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Abnormal expression of the programmed cell death 5 gene in acute and chronic myeloid leukemia

Ruan Guo-Rui [a], Qin Ya-Zhen [a], Chen Shan-Shan [a,*], Li Jin-Lan [a], Ma Xi [b], Chang Yan [a], Wang Ya-Zhe [a], Fu Jia-Yu [a], Liu Yan-Rong [a,*]

[a] *Institute of Hematology and People's Hospital, Peking University, 11 Xi-zhi-men South Street, Beijing 100044, China*
[b] *Department of Immunology, School of Basic Medicine, Peking University, Peking University Center for Human Disease Genomics, Beijing 100083, China*

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Abstract

To clarify whether expression of the programmed cell death 5 (PDCD5) gene in leukemic cells is abnormal, real-time quantitative reverse transcription polymerase chain reaction (RQ-RT-PCR) was used to examine its expression in marrow cells from leukemia patients. We found lower PDCD5 in both AML and CML marrow cells than in normal donor marrow cells. A negative correlation was found between relative levels of PDCD5 and BCR/ABL expression in all CML patients and in CML patients in the advanced phase. Treatment with the ABL tyrosine kinase inhibitor Imatinib mesylate increased PDCD5 expression in K562 and MEG-01 cells. These findings suggest that abnormal expression of PDCD5 in leukemia may be involved in the pathomechanism of AML and CML.

Keywords: Acute myeloid leukemia (AML); Chronic myeloid leukemia (CML); Programmed cell death 5 (PDCD5); Real-time quantitative reverse transcription polymerase chain reaction (RQ-RT-PCR); Apoptosis; Imatinib mesylate

1. Introduction

The development of a malignant cell clone will disrupt the balance between cell proliferation and programmed cell death (e.g. apoptosis). Many types of antitumor therapy, including radiotherapy, exert their effects by activating or inducing apoptosis. It is therefore important to study the alteration of apoptosis in leukemia, which may contribute to the accumulation and drug resistance of the malignant cell clone [1,2].

The programmed cell death 5 (PDCD5) gene, previously named TF-1 apoptosis-related gene 19 (TFAR19), is a novel apoptosis-related gene cloned in 1999 from TF-1 human leukemic cell line undergoing apoptosis (accession number AF014955 in GenBank) [3]. The human PDCD5 gene encodes a protein that shares significant homology with the corresponding proteins of species ranging from yeast to mice. The level of PDCD5 protein expressed in cells undergoing apoptosis is significantly increased compared with control cells. The protein then translocates rapidly from the cytoplasm to the nucleus. The appearance of PDCD5 in the nuclei of apoptotic cells precedes the externalization of phosphatidylserine and fragmentation of chromosomal DNA. The nuclear translocation of PDCD5 is a universal early event of the apoptotic process, and may be a novel early marker for apoptosis [4].

We previously showed that the expression levels of the PDCD5 gene in nucleated marrow cells measured by flow cytometry were lower in cells taken from untreated chronic myeloid leukemia (CML) patients' bone marrow versus normal donors' bone marrow. This suggests that the abnormal PDCD5 expression may play an important role in CML pathogenesis [5]. In the present study, by means of real-time quantitative reverse transcription polymerase chain reaction

* Corresponding author. Tel.: +86 10 68792787; fax: +86 10 68344179/86 10 68792787.
E-mail addresses: chenss@public.bta.net.cn, celiar@public3.bta.net.cn (S.-S. Chen).

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(RQ-RT-PCR) we revealed that the PDCD5 gene expression level was markedly lower in AML marrow cells than in control cells from healthy donors. The relationship between the levels of PDCD5 gene expression and BCR/ABL gene expression was also examined.

2. Materials and methods

2.1. Patients and controls

Bone marrow samples from 146 leukemia patients were tested for PDCD5 expression (81 untreated AML with mean bone marrow blasts = 62.4% and a mean age of 35 years; 36 untreated CML in chronic phase (CML-CP) with mean bone marrow blasts = 9.3% and a mean age of 42 years; and 29 CML in accelerated phase/blastic phase (CML-AP/BP) with mean bone marrow blasts = 31.7% and mean age of 38 years). All leukemia cases were diagnosed according to morphology, immunology and cytogenetics (MIC) classification. Twenty-five normal bone marrow samples were obtained from healthy donors with informed consent. Heparinized bone marrow mononuclear cells (MNC) were harvested by Ficoll solution (Shanghai Huangjing Biotechnology Co., China), the pellet was washed twice with phosphate-buffered saline (PBS) to remove platelets.

2.2. Cell lines

MEG-01 cells (derived from CML, blastic crisis), were kindly provided by Cell Bank, Shanghai Institute of Cell Biology, Chinese Academy of Sciences. Kasumi cells derived from *t*(8;21) AML-M2, were kindly provided by Dr. K. Kita (Mie University, Mie, Japan).

The other cell lines were maintained in our laboratory. All the cell lines were cultured in RPMI 1640 medium containing 10% fetal bovine serum (GIBCO, U.S.). In addition, TF-1 cells were complemented with 10 ng/ml granulocyte macrophage-colony-stimulating factor (GM-CSF). All cells were harvested in the exponential growth phase.

2.3. RNA isolation and preparation of cDNA

Total cellular RNA was extracted using the TRIzol® reagent (Invitrogen, U.S.) as described by the manufacturer. Total RNA was incubated at 65 °C for 5 min and then placed on ice. The cDNA synthesis reaction was performed with 4 μg of RNA in a volume of 20 μl containing 0.25 μg/μl of random primers (Promega, U.S.), 1 mM of each dNTP (Pharmecia, U.S.), 10 U/μl of reverse trancriptase (Promega, U.S.), 1 U/μl of ribonuclease inhibitor (Sino-American Biotechnology Co., China), and 5× first-standard buffer 4 μl (final dilution, 1×; Promega, U.S.). After 60 min of cDNA synthesis at 37 °C, the reactions were terminated by incubation at 95 °C for 5 min and then stored at −20 °C.

2.4. Measurement of relative PDCD5 expression levels by RQ-PCR

Quantification was performed on the ABI 7000 Sequence Detection System (Applied Biosystems, U.S.A.) using TaqMan technology. PCR parameters were: 2 min, 50 °C; 10 min, 95 °C followed by 50 cycles with 15 s, 95 °C and 1 min, 60 °C. RQ-PCR reactions were performed in a total volume of 25 μl including 1 × TaqMan Universal PCR Master Mix (Applied Biosystems, containing dNTPs with dUTP, $MgCl_2$, AmpliTaq Gold® DNA Polymerase, ampErase® UNG, Passive Referene), 1 μl of cDNA sample, 400 nM of each primer and 200 nM probe. Using this system, we generated an amplification plot based on the fluorescent reporter signal (Rn). The threshold cycle number (Ct) was defined as the number of cycles producing a signal at least 10 times stronger than the standard deviation of the baseline fluorescent signal (fluorescent signal of the first 3–15 PCR cycles). For quantification of the PDCD5 transcripts, we prepared standard dilutions of the cDNAof K562 cells by 5 serial 10-fold dilutions with DEPC-treated distilled water. We produced the cDNA standard in bulk, divided it into many aliquots, and stored them at −70 °C. For each assay, we used an aliquot of the frozen standard cDNA after thawing it. A standard curve was constructed from the results of amplification of serial dilutions of standard cDNA. Each point of the standard curve was measured in duplicate. The expression levels in patient samples and in leukemic cell lines were each determined by reference to the corresponding expression level on the standard curve. The expression level of PDCD5 was normalized relative to the expression level of the ABL gene or the beta-2-microglobulin (β_2-MG) gene and was expressed as a relative value when the expression level of PDCD5 in K562 cells was defined as 1.0.

2.5. RQ-PCR for major bcr-abl was performed in CML patients with the same protocol

The following primer and probe sequences for PDCD5, BCR-ABL and ABL were designed using Primer-Express software (PE Applied Biosystems). PDCD5 forward primer: 5′-GTG ATG CGG CCC AAC AG-3′; PDCD5 reverse primer: 5′-ATC CAG AAC TTG GGC TAA GAT ACT G-3′; PDCD5 probe: 5′-FAM-TCT CAT TTC TGC TTC CCT GTG CTT TGC T-TAMARA-3′; BCR-ABL forward primer: 5′-CCG CTG ACC ATC AAT AAG GAA-3′; BCR-ABL reverse primer: 5′-CTC AGA CCC TGA GGC TCA AAG T-3′; BCR-ABL probe: 5′-FAM-AGC CCT TCA GCG GCC AGT AGC ATC T-TAMARA-3′; ABL forward primer for Ia: 5′-TCC TCG TCC TCC AGC TGT TAT C-3′, for Ib: 5′-TTA TCA AAG GAG CAG GGA AGA AG-3′. ABL reverse primer and probe is the same as that of BCR-ABL. The primer and probe sequences for β_2-MG are as follows [6]: forward primer: 5′-GAG TAT GCC TGC CGT GTG-3′; β_2-MG reverse primer: 5′-AAT CCA AAT GCG GCA TCT-3′; β_2-MG probe: 5′-FAM-CCTCCATGATGCTGCTTACATGTCTC-TAMARA-3′. The product size was 74 bp for PDCD5, 146 bp