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The expression of iASPP in acute leukemias

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Abstract

Inhibitory member of the ASPP family (iASPP) is an evolutionarily conserved inhibitor of p53, and its expression is upregulated in human breast carcinomas expressing wild-type p53. To examine the role of iASPP in acute leukemia (AL), we analyzed iASPP mRNA expression in AL by semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR). PCR products were confirmed by restriction endonuclease *Bst*X I digestion and sequencing analysis. The results showed that median levels of iASPP gene expression in cells of AL were significantly higher than those in cells from normal donors and AL patients in complete remission (CR) ($P = 0.019$, 0.021, respectively). There was no significant difference between acute lymphocytic leukemia (ALL) cells and acute myeloid leukemia (AML) cells ($P = 0.593$). The expression level of iASPP gene and its overexpression in M3 and M4EO were significantly lower than in other subtypes of AML. However, iASPP gene expression in AL cells was not associated with gender, age, initial white blood cell count or p53 type, but was associated with CD34 expression. The results of the present study suggest that iASPP gene overexpression may play an important role in the leukemogenesis and/or disease progression of AL.

Keywords: Acute leukemia; CD34; iASPP

1. Introduction

p53 is considered the "guardian of the genome", and plays a critical role in regulation of cell proliferation mainly through induction of growth arrest or apoptosis. The loss of p53 protein is a key event in the pathogenesis of a wide variety of human malignancies. For example, in more than half of all human cancers, p53 was either lost or mutated [1]. However, the frequency of p53 mutations in leukemia is only 10% [2]. Although wild-type p53 is intact in many tumors, the regulation of p53 pathway is abnormal. The activation of p53 relies on multiple auto-regulatory loops. Among these loops, Mdm2, as transcriptional target of p53, is of particular importance. Mdm2 is able to bind p53, inhibits its activities and promotes p53 degradation through the ubiquitin proteasome system [3]. When over-expressed, Mdm2 eliminates p53 tumor suppressor function. Although Mdm2 gene is amplified in over a third of sarcomas [4], aberrations are rare in leukemias and lymphomas [5].

iASPP was identified previously as a p65 relA binding protein (RAI) [6]. Further study demonstrated that iASPP oncoprotein is a key p53 inhibitor, which is evolutionarily conserved from worm to human. iASPP is predominantly expressed as a nuclear protein and is capable of binding to p53. Moreover, overexpression of iASPP greatly enhanced the transforming activities of Ras together with E7 or E1A. In addition, iASPP expression was upregulated in human breast carcinomas expressing wild-type p53 [7].

Abbreviations: AL, acute leukemia; ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; BM, bone marrow; CR, complete remission; EB, ethidium bromide; HAL, hybrid acute leukemia; iASPP, inhibitory member of the ASPP family; MNCs, mononuclear cells; PB, peripheral blood; RAI, relA binding protein; RT-PCR, reverse transcriptase polymerase chain reaction

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To date, iASPP expression in primary acute leukemia (AL) has not been demonstrated. Therefore, we evaluated iASPP gene mRNA expression in AL by using semi-quantitative RT-PCR. The results showed that the median level of iASPP gene expression in cells of AL is significantly higher than that in cells from normal donors or AL patients in CR, which suggests that iASPP gene overexpression may play an important role in the leukemogenesis and/or disease progression of AL.

2. Patients and methods

2.1. Patients

About 65 patients with de novo AL and eleven with AL in CR from our hospital were studied after receiving informed consent. The median age of patients was 23.0, and other characteristics including gender, age, FAB subtypes are summarized in Table 1. The diagnosis and classification of the leukemia were based on morphological, immunophenotyping, cytochemical, and genetic criteria of the French–American–British system [8,9]. The sample was defined as CD34 positive when greater than 20% blasts were positively stained [10]. CR was defined as the presence of <5% blasts in a normocellular bone marrow (BM) with $>1 \times 10^9$/L neutrophils and $>100 \times 10^9$/L platelets in the peripheral blood (PB) and disappearance of all signs attributable to leukemia [11]. BM cells from 18 healthy donors were used as controls for iASPP expression.

Table 1
Clinical characteristic associated with iASPP gene expression in AL

Parameter	*n*	iASPP/β-actin (*M*)	Overexpression (*O*)	Statistical significance
Status				
De novo	65	0.440	16	
CR	18	0.110		0.021 (*M*)
Healthy	11	0.110		0.019 (*M*)
Gender				
Male	36	0.585	12	0.060 (*M*)
Female	29	0.177	4	0.069 (*O*)
Age				
≤14	19	0.440	5	0.515 (*M*)
>14	46	0.355	11	0.838 (*O*)
Leukemia type				
AML	41	0.470	12	0.593 (*M*)
ALL	22	0.335	3	0.272 (*O*)
AHL	2	0.810	1	
CD34				
Positive	17	0.730	6	0.045 (*M*)
Negative	27	0.380	7	0.507 (*O*)
Initial WBC				
$\geq 50.0 \times 10^9$/L	21	0.420	4	0.559 (*M*)
$<50.0 \times 10^9$/L	44	0.455	12	0.472 (*O*)
FAB subtype				
M2	11	0.750	3	
M3	10	0.055	1	
M4(M4EO)	8 (3)	0.305 (0.130)	2(0)	
M5	11	0.440	5	
M6	1	1.340	1	

2.2. Cell lines

Kasumi-1 cell line is human AML cell line with t(8;21) [12], which was used as positive control for iASPP gene expression in this study.

2.3. mRNA preparation

BM was collected on heparin and the mononuclear cells (MNCs) were immediately obtained using Ficoll density centrifugation and washed twice with phosphate-buffered saline. Total RNA was extracted from 5×10^6 MNCs according to Trizol Reagent protocols (GIBCO). The quality of RNA was detected on a 1% agarose gel stained by ethidium bromide (EB).

2.4. Semi-quantitative analyses of RT-PCR products

The cDNA was synthesized from 2 μg of total RNA by extension with oligo (dT) primer in 20 μl reaction mixture containing 200 U of reverse transcriptase (GIBCO). The RT-PCR amplification was performed using 2 μl cDNA. A pair of specific primers (sence: 5′ -CGTGGATTTCCTCATCACCG-3′, antisense: 5′-TCCTTTGA GGCTTCGCCCTG-3′) were designed to amplify a 452 bp fragment of iASPP. The primers were positioned in different exons of iASPP to avoid false-positive results caused by DNA contamination of the RNA preparation. The total reaction volume was 50 μl containing 2 μl cDNA and 25 μl Premix Taq (Takara). The iASPP amplification reactions consisted of denaturation at 94 °C for 45 s, annealing at 55 °C for 45 s, and extension at 72 °C for 45 s. In order to identify linear range of the amplification, reactions were performed for 25, 28, 31, 34, 37, 40 cycles, respectively. 1:3 serial dilutions of the cDNA mixture from a leukemia cell line Kasumi-1 were used to examine semi-quantitative RT-PCR sensitivity at 32 cycles. As an internal control, 271 bp fragment of β-actin was also amplified using its specific primers, which was performed with 28 cycles of 30 s at 94 °C, 30 s at 58 °C, and 30 s at 72 °C. An amount of 8 μl PCR product was subjected to electrophoresis in a 1% agarose gel containing EB, and then photographed under ultraviolet light. Relative expression of iASPP gene (*R*) was calculated following the formula: *R* = densitometrical units of iASPP/densitometrical units of β-actin.

2.5. Identification of iASPP PCR product

Amplified product of iASPP was digested with *Bst*X I, then electrophoresed in 3% agarose gels and visualized under ultraviolet light with EB staining. PCR products from the Kasumi-1 cell line were sequenced directly by ABI PRISM 377–96.