The leukemogenic role of (iASPP) in acute leukemia

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Submitted: 10 April 2007
Accepted: 5 May 2007

Arch Med Sci 2007; 3, 2: 102-107
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Abstract

Introduction: The ASPP family (**a**poptosis – **s**timulating **p**roteins of **p**53) comprises three proteins, ASPP1, ASPP2 and iASPP, that interact with and modulate the behavior of p53. ASPP1 and ASPP2 enhance the ability of p53 to induce apoptosis by causing p53 to up regulate the expression of proapoptotic genes specifically rather than genes involved in cell cycle arrest. Inhibitory member of the ASPP family (iASPP) acts as inhibitor for the p53, it was originally identified as a nuclear protein that interacts with and inhibits NFκβ p56 RelA and inhibits p53-mediated cell death as well.
Material and methods: To examine the role of iASPP in acute leukemic patients, we analyzed iASPP mRNA expression in acute leukemia by semi quantitative reverse transcriptase polymerase chain reaction (RT-PCR).
Results: The results showed that the median level of iASPP in acute lymphoblastic leukemia and acute myeloid leukemia patients was significantly higher than those in cells from normal donors (p=0.04). The expression of iASPP in ALL and AML patients was not associated with age, gender, hemoglobin, platelets; blasts count in bone marrow, treatment outcome but was associated with blasts count in peripheral blood and total leucocytic count in AL patients.
Conclusions: The results of the present study suggest that iASPP may play a role in leukemogenesis and/or disease progression of acute leukemia.

Key words: acute leukemia, ALL, AML, iASPP, p53, apoptosis.

Introduction

Regulation of genomic stability and protection from malignant transformation are complex processes involving a variety of different mechanisms, including permanent cell cycle arrest after expression of an activated oncogene [1].

The activation of p53 is a guardian mechanism to protect primary cells from malignant transformation; however, the details of the activation of p53 by oncogenic stress are still incomplete [2].

p53 acts to prevent potentially dangerous mutations being passed on during cell division either by halting the cell cycle until the damage has been repaired, or by inducing apoptosis in order to eliminate the damaged cell [3].

The ASPP family (**a**poptosis – **s**timulating **p**roteins of **p**53) is comprised of three proteins, ASPP1, ASPP2 and iASPP. These interact with, and modulate the behavior of p53 [4].

ASPP1 and ASPP2 enhance the ability of p53 to induce apoptosis by causing p53 to up regulate the expression of proapoptotic genes specifically

rather than those genes involved in cell cycle arrest. ASPP1 and ASPP2 also induce apoptosis via p53 homologues p63 and p73 in a similar manner [5].

The third family member, iASPP (**i**nhibitory member ASPP family), originally identified as a nuclear protein that interacts with and inhibits NFκβ p56 RelA, was named Rel-associated inhibitor (RAI) [3]. iASPP/RAI is substantially shorter than ASPP1 and ASPP2 and inhibits p53-mediated cell death [6].

The over expression of iASPP greatly enhances the transforming activities of Ras. In addition, iASPP expression is up regulated in human breast carcinomas and in acute myeloid and lymphatic leukemia [7].

The aims of this study were to investigate iASPP expression in Egyptian cases of *de novo* acute leukemia using semi-quantitative RT-PCR and correlate the findings with the clinical and laboratory data of the patients.

Material and methods

Forty patients with *de novo* acute leukemia were selected from the Pediatric and Medical Oncology Department of the National Cancer Institute of Cairo University. The study also included a control group with twenty age and sex matched subjects. Samples were collected from December 2005 to February 2006. The study was approved by the Ethics Committee of the National Research Center, and all participants gave their written informed consent.

All patients had the following carried out:

History taking and a physical examination with careful notation and assessment of clinical signs relevant to leukemia such as hepatomegaly, splenomegaly, lymphadenopathy, gums or skin infiltration.

Laboratory investigations including complete blood count, routine biochemical profile, bone marrow (BM) examination for: FAB classification, cytochemistry and immunophenotyping study by flowcytometry to determine the ALL and AML subtypes, lumbar puncture for CSF examination and radiological examination.

Detection of the expression of iASPP by RT-PCR was carried out through two steps:

1. mRNA preparation

Total RNA was extracted from whole blood using QIA AMP RNA kit (QIAGEN) in which erythrocytes were selectively lysed and leucocytes were recovered by centrifugation. The leucocytes were then lysed using highly denaturizing conditions allowing the isolation of intact RNA. After homogenization of the lysate by a brief centrifugation through a KIA shredder spin column, ethanol was added to adjust binding conditions and the sample was applied to the QIA amp spin column.

RNA was bound to the silica gel membrane during a brief centrifugation step. Contaminants were washed away and total RNA was eluted in 30 µl or more of RNase free water for direct use in any downstream application.

2. Semi-quantitative analysis of RT-PCR products

Amplification was carried out in Pekrin Elmer Thermocycler 9600 with a final volume of 50 µl (Applied Biosystems). Each PCR sample contained 8 µl of template RNA in: 10 µl of 5x Qiagen one step RT-PCR buffer (Tris-CL, KCL, (NH4)2SO4, 12.5mM MgCL2, DTT; Ph 8.7), 2 µl of Qiagen one step RT-PCR enzyme mix, 2 µl of dNTP, 26 µl of RNase free water and a pair of 1 µl of specific primers (sense: 5'CGTGGATTTCCTCATCACCG 3', anti-sense: 5' TCCTTTGAGGCTTCACCCTG 3'). These were designed to amplify a 452 bp fragment of iASPP. All reaction tubes were transferred to a thermal cycler and incubated at 50°C for 30 min for cDNA synthesis then, incubated at 95°C for 15 min to inactivate the reverse transcriptase and to completely denaturize the template. The iASPP amplification reactions consisted of 32 cycles of denaturation at 94°C for 45 sec, annealing at 55°C for 45 sec, and extension at 72°C for 60 sec. As an internal control, a 271 bp fragment of β-actin was also amplified using its specific primers (sense: 5' TCATGTTTGAGACCTTCA 3', anti-sense: 5' GTCTTTGCATGTCCACG 3') which was performed with 28 cycles of 60 sec at 94şC for denaturation, 60 sec at 53şC for annealing and 60 sec at 72şC for extension. An amount of 15 µl PCR product with a DNA marker (OX174/Hae III promega) were subjected to electrophoresis in a 1% agarose gel containing ethidium bromide, and then photographed by ultraviolet light (Figure 1). Relative expression of iASPP was calculated according to the following formula: R=Densiometric units of iASPP/Densiometric units of β-actin. Considering that the 95% confidence interval of iASPP expression in normal specimens ranged between 0-0.53 (Figure 1B), we defined patients over 0.53 as over expressed patients (Figure 1A).

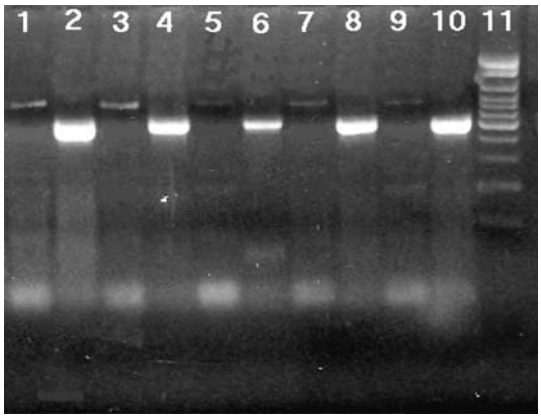
Statistical analysis

Statistical analysis of results was performed using SPSS 7.5 for Windows. Non parametric data were described in the form of median and range, (SD) while qualitative data was presented in the form of number and percentage. Chi-square test was used to test association between two qualitative variables. Quantitative data compared using two sided Student t-test, where a p-value of ≤0.05 was considered significant.

Results

The main presenting signs of leukaemia were easily fatigability and pallor in all patients in the ALL and AML groups. Splenomegaly and hepatome-

galy were found in 35% of AML patients and about 50-55% in ALL groups (Table I).



Lane 1: iASPP in ALL patients shows iASPP over expression
Lane 2: β-actin expression in ALL patients with an iASPP over expression
Lane 3: iASPP in AML patients shows iASPP over expression
Lane 4: β-actin expression in AML patients with an iASPP over expression
Lane 5: iASPP in ALL patients showing iASPP normal expression
Lane 6: β-actin of ALL patients having iASPP normal expression
Lane 7: iASPP in AML patient showing iASPP normal expression
Lane 8: β-actin of AML patient having iASPP normal expression
Lane 9: iASPP expression of normal control
Lane10: β-actin expression of normal control
Lane 11: Marker ladder (1353, 1078, 872, 603, 410, 281, 271, 234, 194, 118, 72)

Figure 1. PCR product of iASPP (452 bp) and β-actin (271bp)

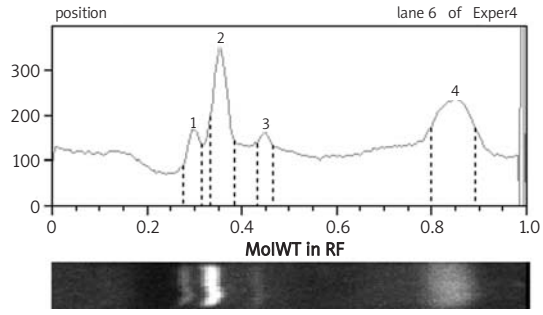


Figure 1A. Densiometric analysis of iASPP over expressed patient

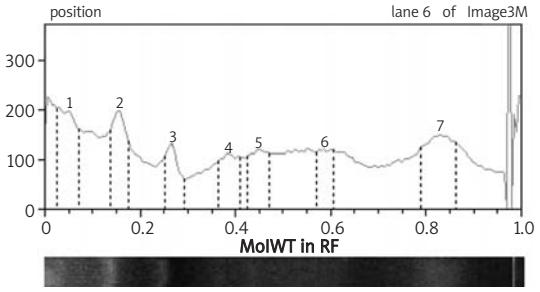


Figure 1B. Densiometric analysis of iASPP expression in control

There were no significant differences between the ALL and AML groups, but a significant difference between AL groups and the controls with respect to Hb, platelets and TLC (Table IIA).

Among the ALL group, 6 cases (30%) were pre-B ALL (μ chain), 8 cases (40%) were common ALL (CD 10), 4 cases (20%) were mature B ALL (surface Ig), 2 cases (10%) were T-ALL (CD 3,7) (Table IIB). In the AML group, 4 cases (20%) were M_1 (CD 13,33, HLA-DR+), 7 cases (35%) were M_2 (CD 13,33, HLA-DR+), 4 cases (20%) were M_3 (CD 13,33, HLA-DR–), 3 cases (15%) were M_4 (CD 13,33,14,15), 1 case (5%) was M5 (CD 14), 1 case (5%) was M6 (Glycophorin A) (Table IIB).

iASPP expression in ALL and AML patients was significantly greater than in the control group (p=0.04), while no difference was present between AML and ALL groups (p=0.6). The frequency of iASPP over expression was 32.5% (35% in ALL and 30% in AML patients) (Table IIB).

There was a significant correlation between iASPP expression and peripheral blood blast cells in both ALL and AML patients (p=0.001 and 0.001 respectively).There was no significant correlation between iASPP over expression and outcome of patients (p>0.05).

Discussion

The balance between cell division and cell death is vital for the maintenance of homoeostasis in metazoan organisms, and the disruption of this balance contributes to many pathological conditions [3].

The p53 tumor suppressor exerts anti-proliferative effects, including growth arrest, apoptosis and cell senescence, in response to various types of stress. Tight regulation of p53 activation is imperative for preventing tumor genesis and maintaining normal cell growth.

iASPP has an oncogenic role through its inhibition of p53 and hence could play role in tumor development [10]. Also over expression of iASPP may play an important role in leukemogenesis and may affect the progression of the disease [11, 12].

Both retrospective and prospective reports have studied iASPP as an inhibitor of p53 protein [3, 5, 6, 8, 13-15].

In the current study, iASPP expression was assessed compared to the internal control, β-actin, in each sample.

In our study, the iASPP expression in ALL patients showed a significant increase as compared to the control group (p=0.04) (Figure 2). ALL patients had a mean level of expression of 0.47±0.37, while the control group had a mean level of expression of 0.19±0.16. This is in accordance with Zhang et al., 2005 [11], who reported in his work that the mean level of expression in ALL patients was 0.33.

Table I. Clinical data of ALL, AML and controls. Table shows that easily fatigability and pallor are the main presenting signs (100%) of both ALL and AML groups. Splenomegaly and hepatomegaly were found in 35% of AML patients and about 50-55% ALL group

Parameter	ALL (n=20)	AML (n=20)	Controls (n=20)
Age (years)	23.24±15	31.03±14.6	20.47±13.4
Sex (M/F)	10/10	10/10	10/10
Easy fatigability	20 (100%)	20 (100%)	N/A
Lymphadenopathy	7 (35%)	6 (30%)	N/A
Splenomegaly	11 (55%)	7 (35%)	N/A
Hepatomegaly	10 (50%)	7 (35%)	N/A

N/A - non-applicable

Table IIA. Laboratory results of ALL, AML and controls

Parameter	ALL (n=20)	AML (n=20)	Controls (n=20)	p^1	p^2	p^3
Hb Mean±SD	7.9±1.8	7.74±1.1	11.52±1.4	0.738	0.000	0.000
Platelets Mean±SD	88.9±32	89.7±53	269.46±105	0.939	0.000	0.000
TLC Mean±SD	21.8±7.3	18.33±5.89	7.9±2.02	0.147	0.000	0.000
PB. Blasts Mean±SD	17.7±5.9	20.8±8.01	N/A	0.136	N/A	N/A
BM. Blasts Mean±SD	78.25±16.6	66.11±18.83	N/A	0.054	N/A	N/A

p^1 – t-test between ALL and AML, p^2 – t-test between ALL and controls, p^3 – t-test between AML and controls
p significant ≤0.05
Hb – hemoglobin, TLC – total leucocytic count, PB – peripheral blood, BM – bone marrow
N/A – non-applicable

There was also a statistically significant increase in the iASPP expression in the AML group of patients compared to the controls (p=0.04) (Figure 2). Their mean level of expression was 0.45±0.32 as compared to the control group who had a mean level of expression of 0.19±0.16. Again this corresponds well with Zhang et al., 2005 [11] who reported that the mean level of expression in AML patients was 0.43.

However there was no significant difference in iASPP expression between acute lymphoblastic leukemic cells and acute myeloid leukemic cells (p=0.6). This could be explained by the fact that the main role of iASPP is inhibiting the p53 mediated apoptosis, thus leading to down regulation of p53 in cases of acute leukemia. Several authors have documented the fact that dysregulation of p53 is found in both types of acute leukemia, whether ALL or AML [16-18].

On comparing iASPP expression in the studied AL patients and the different laboratory parameters, no association was detected with age, gender, hemoglobin level, platelet count, total leucocytic count and blasts count in BM. A significant correlation was only detected with the peripheral blood blast count (p=0.001) (Figure 3-4). However, it has been reported that iASPP gene expression in newly diagnosed ALL patients, was not related with any parameter other than CD34 expression [11].

Regarding the subtypes of each of the acute leukemia cases, no correlation was found between the different subtypes and iASPP expression due to

Table IIB. Laboratory results of ALL and AML (continue)

Parameter/ Classification	Frequency	Percent (%)
AML (FAB)		
M_1	4	20
M_2	7	35
M_3	4	20
M_4	3	15
M_5	1	5
M_6	1	5
ALL (IPT)		
c-ALL	8	40
pre-B ALL	6	30
mature B ALL	4	20
T- ALL	2	10
iASPP expression		
normal	27	67.5
over expression	13	32.5

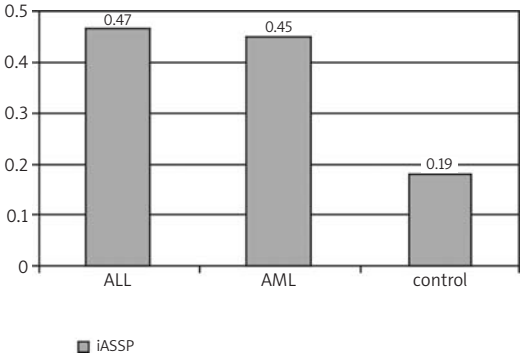


Figure 2. The expression of iASPP in ALL, AML and controls. Figure shows that iASPP expression in ALL and AML patients showed significant increase than in control group (p=0.04), while no difference was present between AML and ALL groups (p=0.6)

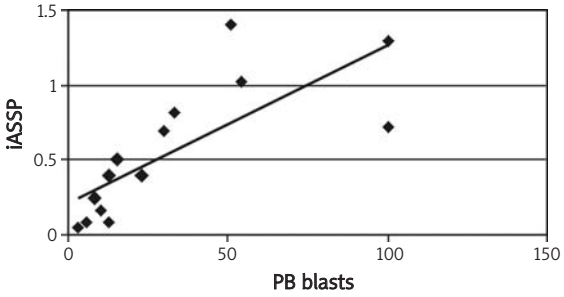


Figure 3. Correlation of iASPP expression and PB blasts in ALL patients. Figure represent the correlation of iASPP expression and peripheral blood blast cells in ALL patients where there was a strong significant correlation (p=0.001)

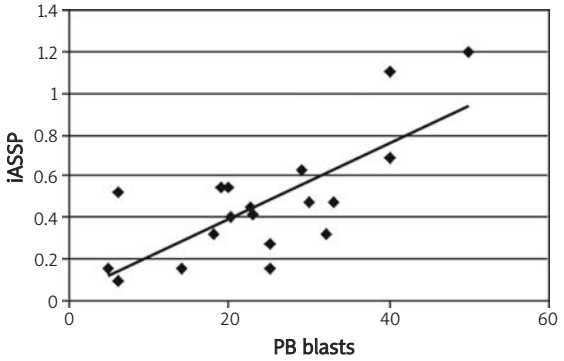


Figure 4. Correlation between iASPP expression and PB blasts in AML patients. Figure represent the correlation of iASPP expression and peripheral blood blast cells in AML patients where there was a strong significant correlation (p=0.001)

the small number of cases in each group. It was reported that iASPP gene expression in M_3 and M_4 were significantly lower than in other subtypes of AML [11]. However, we found that iASPP is over expressed more frequently in M_2 cases (5/7) than in other subtypes of AML patients.

On investigating the treatment outcome and the level of iASPP expression in the different types of acute leukemia, 70% of over expressed cases showed complete remission, while 30% died during induction. In patients expressing normal levels of iASPP, 70% of them entered into complete remission after the first phase of induction. On the other hand, 30% died during the first induction. There was no correlation between iASPP over expression and outcome of the patients (p>0.05). However, others have mentioned that high levels of iASPP expression caused cells to become more resistant to the destructive effects of chemotherapeutic drugs [19]. This finding could not be confirmed in our work due to the small number of patients included in the study. More studies with larger number of patients need to be carried out to clarify the relationship between iASPP over expression and the outcome of patients.

Conclusions

Previous results have shown that iASPP contributes to leukemogenesis and enhanced cellular proliferation. We agree with others [20] that this may open the field of research for new therapeutic drugs with new targets of treatment.

Acknowledgments

This work was supported by Department of Clinical and Chemical Pathology and Department of Child Health at National Research Center (NRC), Cairo, Egypt. We also thank Pediatric and Medical Oncology Department of the National Cancer Institute of Cairo University for their cooperation with us in collecting samples.

References

1. Bringold F, Serrano M. Tumor suppressors and oncogenes in cellular senescence. Exp Gerontol 2000; 35: 317-29.
2. Bulavin DV, Kovalsky O, Hollander MC, Fornace AJ. Loss of oncogenic H-ras-induced cell cycle arrest and p38 mitogen-activated protein kinase activation by disruption of Gadd45a. Mol Cell Biol 2003; 23: 3859-71.
3. Slee EA, Gillotin S, Bergamashi D, et al. The N-terminus of a novel isoform of human iASPP is required for its cytoplasmic localization. Oncogene 2004; 23: 9007-16.
4. Slee EA, Lu X. The ASPP family: deciding between life and death after DNA damage. Toxicol Lett 2003; 139: 81-7.
5. Bergamaschi D, Samuels Y, Jin B, Duraisingham S, Crook T, Lu X. ASPP1 and ASPP2: common activators of p53 family members. Mol Cell Biol 2004; 24: 1341-50.
6. Bergamaschi D, Samuels Y, Zhong S, Lu X. Mdm2 and mdmX prevent ASPP1 and ASPP2 from stimulating p53 without targeting p53 for degradation. Oncogene 2005; 24: 3836-41.
7. Zhang JB, Sun Y, Dong J, Liu LX, Ning F. Expression of lung resistance protein and multidrug resistance-associated protein in naive childhood acute leukemia and their clinical significance. Ai Zheng 2005; 24: 1015-7.
8. Fogal V, Kartasheva NN, Trigiante G, et al. ASPP1 and ASPP2 are new transcriptional targets of E2F. Cell Death Differ 2005; 12: 369-76.

9. Sullivan A, Lu X. ASPP: a new family of oncogenes and tumor suppressor genes. Br J Cancer 2007; 96: 196-200.
10. Mihara M, Erster S, Zaika A, et al. P53 has a direct apoptogenic role at the mitochondria. Mol Cell 2003; 11: 577-90.
11. Zhang X, Wang M, Zhou C, Chen S, Wang J. The expression of iASPP in acute leukemias. Leuk Res 2005; 29: 179-83.
12. Zhang X, Diao S, Rao Q, et al. Identification of a novel isoform of iASPP and its interaction with p53. J Mol Biol 2007; 368: 1162-71.
13. Samuels-Lew Y, O'Connor DJ, Bergamaschi D, et al. ASPP proteins specifically stimulate the apoptotic function of p53. Mol Cell 2001; 8: 781-94.
14. Ryan K, Bell H, Crighton D, Prey J, Bell L, Helgason V. Tumour cell death 2004. Beatson Laboratories Scientific Report.
15. Liu ZJ, Lu X, Zhong S. ASPP-apoptotic specific regulator of p53. Biochim Biophys Acta 2005; 1756: 77-80.
16. Casas S, Ollila J, Aventín A, Vihinen M, Sierra J, Knuutila S. Changes in apoptosis-related pathways in acute myelocytic leukemia. Cancer Genet Cytogenet 2003; 146: 89-101.
17. Zhou M, Gu L, Zhu N, Woods WG, Findley HW. Transfection of a dominant-negative mutant NF-kB inhibitor (IkBm) represses p53-dependent apoptosis in acute lymphoblastic leukemia cells: interaction of IkBm and p53. Oncogene 2003; 22: 8137-44.
18. Yin B, Kogan SC, Dickins RA, Lowe SW, Largaespada DA. Trp53 loss during in vitro selection contributes to acquired Ara-C resistance in acute myeloid leukemia. Exp Hematol 2006; 34: 631-41.
19. Lu Y, Jin J, Xu WL. Expression of WT1 gene in patients with myelodysplastic syndrome and acute leukemia. Zhongguo Shi Yan Xue Ye Xue Za Zhi 2004; 12: 464-6.
20. Bell HS, Dufes C, O'Prey J, et al. A p53-derived apoptotic peptide derepresses p73 to cause tumor regression in vivo. J Clin Invest 2007; 117: 1008-18.