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The study of long noncoding RNA *TUG 1* and *ZEB2-AS1* expression in newly diagnosed Egyptian adult acute myeloid leukemia patients

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Abstract

Background The hematopoietic malignancy acute myeloid leukemia is a fatal disease with poor clinical prognoses. Long non-coding RNA *taurine-upregulated gene1* (*lncRNA TUG1*) and *zinc finger E-box binding homeobox 2 antisense RNA1* (*lncRNA ZEB2-AS1*) are reported to participate in the development and progression of different types of malignancies. The goal of the current study was to evaluate the prognostic value of the *lncRNAs TUG1* and *ZEB2-AS1* as well as their various expression patterns in newly diagnosed Egyptian adult acute myeloid leukemia patients.

Methods We assessed the expression levels of both *lncRNA TUG1* and *lncRNA ZEB2-AS1* using the quantitative real-time reverse transcription polymerase chain reaction technique (qRT-PCR) in 80 newly diagnosed AML patients and 20 healthy subjects.

Results *lncRNA TUG1* expression was significantly higher in the AML cases compared to the controls ($P<0.001$), whereas *lncRNA ZEB2-AS1* expression was considerably lower in the AML cases in comparison with the controls ($P<0.001$). The expression levels of the *lncRNAs ZEB2-AS1* and *TUG1* exhibited a significantly positive association in the AML group ($P<0.001$). There was no difference in overall survival (OS) and disease-free survival (DFS) between the groups with low and high *lncRNA TUG1* expression ($P=0.139$ and 0.918, respectively). Furthermore, the AML cases with higher *lncRNA ZEB2-AS1* expression levels had shorter DFS than patients with lower *lncRNA ZEB2-AS1* expression levels ($P=0.014$), while OS did not significantly differ between the studied cases with lower and higher *lncRNA ZEB2-AS1* expression ($P=0.589$).

Conclusion Overexpression of *lncRNA TUG1* could serve as a diagnostic biomarker for Egyptian adult AML cases, while *lncRNA ZEB2-AS1* high expression could be regarded as an indicator of poor outcome in Egyptian adult AML studied cases.

Keywords AML, *lncRNA TUG1*, *lncRNA ZEB2-AS1*, qRT-PCR, Prognosis

Background

The hematological malignancy known as acute myeloid leukemia (AML) is distinguished by the aberrant proliferation of primitive myeloid cells in the bone marrow and peripheral blood, along with severe inhibition of normal hematopoiesis [1]. Previous studies have shown that genetic aberrations are crucial to the development and spread of AML [2]. Increasing data suggest that AML is

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caused by epigenetic dysregulations including acetylation, DNA methylation, and noncoding RNAs [3].

A family of non-protein coding transcripts known as long non-coding RNAs (*lncRNA*) are > 200 nucleotides and less than 100 kb long. They cannot be translated into proteins because they lack open reading frames [4]. The main mechanisms of *lncRNA* action include their involvement in the process of histone modification [5, 6], their direct interaction with proteins to change their function or localization, or their use as scaffolds to encourage protein binding to other proteins (or DNA) [7]. By serving as a "sponge," they compete for miRNAs, bind to them, and subsequently control the expression of the target genes [8, 9].

The 7.1-kb *lncRNA taurine-upregulated gene 1* is found on chromosome 22q12. Aberrant expression of *lncRNA TUG1* may have an impact on a number of biological events, including cell proliferation, differentiation, apoptosis, invasion, drug resistance, blood tumor barrier permeability regulation, and epithelial-mesenchymal transition (EMT) [10, 11]. According to several studies, *lncRNA TUG1* is an oncogene that contributes to the development and progression of several cancers, including adult Philadelphia-negative acute lymphoblastic leukemia, breast cancer, colon cancer, renal cell carcinoma, and multiple myeloma [12–15].

Long non-coding *RNA zinc finger E-box-binding homeobox 2 antisense RNA 1* (*lncRNA ZEB2-AS1*) is located on chromosome 2q22.3 that is overlapping and antisense to the *ZEB2* gene, which has been identified as an essential component in the process of epithelial-mesenchymal transition (EMT). *lncRNA ZEB2-AS1* had the ability to activate *ZEB2* expression [16]. Also, it has been described to affect cell proliferation, migration, invasion, and cell cycle regulation [17]. Additionally, it has been demonstrated to play a role in the development of a number of cancers, including lung and stomach cancers [18, 19].

Unfortunately, the *lncRNAs ZEB2-AS1* and *TUG1* prognostic implications in AML are poorly understood, and it is still unclear how those *lncRNAs* operate in leukemogenesis [20, 21]. Therefore, our aim was to investigate various expression patterns and the prognostic significance of the *lncRNAs TUG1* and *ZEB2-AS1* in newly diagnosed Egyptian adult acute myeloid leukemia patients.

Methods

This study included 80 Egyptian patients newly diagnosed with AML with a mean age of 48 ± 14 years at diagnosis (49 males and 31 females). Additionally, 20 healthy donors of BM transplantation were included as a control group with a mean age of 50 ± 15 years (13 males and 7 females).

The study was conducted at Clinical Pathology Department, National Cancer Institute, Cairo, Egypt, from 2019 to 2022 and was performed according to the Helsinki declaration guidelines and approved by Benha University ethical scientific committee.

The included cases in this study were Adult cases ≥ eighteen years old newly diagnosed with AML. The following were the exclusion criteria: patients who began therapy, those under the age of 18, patients with secondary AML, patients with AML with myelodysplasia-related changes, or with other malignancies.

Patients were subjected to full history taking, complete clinical examination, and laboratory investigations including complete blood picture, bone marrow aspiration, immunophenotyping, and cytogenetic. Also, molecular genetic analysis was carried out for detection of chromosomal abnormalities, *NPM1* and *FLT3-ITD* mutations by reverse transcriptase PCR and real-time PCR, respectively. Real-time reverse transcription polymerase chain reaction (qRT-PCR) was carried out for all participants in the study to quantitatively estimate the expression of the long noncoding *RNAs ZEB2-AS1* and *TUG1*.

AML diagnosis and classification were performed according to French-American-British [22] and World Health Organization criteria [23]. Genetic risk stratification of the AML cases was performed based on 2017 European Leukemia Net (ELN) recommendations [24].

The AML studied cases received induction chemotherapy in the form of standard 3 + 7 regimens (Doxorubicin 30 mg/m2 on days 1–3; Cytarabine 100 mg/m2 on days 1–7). Patients with PML/RAR-alpha gene expression acute promyelocytic leukemia received induction cycle in the form of tretinoin 22.5 mg/m^2 and arsenic trioxide 0.15 mg/kg therapy.

The presence of complete remission (CR)—defined as bone marrow with at least twenty percent cellularity and BM blasts below five percent, without need for transfusions, an absolute neutrophil count greater than 1×10^9/l, and a platelet count greater than 100×10^9/l—was evaluated after induction chemotherapy was completed. In the present study, long-term prognosis evaluations were conducted using disease-free survival (DFS) and overall survival (OS). DFS was defined as the period from the start of treatment to the time of recurrence, progression, or death, while OS is the period from the time of diagnosis until death or the last follow-up [25].

Quantitative reverse transcriptase-PCR for *lncRNA TUG1* and *incRNA ZEB2-AS1*

RNA extraction and cDNA synthesis

One milliliter of bone marrow aspiration was obtained from both the AML studied cases and the controls and

then evacuated into tubes containing ethylene diamine tetra-acetic acid (1.2 mg/mL).

Following the manufacturer's instructions, total RNA was extracted from bone marrow cells using the QIAamp RNA Blood Mini Kit for total RNA purification (QIAGEN® Austin, Texas, USA catalog no. 52304). The concentration and purity of the extracted RNA were measured using a Spectrophotometer (NanoDrop, Q-500, Scribner, USA) and then stored at − 80 °C until use. Thermo Fisher Scientific's High-Capacity cDNA Reverse Transcription Kit (USA; catalog number 4374966) was used to reverse transcribe RNA into complementary DNA, which was then stored at − 20 °C until its use in quantitative real-time PCR.

Gene expression analysis

Quantitative real-time PCR was carried out to measure the expression levels of *lncRNA TUG1* and *lncRNA ZEB2-AS1*. Real-time PCRs were performed in twenty µL volume using TaqMan® Universal PCR Master Mix II (Catalog no.: 4440043, Thermo Fisher Scientific, Applied Biosystems, USA) and TaqMan primer probes for *lncRNA TUG1* (Hs00215501_m1; Thermo Fisher Scientific, USA, Catalog number: 4448892), *lncRNA ZEB2-AS1* (Hs04274848_g1; Thermo Fisher Scientific, USA, Catalog number: 4426961) and *B-actin* as a reference gene (Hs03929097_g1; Thermo Fisher Scientific, USA, Catalog no: 4331182). These were the primer sequences that were used: *lncRNA ZEB2 AS1* forward, 5′-GGC TGG ATAGCAAAGGAC-3′ and reverse, 5′-ACACTC TTGGCGAGG-3′; *lncRNA TUG1*forward, 5′-TAGGAG TGGATGTGTTCTGTAGCA-3′ and reverse, 5′-TGG TCGTGGAATATGGTCAATGAG-3′; *B-actin* forward, 5′-ATGTTTGAGACCTTCAACACC-3′ and reverse, 5′-GCCATCTCCTGCTCGAAGTCT-3′. The thermal reaction conditions were as follows: polymerase activation at 95 °C for ten minutes followed by forty cycles of 95 °C for 15 s (denaturation), and 60 °C for one min (annealing and extension). Applied Biosystems' Step One plus™ Real-Time PCR System was used to measure the resulting fluorescence. *lncRNAs TUG1* and *ZEB2-AS1* relative expression was assessed using Schmittgen and Livak's comparative Ct method (2-ΔΔCt) [26].

Statistical methods

Statistical analysis and data management were carried out using SPSS version 28 (IBM, Armonk, New York, USA). To check for normalcy in quantitative data, the Kolmogorov–Smirnov test, the Shapiro–Wilk test, and direct data visualization approaches were used. Means, standard deviations, medians, and ranges were used to summarize quantitative data in a manner consistent with normality. Numbers and percentages were used for displaying categorical collections of data. Independent t-tests or Mann–Whitney U tests were employed to compare the data among the study groups based on gene expression, depending on whether or not the quantitative data had been normally distributed. If necessary, Fisher's exact test or Chi-square test was used to compare the categorical data. Correlation analyses were carried out using Spearman's correlation. ROC analyses were carried out to evaluate the role of the studied lncRNAs in AML diagnosis. Areas under the curve, best cutoff points, and diagnostic indices were calculated. To evaluate the overall and disease-free survival of the cases under study, Kaplan–Meier analyses were carried out. The log-rank test compared the overall and the disease-free survival according to lncRNAs expression. There was a two-sided design for each statistical test. P values under 0.05 were considered significant.

The results

Demographic, clinical, and laboratory features of the AML cases are presented in Table 1.

Relative expression of the *lncRNAs TUG1 and ZEB2-AS1* in AML patients and controls

The studied AML patients showed significantly higher *lncRNA TUG1* expression levels compared to controls (6.78 vs. 0.84, $P < 0.001$). On the other hand, they showed lower *lncRNA ZEB2-AS1* expression compared to controls (0.16 vs. 0.88, $P < 0.001$) (Table 2; Figs. 1 and 2).

ROC analysis for *lncRNA TUG1* and *lncRNA ZEB2-AS1* (Fig. 3)

ROC analysis was carried out to compare the expressions of the *lncRNAs ZEB2-AS1* and *TUG1* in AML patients to those in the control group. It verified that the *lncRNA TUG1* revealed significant AUC of 0.970 ($P < 0.001$), with ninety-five percent confidence interval ranging from 0.953 to 1. The best cutoff value was > 2.72, at which the sensitivity, specificity, positive predictive value, and negative predictive value were 88.6%, 100%, 100%, and 67.9%, respectively. Additionally, for *lncRNA ZEB2-AS1*, the Roc curve analysis showed significant AUC of 0.820 ($P < 0.001$), with ninety-five percent confidence interval ranging from 0.732 to 0.908. The best cutoff was ≤ 0.26, at which the sensitivity, specificity, PPV, and NPV were 60.9%, 100%, 100%, and 38.6%, respectively.

Relationship between *lncRNAs ZEB2-AS1* and *TUG1* expression in AML cases

The *lncRNAs ZEB2-AS1* and *TUG1* had a highly significant positive correlation in the studied AML cases ($P < 0.001$) (Fig. 4).

Table 1 Baseline features of AML studied cases

Patient characteristics	Frequency (%)	Patient characteristics	Frequency (%)
Age (years)	48 ± 14	**Immunophenotyping**	
Gender (male/female)	49/31	Positive CD34	43 (53.8)
Initial TLC (median and range) × 10^3/mm^3	22.5 (0.3–403)	Positive HLA-DR	45 (56.3)
Initial HGB (median and range) g/dl	7.8 ± 1.9	Positive MPO (cyto)	68 (85)
Initial PLT (median and range) × 10^3/mm^3	45 (4–208)	Positive CD33	71 (88.8)
Initial PB Blasts (median and range)%	60 (0–100)	Positive CD13	67 (83.8)
Initial BMA Blast (median and range)%	73 (20–98)	Positive CD117	59 (73.8)
Hepatomegaly	16 (20)	Positive CD14	16 (20)
Splenomegaly	16 (20)	Positive CD4	7 (8.8)
Lymphadenopathy	27 (33.8)	Positive CD11c	19 (23.8)
Fever	37 (46.3)	Positive CD64	25 (31.3)
Initial cellularity		Positive CD61	2 (2.5)
Hypocellularity	2 (2.5)	Positive CD7	5 (6.3)
Hypercellularity	67 (83.8)	**Cytogenetics**	
Normocellularity	11 (13.8)	Abnormal Cytogenetics	37 (53.6)
FAB subtypes		Negative FLT-ITD	61 (76.3)
M0	2 (2.5)	Positive FLT-ITD	19 (23.8)
M1	13 (16.3)	Wild NPM	74 (92.5)
M2	30 (37.5)	Mutant NPM	6 (7.5)
M3	8 (10)	*t*(8;21)	5 (6.3)
M4	18 (22.5)	PML/RARA	7 (8.8)
M5	7 (8.8)	*t*(16;16)/inv16	2 (2.5)
M7	2 (2.5)	*t*(9;22)	2 (2.5)
Genetic risk (ELN 2017)		**Treatment response**	
Favorable	19 (23.8)	BMA blast on day 14 Median (min–max)	2 (0–90)
Intermediate	36 (45)	BMA blast on day 28 Median (min–max)	2 (0–88)
Poor	25 (31.3)		

Table 2 lncRNA ZEB2-AS1 and lncRNA TUG1 in studied groups

	Patients ($n=80$)	Controls ($n=20$)	*P* value
ZEB2-AS1 RQ	0.16 (0–4.46)	0.88 (0.3–2.9)	*< 0.001*
TUG1 RQ	6.78 (0.86–93.13)	0.84 (0.53–2.72)	*< 0.001*

Italics: Significant *P* value

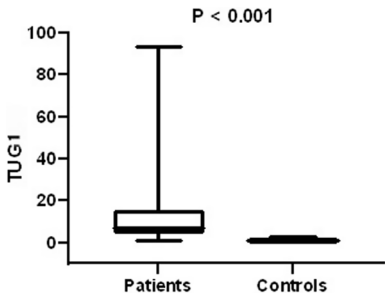

Fig. 1 *lncRNA TUG1* expression in the studied groups

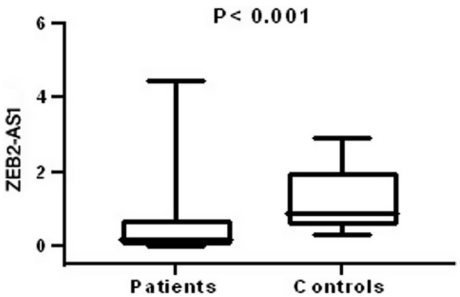

Fig. 2 *lncRNA ZEB2-AS1* expression in the studied groups

Correlation of *lncRNA TUG1* and *lncRNA ZEB2-AS1 expression* with initial blast count

lncRNA TUG1 expression showed a significant positive correlation with the initial PB blast ($P=0.018$) and initial BMA blast ($P=0.002$) (Fig. 5b, c). Additionally, *lncRNA ZEB2-AS1* expression showed a significant

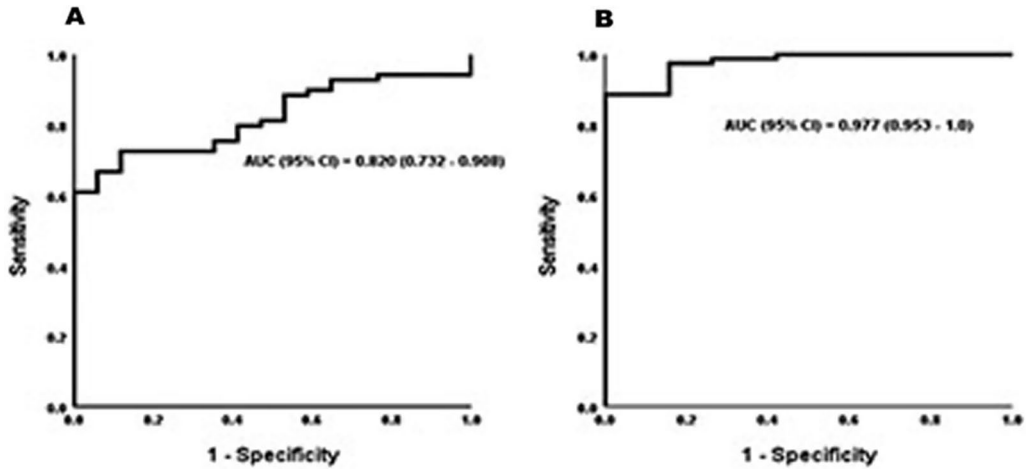

Fig. 3 ROC analysis for a *lncRNA ZEB2-AS1*; b *lncRNA TUG1* expressions to diagnose AML

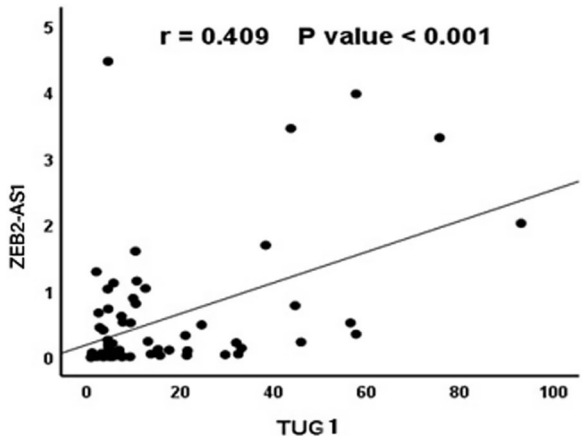

Fig. 4 Correlation between *lncRNA ZEB2-AS1* and *lncRNA TUG* expression

positive correlation with the initial BMA blast ($P=0.027$) (Fig. 5a).

Correlation analysis between the expression of *lncRNAs TUG1, ZEB2-AS1*, and clinicopathological features of AML studied patients (Tables 3 and 4)

Patients were classified into high and low expression groups according to *lncRNA TUG1* and *lncRNA ZEB2-AS1* cutoff. For *lncRNA TUG1* cases with a cut-off > 2.72 were considered high expression while for *lncRNA ZEB2-AS1* cases with cutoff more than 0.26 were deemed high expression.

The results of this study demonstrated that 70 from 80 patients showed high *lncRNA TUG1* gene expression, while only 27 patients showed high *lncRNA ZEB2-AS1* expression.

There were no significant differences between the high and low *lncRNA TUG1 and lncRNA ZEB2-AS1* expression groups regarding clinical findings, initial (TLC, hemoglobin, platelets, PB blasts, and cellularity), FAB subtypes, cytogenetics, and cytogenetic risk stratification (ELN 2017). Patients with higher levels of *lncRNA TUG1* expression ($n=70$) displayed more positive CD13 (88.6%) than patients with lower levels (55.6%) ($P=0.009$). Additionally, patients with high *lncRNA TUG1* expression had median initial BMA blasts that were considerably greater (76%) than those of patients with low *lncRNA TUG1* expression (64%) ($P=0.034$), while patients with high levels of *lncRNA ZEB2-AS1* expression ($n=27$) showed significantly higher CD14 than patients with low levels of *lncRNA ZEB2-AS1* expression ($P=0.029$). However, there was not a significant difference in initial blast count between the groups with high and low *lncRNA ZEB2-AS1* expression.

Overall survival and disease-free survival assessment (Fig. 6)

To evaluate the overall and disease-free survival of the cases under study, Kaplan–Meier analysis was used. The estimated overall survival rate was 26.7% at 6 months and 21.6% at 12 and 24 months. The median survival time was 0.9 month, with a ninety five percent confidence interval ranging from 0.543 to 1.257. The estimated disease-free survival rate was 46% at six months, 21.9% at twelve months, and 16.4% at twenty-four months. The median disease-free survival time was 3.567 months, with ninety-five percent confidence interval ranging from 0 to 9.526.

Table 3 Characteristics of AML studied cases in comparison to *lncRNA TUG1* expression groups

TUG1 expression			
Characteristic	**Low expression (*n* = 9)**	**High expression (*n* = 70)**	***P* value**
Age (years)	39 ± 14	49 ± 14	***0.053***
Sex			
Males	7 (77.8)	42 (60)	*0.301*
Females	2 (22.2)	28 (40)	
Initial TLC ($\times 10^3$)	25 (0.7–316)	20 (0.3–403)	*0.982*
Initial HGB	7.5 ± 1.1	7.8 ± 1.9	*0.620*
Initial PLT ($\times 10^3$)	70 (6–208)	45 (4–166)	*0.537*
Initial PB blasts (%)	25 (4–92)	60 (0–100)	*0.222*
Initial BMA blast (%)	64 (21–88)	76 (20–98)	***0.034***
Initial cellularity			
Hypocellularity	0 (0)	2 (2.9)	*1.0*
Hypercellularity	8 (88.9)	58 (82.9)	
Normocellularity	1 (11.1)	10 (14.3)	
Hepatomegaly	0 (0)	16 (22.9)	*0.108*
Splenomegaly	0 (0)	15 (21.4)	*0.123*
Lymphadenopathy	2 (22.2)	24 (34.3)	*0.468*
Fever	4 (44.4)	32 (45.7)	*1.0*
Positive CD34	7 (77.8)	35 (50)	*0.162*
Positive HLA-DR	5 (55.6)	40 (57.1)	*0.928*
Positive MPO (cyto)	7 (77.8)	60 (85.7)	*0.532*
Positive CD33	8 (88.9)	62 (88.6)	*0.977*
Positive CD13	5 (55.6)	62 (88.6)	***0.009***
Positive CD117	7 (77.8)	52 (74.3)	*0.821*
Positive CD14	2 (22.2)	13 (18.6)	*0.793*
Positive CD4	1 (11.1)	5 (7.1)	*0.672*
Positive CD11c	3 (33.3)	16 (22.9)	*0.489*
Positive CD64	2 (22.2)	22 (31.4)	*0.572*
Positive CD61	0 (0)	2 (2.9)	*1.0*
Positive CD7	1 (11.1)	4 (5.7)	*0.463*
Abnormal cytogenetics	6 (75)	30 (50)	*0.266*
Negative FLT-ITD	8 (88.9)	52 (74.3)	*0.335*
Positive FLT-ITD	1 (11.1)	18 (25.7)	
Wild NPM	8 (88.9)	66 (94.3)	*0.463*
Mutant NPM	1 (11.1)	4 (5.7)	
t(8:21)	1 (11.1)	4 (5.7)	*0.463*
PML/RARA	0 (0)	7 (10)	*1*
t(16:16)/inv16	1 (11.1)	1 (1.4)	*0.216*
t(9:22)	1 (11.1)	1 (1.4)	*0.216*
FAB			
M0	0 (0)	2 (2.9)	*0.529*
M1	0 (0)	13 (18.6)	
M2	4 (44.4)	26 (37.1)	
M3	1 (11.1)	7 (10)	
M4	4 (44.4)	13 (18.6)	
M5	0 (0)	7 (10)	
M6	0 (0)	0 (0)	
M7	0 (0)	2 (2.9)	
Genetic risk (ELN 2017)			
Favorable	3 (33.3)	15 (21.4)	*0.282*
Intermediate	2 (22.2)	34 (48.6)	
Poor	4 (44.4)	21 (30)	
Treatment response			
BMA blast on day 14	3 (1–70)	2 (0–90)	*0.415*
BMA blast on day 28	4 (0–30)	2 (0–88)	*0.1*

Bold, italics: Significant *P* value

OS and DFS in relation to *lncRNA TUG1* and *lncRNA ZEB2-AS1* expression groups

No significant association was observed between the OS and DFS rates regarding the *lncRNA TUG1* expression groups ($P = 0.139$ and 0.918, respectively) (Fig. 7a, b). However, a lower level of *lncRNA ZEB2-AS1 expression* has been associated with a better DFS than a higher level of expression ($P = 0.014$). Between groups with high and low *lncRNA ZEB2-AS1* expression, OS did not significantly differ ($P = 0.589$) (Fig. 8a, b).

Multivariate comparison for DFS on *lncRNA ZEB2-AS1* expression and other prognostic factors

Multivariate cox regression analysis was carried out to predict disease-free survival. The model was built clinically, including all factors that may affect prognosis, including age, initial TLC, initial hemoglobin, initial platelets, initial blasts, ELN genetic risk, *t*(8;21), *t*(9;22), *t*(16;16), *PML/RARA*, and high expression of *lncRNA ZEB2-AS1*. Stepwise cox regression analysis was conducted, and the only significant variable that remained in the model was high *lncRNA ZEB-AS1* expression (HR 3.107, 95% CI 1.193–8.094, $P = 0.02$).

Discussion

Acute myeloid leukemia is characterized by a wide variety of prognosis and multiple pathogenic pathways [27]. The clinical outcomes of AML remain dismal despite the breakthroughs in therapeutic approaches, such as intense chemotherapy and hematopoietic stem cell transplantation, especially in older patients (> 60 old). In order to find effective treatment options for AML, it is crucial to look at the molecular mechanisms behind its development and progression [28].

Table 4 Characteristics of AML studied cases in comparison with lncRNA ZEB2-AS1 expression groups

lncRNA ZEB2-AS1 expression			
Characteristic	**Low expression (*n* = 42)**	**High expression (*n* = 27)**	***P* value**
Age (years)	47 ± 13	49 ± 16	*0.581*
Sex			
Males	24 (57.1)	16 (59.3)	*0.862*
Females	18 (42.9)	11 (40.7)	*0.581*
Initial TLC ($\times 10^3$)	17.4 (0.6–316)	32 (0.3–403)	*0.376*
Initial HGB	7.9 ± 1.9	7.9 ± 2	*0.998*
Initial PLT ($\times 10^3$)	43 (5–208)	54 (6–135)	*0.966*
Initial PB blasts (%)	55 (0–100)	70 (0–90)	*0.279*
Initial BMA blast (%)	70 (20–98)	75 (20–96)	*0.226*
Initial cellularity			
Hypocellularity	1 (2.4)	1 (3.7)	*1.0*
Hypercellularity	35 (83.3)	22 (81.5)	
Normocellularity	6 (14.3)	4 (14.8)	
Hepatomegaly	7 (16.7)	7 (25.9)	*0.351*
Splenomegaly	6 (14.3)	6 (22.2)	*0.396*
Lymphadenopathy	11 (26.2)	13 (48.1)	*0.062*
Fever	20 (47.6)	13 (48.1)	*0.966*
Positive CD34	19 (45.2)	17 (63)	*0.150*
Positive HLA-DR	26 (61.9)	14 (51.9)	*0.409*
Positive MPO (cyto)	36 (85.7)	23 (85.2)	*0.951*
Positive CD33	37 (88.1)	25 (92.6)	*0.697*
Positive CD13	36 (85.7)	23 (85.2)	*0.951*
Positive CD117	28 (66.7)	21 (77.8)	*0.321*
Positive CD14	6 (14.3)	10 (37)	***0.029***
Positive CD4	3 (7.1)	4 (14.8)	*0.42*
Positive CD11c	11 (26.2)	8 (29.6)	*0.755*
Positive CD64	13 (31)	9 (33.3)	*0.836*
Positive CD61	1 (2.4)	1 (3.7)	*1.0*
Positive Cd 7	2 (4.8)	1 (3.7)	*1.0*
Abnormal cytogenetics	21 (53.8)	11 (52.4)	*0.914*
Negative FLT-ITD	34 (81)	19 (70.4)	*0.309*
Positive FLT-ITD	8 (19)	8 (29.6)	
Wild NPM	41 (97.6)	23 (85.2)	*0.073*
Mutant NPM	1 (2.4)	4 (14.8)	
t(8;21)	4 (9.5)	1 (3.7)	*0.641*
PML/RARA	3 (7.1)	2 (7.4)	*1.0*
t(16;16)/inv16	1 (2.4)	0 (0)	*1.0*
t(9:22)	2 (4.8)	0 (0)	*0.517*
FAB subtypes			
M0	0 (0)	1 (3.7)	*0.778*
M1	8 (19)	5 (18.5)	
M2	16 (38.1)	7 (25.9)	
M3	3 (7.1)	3 (11.1)	
M4	9 (21.4)	8 (29.6)	
M5	5 (11.9)	2 (7.4)	
M6	0 (0)	0 (0)	
M7	1 (2.4)	1 (3.7)	
Genetic risk (ELN 2017)			
Favorable	9 (21.4)	6 (22.2)	*0.966*
Intermediate	20 (47.6)	12 (44.4)	
Poor	13 (31)	9 (33.3)	
Treatment response			
BMA blast on day 14	2 (0–77)	1 (0–90)	*0.304*
BMA blast on day 28	3 (0–80)	2 (0–88)	*0.398*

Bold, italics: Significant *P* value

LncRNAs had previously been thought to be transcription waste products. Nevertheless, it is now well defined that lncRNAs have a role in cell proliferation and differentiation as well as pathophysiology of many illnesses, including cancer [29].*lncRNA TUG1* is a rising star among all cancer-related lncRNAs [30]. Increasing evidence has found that the *lncRNA TUG1* is implicated in the pathogenesis of different cancers, such as osteosarcoma, bladder cancer, and glioma [31]. Also, *lncRNA ZEB2-AS1* upregulation was observed in human cancers like pancreatic cancer [32] and bladder cancer [33]. Unfortunately, the functional importance and underlying mechanisms of the *lncRNAs TUG1* and *ZEB2-AS1* in AML are poorly understood [20, 21]. So, we aimed in this study to assess the expression levels of *lncRNA TUG1* and *lncRNA ZEB2-AS1* in newly diagnosed AML Egyptian patients (Fig. 5).

Our results demonstrated that the AML patient group had considerably greater levels of *lncRNA TUG1* expression than the control group (*P* 0.001), which suggests its role in AML pathogenesis. The results of our study are consistent with previously published studies that detected *lncRNA TUG1* upregulation in AML patients by using SYBR Green RT-qPCR [20, 34–36]. These reported results were supported by studies suggesting that the overexpression of the *lncRNA TUG1* in AML was associated with increased cell proliferation, migration, and invasion, while knockdown of *TUG1* might suppress AML development [34, 37].*lncRNA ZEB2-AS1* is an oncogene that was known to be associated with human cancers such as lung cancer and hepatocellular carcinoma [32, 38], but there are limited research studies about its role in AML. Guan et al. [39] detected that *lncRNA ZEB2-AS1* was upregulated in bone marrow bulk cells from AML cases compared to control by using SYBR Green RT-qPCR and also reported that

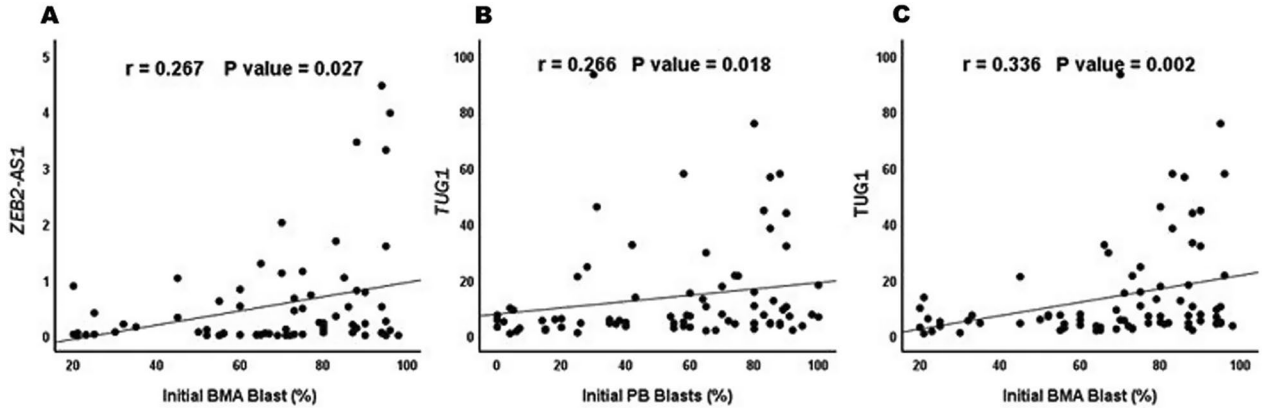

Fig. 5 Correlation of **a** *lncRNA ZEB2-AS1* with initial BMA blast: **b** *lncRNA TUG1* with initial PB blast: **c** *lncRNA TUG1* with initial BMA blast

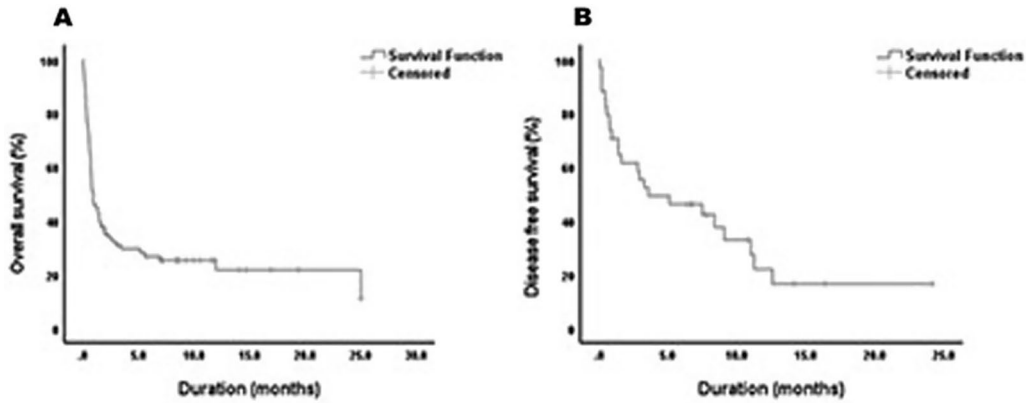

Fig. 6 Kaplan–Meier curve for **a** overall survival; **b** disease-free survival of the studied patients

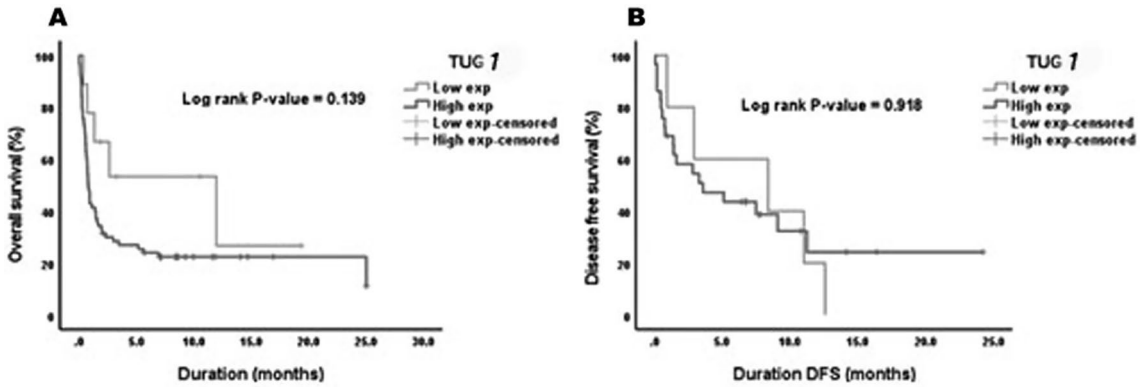

Fig. 7 Kaplan–Meier analysis for **a** overall survival; **b** disease-free survival according to *lncRNA TUG1* expression

lncRNA ZEB2 AS1 overexpression has been correlated with a reduced survival rate in AML cases. Additionally, AML cells' growth was also inhibited, and their rate of apoptosis was enhanced when the *lncRNA ZEB2 AS1* was silenced. Similarly, Shi et al. [21] demonstrated that *lncRNA ZEB2-AS1* expression was higher in bone marrow bulk cells from AML cases than in the control group ($P < 0.001$), as high *lncRNA ZEB2-AS1*expression was detected only in 13 patients out of sixty-two patients.

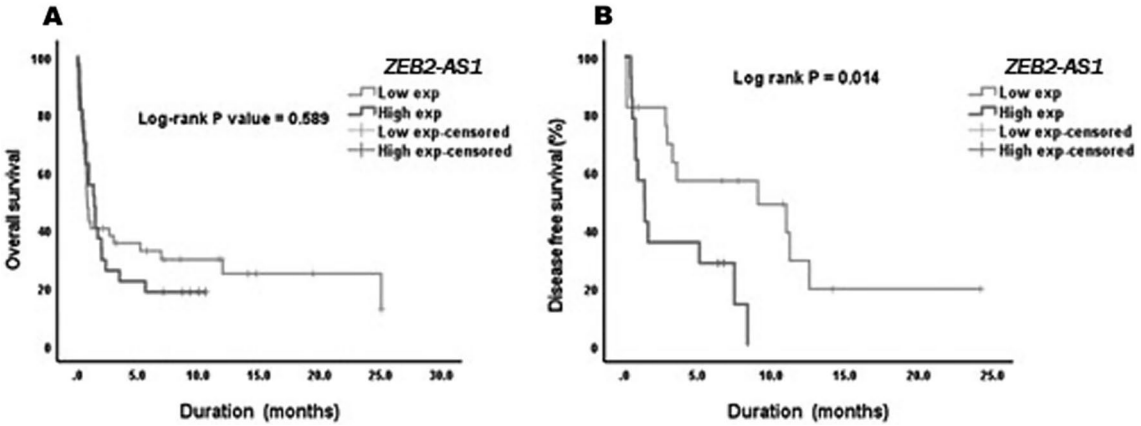

Fig. 8 Kaplan–Meier analysis for **a** overall survival; **b** disease-free survival according to *lncRNA ZEB2-AS1* expression

Contrary to studies that support the oncogenic role of the *lncRNA ZEB2-AS1*, the current study demonstrated significantly lower *lncRNA ZEB2-AS1* expression in acute myeloid leukemia cases than in the normal control group ($P<0.001$). However, the *lncRNA ZEB2-AS1* expression and the initial BMA blast revealed a significant positive association ($P=0.027$). The discrepancy between our results and the results of other studies may be due to ethnic differences, a difference in sample sizes, or different techniques. Also, the precise mechanism of *lncRNA ZEB2-AS1* upregulation in AML cases and its role in the pathophysiology of the illness are still unclear.

This study revealed a significant positive relationship among *lncRNA ZEB2-As1* and *lncRNA TUG* expression levels in AML cases ($P<0.001$), as cases with high *ZEB2-AS1* also showed increased expression of *TUG1.*

The present study revealed a significant difference among high and low *lncRNA TUG1* expression groups regarding age ($P=0.053$), but no significant variation had been reported regarding gender ($P=0.301$). A study performed by Wang et al. [20] revealed non-significant differences between high and low *lncRNA TUG1* expression groups regarding age and gender. Furthermore, no variations were observed among low and high *lncRNA ZEB2-AS1* expression groups regarding age ($P=0.581$) and gender ($P=0.862$). Similar findings were reported by Shi et al. [21].

Regarding laboratory findings, our results demonstrated a significantly higher median initial BMA blast (76%) in studied cases with high *lnc RNA TUG1* expression than patients with low expression (64%) ($P=0.034$). While there were no significant differences observed regarding initial (TLC, hemoglobin, platelets, and PB blasts), FAB subtypes ($P=0.529$), *FLT-ITD* and *NPM* mutation. Li and Wang [35] also reported no relationship among high and low lncRNA *TUG1* expression groups and total leukocytic count, hemoglobin level, platelet count, FAB subtypes, FLT-*ITD*, and *NPM* mutation. However, Wang et al. [20], found that the patients with higher *lncRNA TUG1* expression showed a high white blood cell count.

This study revealed no significant differences between low and high *lncRNA ZEB2-AS1* expression groups regarding initial (TLC, hemoglobin, platelets, PB blasts, BM blasts, and cellularity), FAB subtypes ($P=0.778$), *FLT-ITD* and *NPM* mutation. Contrary to our results, Shi et al. [21] detected significant differences among high and low *lncRNA ZEB2-AS1* expression groups regarding initial TLC ($P=0.046$), initial platelets ($P=0.044$), and FAB subtypes ($P=0.006$).

As a predictor of treatment response, we analyzed the percentage of bone marrow aspirate blast at days 14 and 28 after chemotherapy in relation to low and high *lncRNA TUG1*and *lncRNA ZEB2-AS1* expression groups. Our results demonstrated no statistically significant difference between both groups. In contrast with our finding, Wang et al. [20] reported that *LncRNA TUG1* high expression was associated with a reduced chance of achieving CR ($P=0.001$). Also, Shi et al. [21] observed that the CR rate was lower in the studied AML cases with high *lncRNA ZEB2 AS1* expression compared to low expression subgroup ($P=0.031$).

Regarding the association between *lncRNA TUG1* expression levels and AML outcomes, there was no significant difference between the *lncRNA TUG1* high and low expression groups and the OS and DFS. On the other hand, Li and Wang [35] found that the median DFS and OS in the AML studied cases with high *lncRNA TUG1* expression were shorter than in cases with low *lncRNA TUG1* expression ($P<0.001$, $P=0.002$, respectively). This discrepancy in the results may be attributed to different treatment protocols.

According to our findings, DFS was considerably better in AML cases with lower *lncRNA ZEB2-AS1* expression than it was in those with higher expression ($P=0.014$), while OS did not significantly differ between high and low *lncRNA ZEB2-AS1* expression groups ($P=0.589$). However, Shi et al. [21] demonstrated that AML patients with high *lncRNA ZEB2-AS1* expression had shorter OS ($P=0.036$) and lower DFS rates ($P=0.039$) compared to those with low expression.

Conclusion

In conclusion, for Egyptian adult AML patients, *lncRNA TUG1* overexpression may serve as a diagnostic biomarker, while high levels of the *lncRNA ZEB2-AS1* are associated with lower survival rates and an increased risk of relapse. Further research is recommended on a larger number of cases with different ethnic groups for accurate assessment of the diagnostic and prognostic value of *lncRNA TUG1* and *lncRNA ZEB2-AS1* expression and to understand the molecular pathways linking these long non-coding *RNAs* to leukemia progression in order to target them for therapeutic benefit. Finally, a longer follow-up duration is recommended for better assessment of OS and DFS.

Abbreviations

AML	Acute myeloid leukemia
TUG1	Taurine-upregulated gene1
ZEB2-AS1	Zinc finger E-box binding homeobox 2 antisense RNA1
Ct	Cycle threshold
DFS	Disease-free survival
OS	Overall survival
RQ	Relative quantitation
AUC	Area under the curve
BM	Bone marrow
EMT	Epithelial–mesenchymal transition

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None.

Author contributions

AMA, SMD, HMK, and RN put the idea, study design, and supervised the work. RN shared in the molecular work and data collection. MNA collected the data, performed the molecular work, and drafted the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Data analyzed in the current work are available from the corresponding author upon a reasonable request.

Declarations

Ethics approval and consent to participate

The study was approved by the Benha University research ethics committee, and the included patients gave the consent to participate.

Consent for publication

Not applicable.

Competing interests

None.

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