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Prognostic value of *CIP2A* gene expression in adult Egyptian acute myeloid leukemia patients

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Abstract

Background: Protein phosphatase 2A (*PP2A*) functions as a tumor suppressor in many cancers. Cancerous inhibitor of protein phosphatase 2A (*CIP2A*) inhibits *PP2A* proteolytic degradation of *c-Myc* and enhances cell growth and tumor formation in several tissues. *CIP2A* expression in acute myeloid leukemia (AML) and its effect on outcome of treatment were not reported in Egyptian patients.

Results: No significant difference was detected in *CIP2A* expression between AML and control groups ($P = 0.48$). However, in AML patients, those with low *CIP2A* expression had a longer median overall survival than those with high expression ($P = 0.059$). *CIP2A* expression was not related to the clinical and laboratory variables and did not affect response to treatment.

Conclusion: High-*CIP2A* expression was associated with a trend of shorter overall survival in adult Egyptian AML patients. It might serve as a useful marker to predict poor prognosis. *CIP2A* may represent a potential target for cancer therapy.

Keywords: AML, *CIP2A*, RT-qPCR

Background

Acute myeloid leukemia (AML) encompasses a heterogeneous group of biologically and clinically aggressive disorders that result from genetic and epigenetic changes in hematopoietic progenitors. Despite considerable advances in the understanding of the biology of AML, overall survival (OS) remains poor because of the high rate of relapse after achievement of complete remission (CR) and failure of primary induction chemotherapy [1, 2].

Patients with cytogenetically normal AML (CN-AML) are usually classified in the intermediate-risk prognostic category because their CR rate, relapse risk, and survival are worse in comparison to patients with favorable aberrations such as *t*(8;21), inv(16)/*t*(16;16), or *t*(15;17). However, performance of CN-AML is better than patients with unfavorable cytogenetic findings such as monosomy 7, inv(3;3), balanced translocations involving 11q23 other than t(9;11), or complex karyotype [3, 4]. Of note, the outcomes of therapeutic approaches differ among patients with CN-AML probably because this cytogenetic subset is heterogeneous at the molecular level. Numerous mutations and changes in gene expression that affect clinical outcomes have been discovered in CN-AML patients [5–9].

Protein phosphatase 2A (*PP2A*) is a major serine/threonine phosphatase that functions as a tumor suppressor in several cancers. It negatively regulates numerous signal-transduction pathways [10–12]. Cancerous inhibitor of protein phosphatase 2A (*CIP2A*) is an endogenous protein that interacts with both *PP2A* and the oncogenic transcription factor *c-Myc*. *CIP2A* inhibits dephosphorylation of *c-Myc* serine 62 by *PP2A*, leading to prevention of proteolytic degradation of *c-Myc*. *CIP2A* activity enhances anchorage-independent cell growth

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and promotes tumor formation in vivo. Upregulation of *CIP2A* expression is seen in patients with head and neck squamous-cell carcinoma, gastric cancer, breast cancer, or colon cancer, and is associated with aggressive tumor behavior [13, 14].

Our aim in this study was to investigate *CIP2A* gene expression in Egyptian AML patients and to determine its effect on treatment response and survival.

Methods

The present study was conducted at Medical Oncology Department, National Cancer Institute (NCI), Cairo University, Cairo, Egypt. Ethical approval was obtained from the NCI Institutional Review Board, and written informed consent was obtained from all participants.

The study population comprised 60 adult consecutive patients aged 18–65 years with newly diagnosed AML over a period of 1 year. In addition, 22 age- and gender-matched healthy individuals who were donors for bone-marrow transplantation were included as a control group.

Diagnosis of AML was established after clinical, morphological, cytochemical, flow-cytometric, and cytogenetic analyses. All cases met the AML diagnostic standards including involvement of more than 20% of the blood and/or bone marrow by leukemic myeloblasts.

Clinical end points

CR was defined by the following criteria: < 5% bone-marrow blasts; the absence of blasts with Auer rods; the absence of extramedullary disease; absolute neutrophil count > 1.0 × 10^9/L; platelet count > 100 × 10^9/L; and independence from red blood cell transfusions. Treatment failure was defined as either resistant disease or relapse. Resistant disease was defined as the failure to achieve CR following completion of initial treatment, with evidence of persistent leukemia in examinations of blood and/or bone marrow. Relapse was defined as ≥ 5% bone-marrow blasts, reappearance of blasts in the blood, or development of extramedullary disease.

Overall survival (OS) was defined as the time from diagnosis to death from any cause. Data from patients who were alive on the date of the last follow-up were censored on that date. Progression-free survival (PFS) was defined as the time from the initiation of therapy until documented progression or death. For patients without disease progression at the time of analysis, data were right-censored at the date of their last follow-up [15].

Sample collection, RNA extraction, and cDNA synthesis

Bone-marrow samples (1 mL) from patients with AML were collected in a solution containing ethylenediaminetetraacetic acid and treated with erythrocyte-lysis solution. Leukocytes (1 × 10^7) were collected and stored in RLT buffer at − 80 °C prior to RNA extraction. Total RNA was extracted using the QIAamp RNA extraction blood mini kit (QIAGEN® Austin, TX, USA, catalog no. 52304), following the manufacturer's instructions. The amount of RNA was measured with a nanodrop spectrophotometer at 260 nm and 280 nm. A ratio of 1.8–2.0:1 indicated good RNA purity. Subsequently, 1.0 μg of RNA was reverse transcribed into cDNA in a 20 μL reaction with random primers and a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA, catalog no. 4368814) according to the manufacturer's instructions. cDNA was stored at − 20 °C until required.

Molecular detection of CIP2A expression

Quantitative assessment of gene expression levels was performed with the TaqMan Universal PCR Master Mix with no UNG (Applied Biosystems, Foster City, CA. P/N 4440043) as recommended by the manufacturer. The StepOne Real-Time PCR system (Applied Biosystems) was used for real-time analysis. The relative expression levels of the *CIP2A* gene were analyzed by the comparative CT method ($2^{-\Delta\Delta Ct}$) [16], with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the endogenous control. Data are expressed as the fold change in *CIP2A* gene expression in patients with AML and normalized to the expression levels of the endogenous control and relative to the healthy controls. Median *CIP2A* expression in the control group was 28. This value was taken as the cut-off value. Patients with values above median expression were considered to have high expression of *CIP2A*, and patients with values below median expression were considered to have low expression of *CIP2A*.

Statistical methods

Data were analyzed with SPSS version 20. Categorical data are summarized as frequencies and percentages. Continuous numerical data are summarized as mean ± standard deviation (SD), or median and range. Chi-square test was used to compare categorical data and *t* test or Mann-Whitney *U* test were used to compare continues data.

OS and PFS were estimated by Kaplan–Meier analysis. The log-rank test was used for comparison of survival curves. All tests were conducted at the 0.05 alpha level, with 95% confidence intervals (CI).

Results

In this case-control study, we enrolled 60 patients with AML into the study group and 22 healthy bone-marrow donors into the control group. The baseline demographic and laboratory characteristics of participants are summarized in Table 1.

Table 1 Clinical and laboratory characteristics of 60 adults with acute myeloid leukemia

Parameter	Value (mean ± SD) or distribution (*n* (%))
Age (years)	36.8 ± 1.6
Gender	
Male	27 (45.0)
Female	33 (55.0)
Hepatomegaly	25 (41.6)
Splenomegaly	23 (38.3)
Lymphadenopathy	12 (20.0)
Total leukocytic count ($\times 10^9$/L)	49.8 ± 7.6
Hemoglobin (g/dL)	8.1 ± 0.2
Platelets ($\times 10^9$/L)	56.7 ± 9.8
Peripheral-blood blasts (%)	50.4 ± 26.4
Bone-marrow blasts (%)	62.3 ± 20.7
Bone-marrow cellularity[a]	
Normocellular	9 (15.0)
Hypercellular	51 (85.0)
Cytogenetics and molecular classification	
Normal karyotype	9 (15.0)
t(5:17)	6 (10.0)
t(8;21)	6 (10.0)
t(9;12)	5 (8.3)
Trisomy 21	2 (3.3)
Inversion 16	1 (1.7)
Trisomy 3	1 (1.7)
Multiple	9 (15.0)
Cytogenetics not performed	21 (35.0)
FLT3 status	
Wild type	51 (85.0)
Mutant	9 (15.0)
French–American–British classification	
M1	7 (11.7)
M2	20 (33.3)
M3	5 (8.3)
M4	14 (23.3)
M5a	4 (6.7)
M7	1 (1.7)
CD34 status	
Positive	35 (58.3)
Negative	25 (41.6)

[a]Cellularity is the volume ratio of hematopoiesis and fat. Normal of an adult 30 to 70% and hypercellular over 70%

To define high expression of *CIP2A* a cut-off value of 28 was taken, which corresponds to the median value of *CIP2A* expression in the control group.

CIP2A was over-expressed in 21 patients with AML (35%), and under-expressed in 39 patients (65%). The proportions with over-expression and under-expression of *CIP2A* did not significantly differ between the study and control groups (P = 0.31). No significant relations were found between *CIP2A* expression and the clinical characteristics (Table 2).

In patients with high-*CIP2A* expression levels, 13 patients (61.9%) achieved CR and 8 patients (38.1%) were

Table 2 Association between *CIP2A* expression in patients with acute myeloid leukemia and demographic characteristics, clinical and laboratory data, and risk stratification

Parameter	*CIP2A* expression		*P* value
	High (n = 21)	Low (n = 39)	
	Value (mean ± SD) or distribution (n (%))	Value (mean ± SD) or distribution (n (%))	
Age (years)	33.8 ± 2.7	38.5 ± 2.0	0.17
Gender			0.83
Male	9 (33.3)	18 (66.7)	
Female	12 (36.3)	21 (63.7)	
Hepatomegaly	7 (28.0)	18 (72.0)	0.28
Splenomegaly	5 (21.7)	18 (78.2)	0.11
Lymphadenopathy	1 (8.3)	11 (91.6)	0.04
Total leukocyte count ($\times 10^9$/L)	41.6 ± 10.6	54.3 ± 10.2	0.43
Hemoglobin (g/dL)	8.2 ± 0.4	8.0 ± 0.3	0.81
Platelets ($\times 10^9$/L)	45.5 ± 9.6	62.8 ± 14.3	0.41
Peripheral-blood blasts	45.8 ± 5.6	47.9 ± 4.5	0.78
Bone-marrow blasts	58.7 ± 4.3	61.7 ± 3.6	0.61
FLT3			0.38
Wild type	19 (90.5)	32 (82.1)	
Mutant	2 (9.5)	7 (17.9)	
Risk stratification			0.94
Favorable	5 (23.8)	9 (23.1)	
Intermediate	3 (14.3)	8 (20.5)	
Poor	5 (23.8)	9 (23.1)	
Not performed	8 (38.1)	13 (33.3)	

resistant to therapy. In patients with low-*CIP2A* expression, 27 patients (69.2%) achieved CR and 12 (30.8%) were resistant with no statistically significant differences between subgroups. Also, no statistically significant difference was observed in relapse rates between patients with high- and low-*CIP2A* expression (RR = 0.46; 95% CI 0.11–1.99) (Table 3).

The median duration of follow-up was 21.9 months (24.0–27.3 months). There was no statistically significant difference in the estimated PFS between patients with high- and low-*CIP2A* expression (P = 0.28) (Fig. 1). OS showed a trend towards longer survival in patients with low-*CIP2A* expression. The median OS for all patients with AML was 15.6 months (95% CI 10.71–20.48 months). The median OS for patients with low-*CIP2A* expression was 16.0 months (95% CI 9.0–23.2 months), and that for patients with high-*CIP2A* expression was 9.67 months (95% CI 5.1–14.3 months), although this difference did not reach statistical significance (P = 0.059) (Fig. 2).

Discussion

PP2A inhibition is a common event in AML. Restoration of *PP2A* activity induces both arrest of cell growth and caspase-dependent apoptosis, suggesting that *PP2A* inactivation has an important role in AML and can be a promising therapeutic target in hematological malignancies [17].

CIP2A is an endogenous *PP2A* inhibitor that interacts directly with the oncogenic transcription factor *c-Myc*, inhibiting *PP2A*-mediated dephosphorylation of *c-Myc* and preventing its proteolytic degradation, resulting in *c-Myc* stabilization, promotion of cell growth, and tumor formation [18]. *CIP2A* is expressed in few normal tissues, but it is overexpressed in many human cancers, and is associated with clinically aggressive cancer behavior. *CIP2A* is known to be overexpressed in head and neck squamous-cell carcinoma, breast cancer, colon cancer, and gastric cancer [14].

To date, *CIP2A* expression levels in patients with AML have been investigated in two studies. In a study involving Asian patients with AML, conventional RT-PCR identified higher expression levels (77.14%) of *CIP2A* in newly diagnosed AML patients as well as in patients with relapsed AML (70.86%) compared to 6.25% in AML patients in CR and 2.86% in healthy controls. This finding points to a relation between *CIP2A* overexpression and disease activity in AML [18].

Table 3 Association between *CIP2A* expression in patients with acute myeloid leukemia and the response to induction therapy and the relapse rate

	High *CIP2A* (n = 21) Distribution [n (%)]	Low *CIP2A* (n = 39) Distribution [n (%)]	*P* value	Relative risk (95% CI)
Response to induction therapy			0.57	1.2 (0.6–2.54)
Complete remission	13 (61.9)	27 (69.2)		
Resistance	8 (38.1)	12 (30.8)		
Relapse			0.27	0.4 (0.11–1.99)
Still in complete remission	19 (90.5)	31 (79.8)		
Relapsed	2 (9.5)	8 (20.2)		

In a cohort of 203 European patients diagnosed with a normal karyotype AML, qRT-PCR was done to find out the prevalence of *CIP2A* in patients with NK-AML. In that study, the median expression of *CIP2A* was 0.005 (range 0.0027–0.007) in the control group and 0.004 (range 0.00012–0.59) in the AML group. The authors chose a cut-off value of 0.0072 to define high-*CIP2A* expression which corresponds to the mean value in the control [9].

In this study, qRT-PCR was used to detect the expression of *CIP2A* in de novo AML patients. The median expression in the control group which we used as a cut-off was much higher than the value reported by Barragan et al. [9].

Based on this cut-off value, we found that 35% of the patients had a high-*CIP2A* level; a value higher than that reported by Barragan et al. [9], and much less than that reported by Wang et al. [18] who used conventional RT-PCR. This may be explained by the fact that the relative quantitation method and the threshold for determining high and low expression in our study were different from those used by the other two studies.

In the current study, no significant relations was found between *CIP2A* expression and the clinical and laboratory data; a finding consistent with previous findings [9]. Similarly, we found that the level of expression of *CIP2A* did not have a significant effect on the response to induction chemotherapy or relapse rate (P = 0.57 and P = 0.27, respectively), which is in line with previous findings [9].

Noteworthy, we found a trend towards improved survival in patients with low-*CIP2A* expression with a 3-year survival probability of 36% and 25% for patients

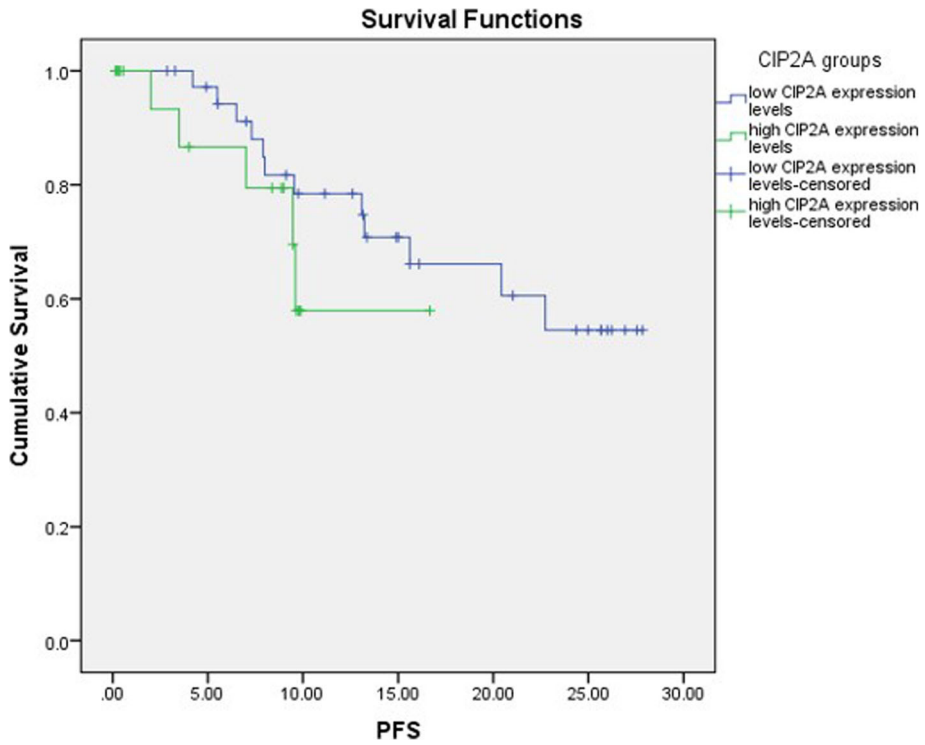

Fig. 1 Progression-free survival in patients with acute myeloid leukemia and high or low levels of *CIP2A* expression

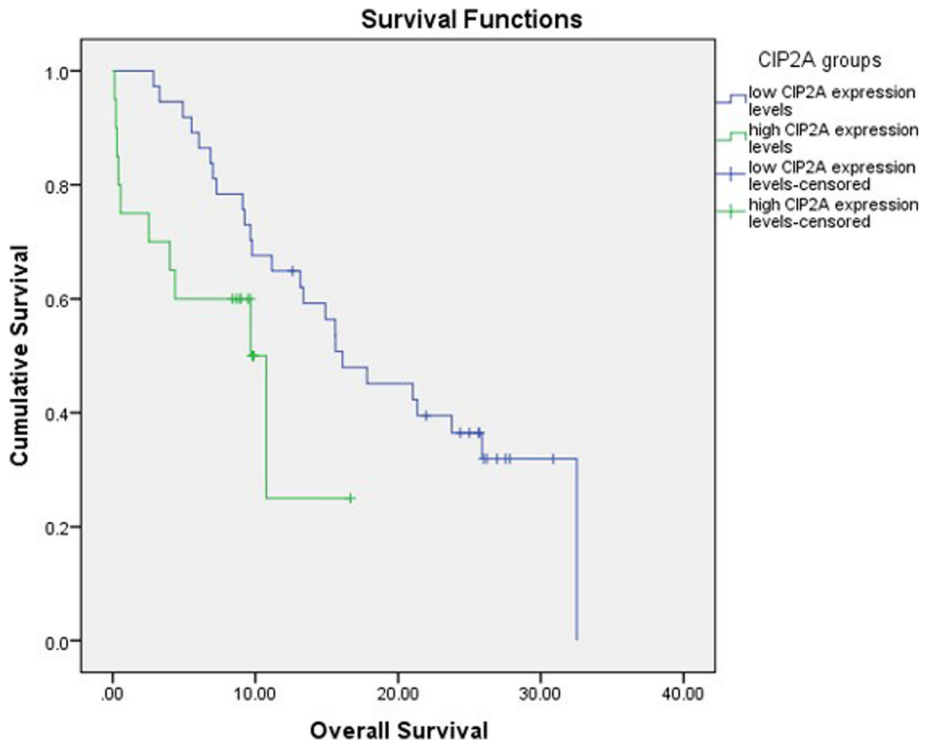

Fig. 2 Overall survival in patients with acute myeloid leukemia and high or low levels of *CIP2A* expression

with low- and high-*CIP2A* expression, respectively. Barragan et al. [9] reported a close percentage (27%) in patients with high *CIP2A*, but a much higher percentage (50%) in patients with low expression.

At 3 years, the current study recognized relapse in 54.5% and 57.9% of patients with low- and high-*CIP2A* expression, respectively. These values are higher than those reported by Barragan *et al.* [9] (30% and 47% for low- and high-*CIP2A* expression)

The lower PFS and OS reported in this study compared to those reported by Barragan et al. [9] may be attributed to the fact that the European trial was larger and only patients with NK-AML were allowed to participate.

Conclusion

High-*CIP2A* expression was associated with a trend towards shorter OS in adult Egyptian AML patients. It might serve as a marker to predict poor prognosis. However, larger studies are needed to determine the most suitable cut-off for defining high versus low *CIP2A* level and its impact on response to induction chemotherapy as well as its effect on PFS and OS.

Abbreviations

AML: Acute myeloid leukemia; CI: Confidence intervals; *CIP2A*: Cancerous inhibitor of protein phosphatase 2A; CN-AML: Cytogenetically normal AML; CR: Complete remission; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; OS: Overall survival; PCR: Polymerase chain reaction; PFS: Progression-free survival; *PP2A*: Protein phosphatase 2A; RR: Relative risk

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Authors' contributions

Conception and design: R.S, A.I, and F.S. Data acquisition, analysis, and interpretation: N.H, H.E.S, and H.A.S. Drafting: N.H, H.E.S, and H.A.S. Critical revision: R.S, A.I, and F.S. Final approval: all authors. Agreement to be accountable for all aspects of the work: All authors. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Ethical approval was obtained from the National Cancer Institute Institutional Review Board, and written informed consent was obtained from all study participants. Ethical committee approval no.: I-21101h (Ethical committee of Clinical and Chemical Pathology Department, Cairo University Faculty of Medicine).

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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