tumor Inhibition rate of tumor growth was calculated as (1 − average tumor weight of treated group/average tumor weight of saline control group) × 100%.

2.7. Immunohistochemical analysis

K562 tumor xenograft samples were fixed in 10% neutrally buffered formalin for 12–24 h and then embedded in paraffin. Tumor xenograft slides were cut, deparaffinized in xylene and rehydrated in descending grades of ethanol. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in water. The slides were stained with an IgG1 rabbit polyclonal antibody to Ki-67 (Dako Corporation). The Ki-67 antibody was used at an optimal dilution of 1:150. Immunostaining was performed using a variation of the avidin-biotin-peroxidase method. Five randomly selected fields were analyzed in the tumors of each specimen. The percentage of positively stained cells over the total number of nucleated cells in a 400× field (average of five 400× fields) was recorded as the Ki-67 labeling index.

2.8. Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay

Tumor xenograft sections were stained with the In Situ Cell Death Detection Kit, alkaline phosphatase and BCIP/NBT (5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium) according to instructions provided by the manufacturer (Roche). The apoptosis rates were calculated as the mean percentage of TUNEL positive cells over the total number of nucleated cells using a light microscope at 400× magnification. Five different fields per slide were examined.

2.9. Statistical analysis

The statistical analysis was performed using SPSS software version 13.0 (Chicago, IL). Differences between groups were evaluated