Am J Blood Res 2023;13(3):84-93
www.AJBlood.us /ISSN:2160-1992/AJBR0149882

Original Article

Expression of long non-coding RNA UCA1 and its clinical relevance in paediatric acute myeloid leukemia

Christine Wilson[1*], Diwakar Sharma[1*], Priyanka Swaroop[2], Sachin Kumar[1], Sameer Bakhshi[1], Surender K Sharawat[1]

[1]Department of Medical Oncology, All India Institute of Medical Sciences, New Delhi, India; [2]Department of Paediatric Surgery, All India Institute of Medical Sciences, New Delhi, India. [*]Equal contributors.

Received February 28, 2023; Accepted June 15, 2023; Epub June 15, 2023; Published June 30, 2023

Abstract: The underlying mechanisms and clinical significance of long non-coding RNA (lncRNA) urothelial cancer associated 1 (UCA1) is largely unknown in acute myeloid leukemia (AML). We aimed to study the expression of lncRNA UCA1, and its biological and clinical relevance in AML. Expression of lncRNA UCA1 was quantified in peripheral blood (PB) samples of children with *de novo* AML (n=69), post-induction, after achieving complete remission (CR) (n=8), and in patients who had relapsed (n=10). Additionally, two external cohorts were analysed i.e., TCGA-LAML dataset and Leukemia-MILE study. We also quantified expression in four different AML cell lines and analysed expression after cell differentiation. A consistent pattern of low UCA1 expression in AML was observed in our cohort of sixty-nine patients at baseline ($P < 0.0001$) and in the TCGA and Leukemia-MILE datasets. In patients who achieved remission, expression was comparable to healthy individuals, while relapsed patients interestingly had lower levels of UCA1 ($P=0.0002$). Furthermore, lncRNA UCA1 expression was significantly lower in AML cell lines (THP-1, $P=0.0112$; KG-1, $P=0.0168$; and HL-60, $P=0.0112$) and increased when THP-1 cells were differentiated ($P=0.0001$). In our AML patient cohort, lower expression was significantly associated with CR ($P=0.043$), however, the impact on survival (EFS and OS) was not significant. This is the first study wherein the lncRNA UCA1 expression was studied in various AML cell lines along with AML patients at baseline, remission and relapse. In conclusion, we found that UCA1 is significantly downregulated in AML compared to healthy individuals and mature differentiated cells.

Keywords: AML, long non-coding RNA, UCA1, tumor suppressor gene, differentiation

Introduction

Acute myeloid leukemia (AML) results from accumulation of immature myeloid cells or blast cells in the bone marrow and peripheral blood, thereby compromising the normal functioning of the hematopoietic system. Aberrations like chromosomal translocations or mutations that disrupt the normal molecular circuitry leads to the activation of genes involved in cell proliferation and avoidance of leukemic cell death. The current treatment modality for children with AML is intensive chemotherapy which poses significant toxicity, therefore there is a pressing need to explore for better diagnostic and therapeutic biomarkers and understanding of molecular pathobiology of the disease [1, 2].

Knowledge about the biology of paediatric AML pathogenesis is majorly limited to genes that encode proteins, such as transcription factors and signalling molecules [3]. With advancements in the approaches for analysis of big data such as those from transcriptomic studies, it is apparent that a class of genes that were previously considered “junk” genes and contributed to “transcriptional noise”, are in fact dysregulated across several cancers. Remarkably, functional assays that perturb these non-coding genes result in significant effects on leukemia cell phenotypes such as their proliferation, apoptosis rate and their ability to differentiate [4].

One class of non-coding genes gives rise to transcripts termed “long non-coding RNAs” (lncRNAs) due to their size which is greater than 200 nucleotides in length. In recent years, there has been an effort to uncover the functional relevance of lncRNAs in the context of disease biology, which has been very fruitful

leading to the discovery of several lncRNAs that have important roles in the development of cancers such as MALAT1 in prostate, breast and gastric cancer, H19 in breast and liver cancers, and the lncRNA UCA1 in bladder cancer [5].

UCA1 (Urothelial cancer associated 1) is a lncRNA that was first discovered as a bladder carcinoma-specific gene, being absent in normal bladder and other tissues such as liver, muscle, kidney, pancreas, etc. It was found to have high specificity and sensitivity, thereby being a potential non-invasive biomarker for bladder cancer [6]. In 2008 the same group demonstrated that UCA1 acts in an oncogenic fashion in bladder cancer to promote cell growth and invasion [7]. There is significant evidence that points to UCA1 as an oncogenic lncRNA, being upregulated in cancers such as liver, gallbladder, ovarian, glioma, melanoma, etc. [5]. Due to the high evidence of UCA1 derailed expression, we used a top-down approach to shortlist this lncRNA to be investigated in AML. Studies that have described UCA1 as an oncogenic lncRNA in AML demonstrate limited evidence through *in vitro* functional assays like cell proliferation assays, colony formation assays, and apoptosis assays [7-11, 12]. The analysis of UCA1 expression on patient samples has been performed on a cohort of small sample sizes (n=9 to n=27) at baseline or on patients who were already treated with ADR-based chemotherapy [9, 10].

This is the first detailed study wherein we evaluated the expression of UCA1 in paediatric patients of acute myeloid leukemia at baseline, remission, and after the recurrence of disease. Additionally, we studied the expression of lncRNA UCA1 in various AML cell lines. The current study has shown the dysregulated expression of UCA1 in paediatric patients with acute myeloid leukemia and various AML cell lines. The significantly low expression of UCA1 in comparison to healthy controls could possibly indicate its tumor suppressor potential. The current study has paved the way for further understanding of its biological and clinical potential in paediatric AML.

Methods

Patient recruitment

Peripheral blood (PB) samples from children with *de novo* acute myeloid leukemia registered in the medical oncology department of Dr. B.R.A. Institute Rotary Cancer Hospital (IRCH) between November 2017 and February 2021 were included in the study for collection of peripheral blood (n=69). Additionally, unpaired peripheral blood samples of paediatric patients in complete remission (n=8) and peripheral blood samples of paediatric patients at relapse (n=10) were collected for the study. Peripheral blood samples from age-matched healthy individuals were included in the control group (n=19). We excluded patients diagnosed with acute promyelocytic leukemia (APL)/AML M3, secondary AML, biphenotypic leukemia, and Insufficient PB sample, granulocytic sarcoma without marrow involvement and adult AML patients. Institute ethical clearance was obtained for this study and informed consent was taken from the legal guardians of all participants. The treatment regimen provided was uniform and according to the institutional protocol, as previously described [13].

Erythrocyte lysis of PB samples

About 5 ml of peripheral blood was collected from patients as well as healthy individuals in EDTA vials (DBO Phlecocuum®, Indore, India), following which whole blood lysis was performed using red blood cells lysis buffer (155 mM NH_4Cl, 12 mM $NaHCO_3$, 0.1 mM EDTA). The remaining cells were washed with 1X PBS and resuspended in TRI Reagent® (Sigma-Aldrich®, MO, USA).

Cell culture and cell differentiation

Four human AML cell lines (THP-1, KG-1, HL-60, and MOLM-13) were cultured in RPMI-1640 medium (Gibco, ThermoFisher Scientific, Inc., Waltham, MA), supplemented with 10% fetal bovine serum (Gibco) and L-glutamine (2 mM; Gibco), with 100 U/mL penicillin and 100 U/ml streptomycin (Gibco) added. The cells were cultured at 37°C, with 5% CO_2 in a humidified incubator (ThermoFisher Scientific, Inc., MA, USA).

To differentiate THP-1, the cells were grown in 6 well plates along with 10 ng/mL of Phorbol-12-myristate-13-acetate (Sigma-Aldrich®, MO, USA) and allowed to attach to the cell surface, following which they were collected by trypsinization after a period of 24 hours. We validated the differentiation microscopically as well as by flow cytometry (Becton, Dickinson and Company, New Jersey, USA) by assessing the expression of the monocyte cell surface

proteins CD11b and CD14 (Becton, Dickinson and Company, New Jersey, USA).

Isolation of RNA, cDNA synthesis, and quantitative PCR (qPCR)

Total cellular RNA was isolated using TRI Reagent® (Sigma-Aldrich®, MO, USA) by the phenol-chloroform and isopropanol method. RNA concentration was measured using a NanoDrop (ThermoFisher Scientific, Inc., MA, USA). A total of 1.2 µg RNA was reverse transcribed into cDNA using high-capacity reverse transcriptase (Invitrogen, ThermoFisher Scientific, Massachusetts, USA). The Expression of UCA1 was quantified using the TaqMan™ Universal PCR Master Mix (ThermoFisher Scientific, Inc., MA, USA) and gene expression assay (Hs01909129_s1) (ThermoFisher Scientific, Inc., MA, USA) on the LightCycler 480 system (Roche, Basel, Switzerland). We used 18S (Hs03003631_g1, ThermoFisher Scientific) as the internal reference for quantification. UCA1 primer sequences are as follows: UCA1 forward primer: GACCCTCATCTCTTAAGACCTGCC; UCA1 reverse primer: GAGAGTAGGCTTGAGGACACCATG; 18S forward primer: CGATGCTCTTAGCTGAGTGTCC; 18S reverse primer: GTCCTATTCCATTATTCCTAGCTGCG.

The PCR conditions included a Uracil-DNA glycosylase (UNG) hold, at 50 degrees Celsius for 2 minutes, a polymerase activation step at 95 degrees Celsius, for 2 minutes, with 50 cycles of a denaturation step at 95 degrees Celsius for 1 second, and an annealing/extension step at 60 degrees for 20 seconds. This was followed by a final cooling step at 40 degrees Celsius for 30 seconds. Calculation of relative gene expression was performed by the comparative C_T method [14].

Statistical analysis

Expression of UCA1 in patient samples was depicted as median values with an interquartile range. We used the Mann-Whitney U test for comparison between AML and control groups. Expression analysis of UCA1 on cell lines was done using three biological replicates, and data are presented as mean ± standard error of mean (SEM). The student's *t*-test was performed for the analysis between two groups, and a *P*-value of < 0.05 was considered statistically significant. Where more than two groups were involved, comparisons were made using ANOVA followed by Dunn's Multiple Comparison Test for post hoc analysis. A *P*-value of < 0.05 was considered significant. For survival analysis, the patients who did not achieve pathological complete remission (5% bone marrow blast) after one or two cycles of induction chemotherapy were defined as refractory diseases. We defined event-free survival (EFS) as the time between diagnosis and the first event (failure to achieve CR, relapse, or death). Overall survival (OS) was defined as the time between diagnosis and death or the last follow-up. Survival analysis was performed using the Kaplan-Meier method along with the log-rank test. All analysis was performed using GraphPad Prism version 8.0.0 for Windows, GraphPad Software, San Diego, California, USA.

Results

Baseline patient demographics and clinical details

During the study period, total 83 patients were evaluated, 14 were excluded, and 69 eligible were included for final analysis. The baseline demographic and clinical details are provided in **Table 1**. Briefly, the median age of children with AML was 10 years, with a higher proportion of males as compared to females. Core binding factor AML was the most prevalent cytogenetic abnormality, followed by normal karyotype and other cytogenetic aberrations. Among the molecular abnormalities, the FLT3 mutation was the most common. Patients were also classified into the FAB subtypes. There was also no patient characteristic at baseline which significantly associated with UCA1 expression (**Table 2**). Patients who were UCA1 high vs. UCA1 low (based on median expression) did not show any significant difference with respect to characteristics such as age, sex, hematological parameters (Hb, TLC and platelet counts), as well as the percentage of peripheral blood and bone marrow blasts (**Table 2**).

UCA1 expression was downregulated in paediatric patients with AML

The long non-coding RNA UCA1 is reported to be aberrantly expressed in several cancers. To evaluate the expression pattern of lncRNA UCA1 in AML, we used the GEPIA web application to access gene expression levels from the TCGA LAML dataset which has data from bone marrow samples of 173 patients and 70 heal-

Table 1. Baseline characteristics of paediatric patients with AML (n=69)

Characteristics	Median (range/percentage)
Median age (years)	10 (0.5-18)
Sex	
Male	37 (53.62)
Female	32 (46.37)
Hematological parameters	
Median haemoglobin, (g/dL)	7.2 (2.5-13.3)
Median total leukocyte count, ($\times 10^3$/μL)	35 (1-290)
Median platelet count, ($\times 10^3$/μL)	25.4 (1.1-36.42)
Cytogenetics (n=53)	
Normal	10 (18.86)
t(8;21) & inv (16)	30 (56.60)
Complex karyotype	3 (5.66)
Other	6 (11.32)
Failed Cytogenetics	4 (7.5)
Molecular Analysis (n=59)	
FLT3 (*ITD/TKD*)	11 (18.64)
CBFB-MYH11	6 (10.16)
NPM1	2 (3.3)
Negative	40 (67.7)
AML Subtype (n=63)	
M0/M1	17 (26.98)
M2	29 (46.03)
M4	9 (14.28)
M5	7 (11.11)
M6	-
M7	1 (1.58)
PB Blasts (n=63)	50 (8-98)
BM Blasts (n=65)	66 (8-98)

thy controls. UCA1 was significantly downregulated in patient samples, relative to healthy controls (**Figure 1A**). We also analyzed the expression of UCA1 from the GSE13159 dataset which was generated by the leukemia MILE study using the BloodSpot database for visualization of gene expression and found a significantly lower level of expression in various AML subtypes such as CBF-AML, MLL rearranged AML, etc. compared to normal bone marrow (**Figure 1B**). Taking these findings forward, we quantified the expression of UCA1 in our cohort of 69 peripheral blood samples from paediatric AML patients before treatment along with 19 healthy individuals. The expression levels were also quantified in PB samples from patients who had achieved complete remission (CR) (n=8) and from patients who relapsed (n=10). On comparing the expression of UCA1 between AML patients and healthy controls, we obtained similar findings to that of our analysis in the TCGA-LAML cohort, where there was a significant reduction ($P < 0.0001$) of UCA1 expression in the peripheral blood of patients with AML (**Figure 1C**). Among patients who achieved complete remission, the expression of UCA1 was comparable to healthy controls, while patients who relapsed had a significantly lower expression of UCA1 relative to healthy individuals (P=0.0002) (**Figure 1C**).

UCA1 expression is downregulated in AML cell lines

To study UCA1 *in vitro,* we cultured four AML cell lines, each harbouring specific chromosomal translocations and molecular aberrations, and quantified its expression in each cell line. UCA1 was downregulated significantly in THP-1 (P=0.0112), KG-1 (P=0.0168), and HL-60 (P=0.0112), with a fold change of 0.029, 0.12, and 0.29, respectively. However, no significant differences were observed in the expression of lncRNA UCA1 in the MOLM-13 cell line (**Figure 2**).

UCA1 expression is associated with mature and terminally differentiated cells

Since the expression of UCA1 was found to be low in patients with AML and AML cell lines while having high expression in normal bone marrow and peripheral blood, we hypothesised that UCA1 could potentially have a tumor suppressor role, and associated with mature/differentiated cells. We asked whether the expression of UCA1 would change if we induce cell differentiation in THP-1 cells, which leads these cells to a terminally differentiated state. We treated THP-1 cells with phorbol-12-myristate-13-acetate (PMA), which induces their terminal differentiation. We validated phenotypically and characterized the differentiated cells by quantifying changes in levels of genes associated with differentiation i.e., CD11b and CD14, using flow cytometry. CD11b levels significantly

Table 2. Relationship of baseline characteristics with UCA1 expression

Characteristics	Median dCt (Interquartile Range)	*P*-value	UCA1 High (n)	UCA1 Low (n)	*P*-value
Median age (years)					
< 10 (32)	16.62 (6.36)	0.66	15	17	P=0.71
≥ 10 (37)	17.55 (5.20)		19	18	
Sex					
Male (37)	17.73 (5.14)	0.22	20	17	P=0.39
Female (32)	16.58 (5.54)		14	18	
Hematological parameters					
Median haemoglobin, (g/dL)					P=0.07
< 7.2 (34)	17.79 (5.21)	0.24	13	21	
≥ 7.2 (35)	16.00 (5.11)		21	14	
Median total leukocyte count, ($\times 10^3/\mu L$)					P=0.72
< 35 (34)	16.71 (7.72)	0.13	17	19	
≥ 35 (35)	16.99 (4.08)		16	19	
Median platelet count, ($\times 10^3/\mu L$)					P=0.39
< 25.4 (35)	17.46 (4.38)	0.57	15	19	
≥ 25.4 (34)	16.32 (6.35)		19	16	
PB Blasts					
< 50 (26)	17.90 (6.31)	0.79	11	15	P=0.70
≥ 50 (36)	16.45 (5.41)		17	19	
BM Blasts					
< 66 (26)	16.58 (4.56)	0.44	15	13	P=0.39
≥ 66 (37)	16.99 (5.58)		15	20	
Molecular Analysis (n=59)					
FLT3 (*ITD/TKD*)	16.82 (6.68)	0.02			
CBFB-MYH11	18.83 (3.66)				
NPM1	-				
Negative	15.43 (5.80)				
AML Subtype (n=63)					
M0/M1	18.22 (5.65)	0.21			
M2	15.30 (5.72)				
M4	17.55 (2.34)				
M5	15.01 (8.48)				
M6	-				
M7	-				

increased upon treatment with PMA (P=0.001), whereas CD14 expression showed a significant decrease (P=0.04) (**Figure 3A-D**). We also visualized the change in cellular phenotype upon PMA treatment i.e., adherence to the culture plate using light microscopy (**Figure 3E**). Once THP-1 differentiation was validated phenotypically and with molecular markers, we collected differentiated cells and performed qPCR analysis for the expression of UCA1. Interestingly, we found a 15-fold increase in UCA1 expression upon cellular differentiation (P=0.0001) (**Figure 3F**).

UCA1 expression and patient survival outcome

The expression of UCA1 was analysed in patients who achieved complete remission (79.10%) vs. refractory (20.89%), and a significant difference was observed in the expression levels between these two groups (P=0.0431) (**Table 3**). In addition, patients were categorized into UCA1 high and low-expression groups. No significant event-free and overall survival difference existed amongst the high and low UCA1 expression groups (**Table 4**; **Figure 4A**, **4B**).

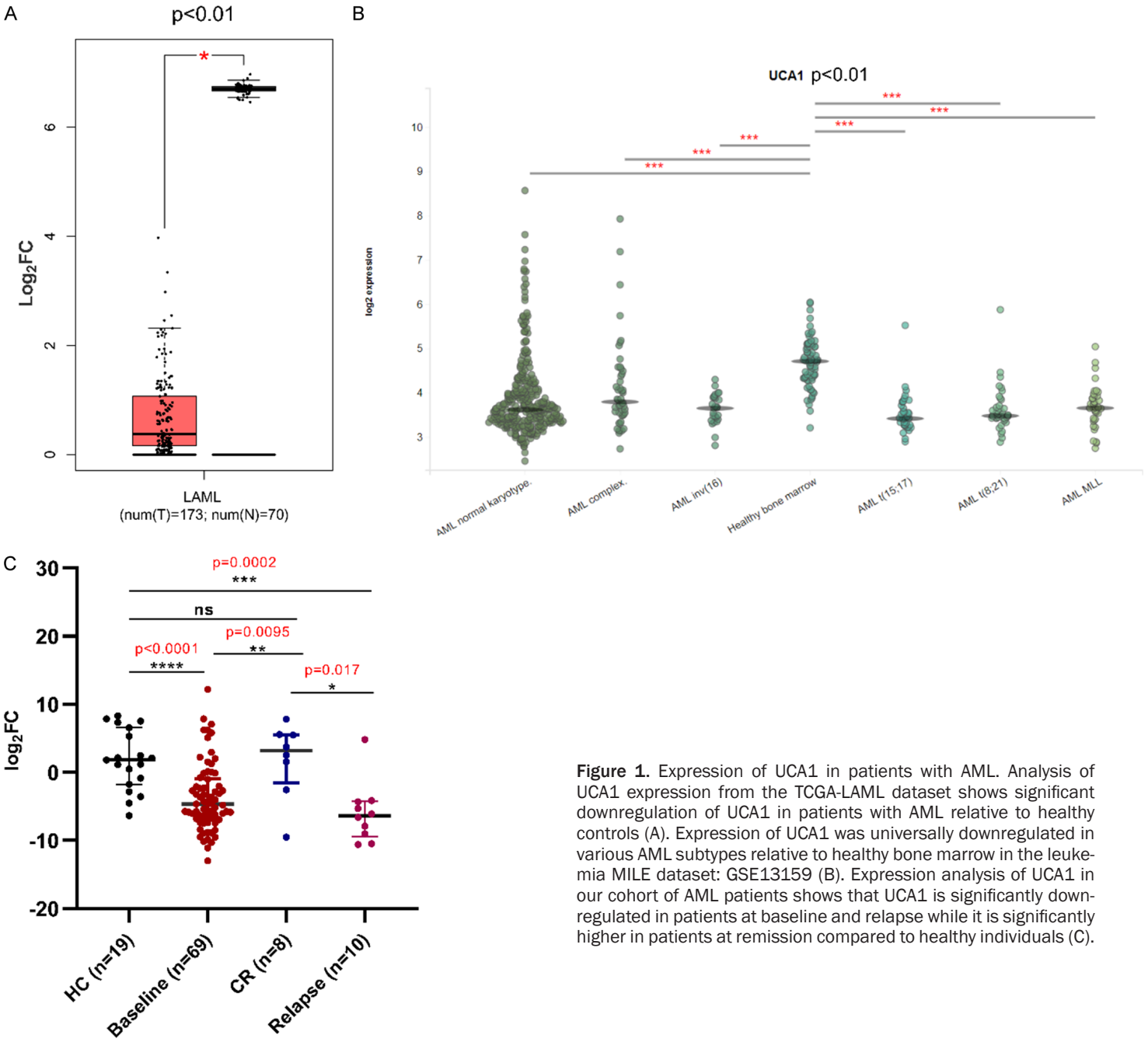

Figure 1. Expression of UCA1 in patients with AML. Analysis of UCA1 expression from the TCGA-LAML dataset shows significant downregulation of UCA1 in patients with AML relative to healthy controls (A). Expression of UCA1 was universally downregulated in various AML subtypes relative to healthy bone marrow in the leukemia MILE dataset: GSE13159 (B). Expression analysis of UCA1 in our cohort of AML patients shows that UCA1 is significantly downregulated in patients at baseline and relapse while it is significantly higher in patients at remission compared to healthy individuals (C).

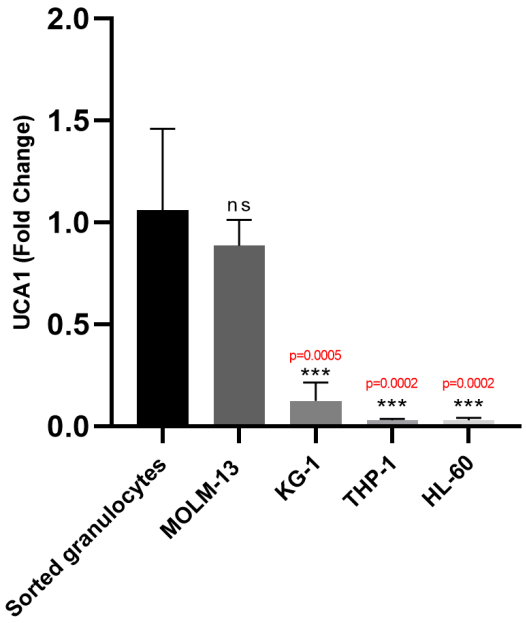

Figure 2. Expression of UCA1 in AML cell lines. In the acute myelogenous leukemia cell line KG-1, UCA1 was downregulated significantly (P=0.0005). A similar pattern of downregulated expression was observed in the monocytic leukemia cell line THP-1 which harbours the MLL-rearrangement (P=0.0002), and in the acute promyelocytic leukemia cell line HL-60 (P=0.0002). Although there was a decrease in UCA1 expression in the MOLM-13 cell line, it was not of significance.

Discussion

The lncRNA UCA1 was first identified as an oncogenic non-coding RNA and linked to the development of urothelial cancer [6]. Several studies have shown that UCA1 is majorly oncogenic, and reducing the levels of UCA1 in various cancer cell lines leads to a reduction in oncogenic phenotypes such as proliferation, migration, and invasion, accompanied by an increase in apoptosis [15]. In the context of AML, a few studies report the upregulation of UCA1 in AML compared to healthy controls and describe an oncogenic role for the lncRNA based on *in vitro* assays such as proliferation assays, migration, invasion, and apoptosis assays [8, 12, 16]. However, the quantification of UCA1 expression in these studies has been performed on a cohort of limited sample size, with the largest cohort being twenty-seven samples [10].

In this current study, we first performed an *in-silico* analysis of UCA1 expression and found that in the TCGA LAML and Leukemia MILE study datasets which contain expression data from a large number of patients (n=173 and n=2096 respectively), UCA1 expression was significantly lower in AML than in healthy controls. Additionally, the reduced expression in AML was universal to all major cytogenetic subtypes, indicating that UCA1 is potentially part of a molecular network that is affected during leukemogenesis in general. This prompted us to analyse our own cohort of patients, and we discovered that similar to the publicly available datasets, UCA1 was significantly downregulated in our data as well (n=69). Since UCA1 is aberrantly expressed in patients before treatment, we then evaluated if expression levels of lncRNA UCA1 change after treatment and on achieving complete remission. Interestingly, UCA1 expression in patients who were in remission was significantly higher than those at baseline and comparable to expression levels of healthy individuals. Additionally, patients who relapsed had significantly lower expression compared to patients in remission and also healthy controls. This pattern indicates that UCA1 is possibly required in mature/differentiated cells, and low expression of UCA1 might be associated with the molecular mechanisms driving leukemogenesis. Keeping this in perspective, we used the THP-1 cell line to model cellular differentiation by treating cells with PMA to induce a terminally differentiated state and activate the molecular pathways involved in the process of differentiation. Once differentiated, cells were profiled for their levels of UCA1, and consistent with our hypothesis, we found that UCA1 expression increases when THP-1 cells are treated with PMA, indicating that the expression of lncRNA UCA1 is induced upon cell differentiation, and required for mature cells, while cells with lower levels of UCA1 are those that are immature or undifferentiated. We have also found significantly low levels of UCA1 expression in three AML cell lines that were cultured compared to mature, healthy cells. Our cell lines harboured various cytogenetic abnormalities to justify the wide spectrum of AML molecular heterogeneity. Further highlighting that the low levels of UCA1 in AML were consistent, irrespective of the kind of molecular aberration the cells harbours.

We also found that among patients who achieved CR, the expression of UCA1 was significantly higher than in refractory patients of AML. Further strengthening our hypothesis for

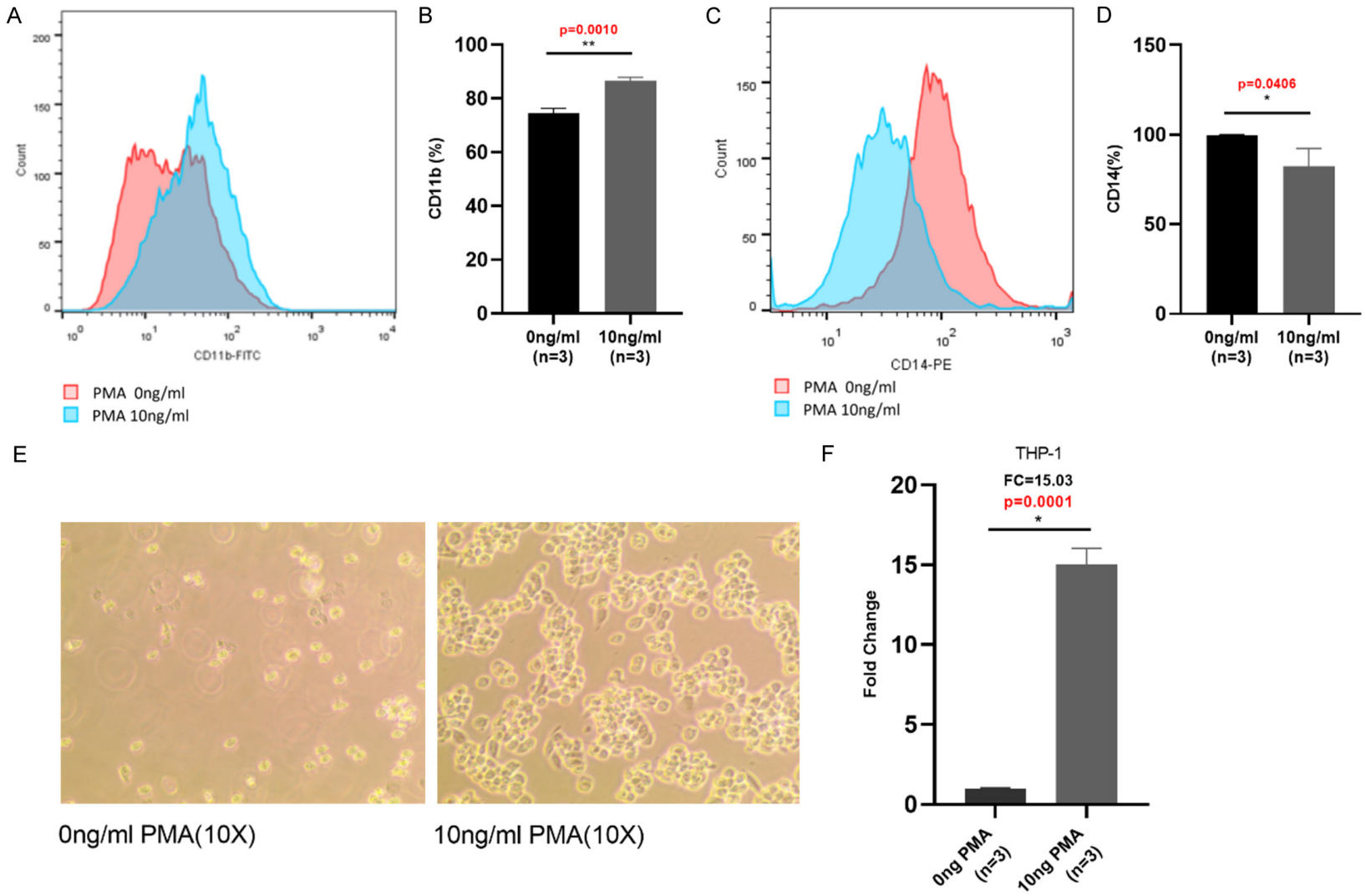

Figure 3. Expression of UCA1 in THP-1 cells differentiated with 10 ng/mL PMA. Flow cytometric analysis post treatment with PMA shows an increase in CD11b indicative of differentiated cells (A), the same was quantified and the increase was found to be significant (P=0.001) (B). Flow cytometric analysis post PMA treatment, shows a significant decrease in CD14 expression indicative of differentiated cells (P=0.04) (C, D). Microscopic images captured after treatment of THP-1 cells with PMA shows adherence of cells which were previously in suspension, to the culture plate, and change in morphology (E). Differentiated THP-1 cells had a 15.03-fold increase in UCA1 expression which was statistically significant (P=0.0001) (F).

Table 3. Relationship of UCA1 expression with outcome

Status	UCA1 Expression (Interquartile range)	*P* value
Complete Remission		
Achieved: n=53 (79.10%)	16.19 (6.21)	0.0431
Failed to achieve: n=14 (20.89%)	18.40 (3.98)	

Table 4. Impact of UCA1 expression on event free survival (EFS) and overall survival (OS)

Event free survival (EFS)		Overall Survival (OS)	
Median survival		Median survival	
Low Expression: 11.15 months		Low Expression: 16.18 months	
High Expression: 13.05 months		High Expression: 21.47 months	
Hazard Ratio (95% CI)	*P*-value	Hazard Ratio (95% CI)	*P*-value
1.38 (0.76-2.49)	0.28	1.147 (0.62-2.10)	0.65

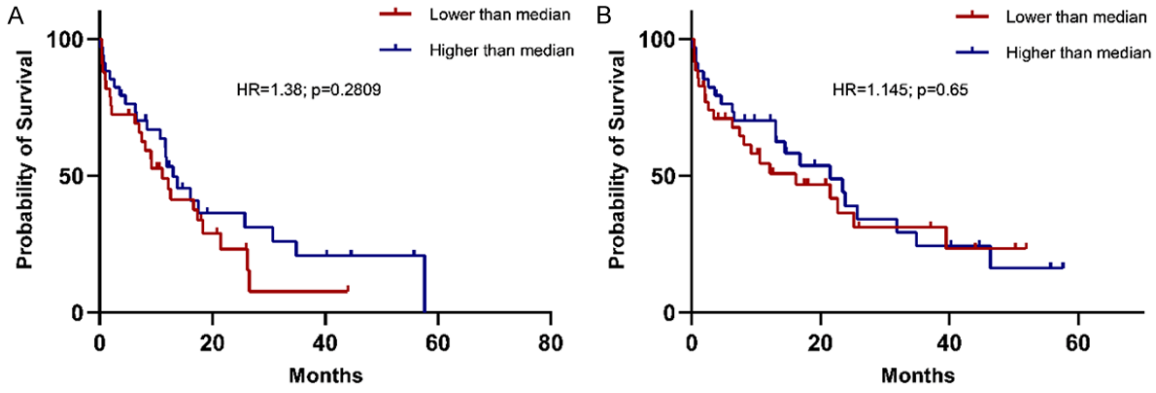

Figure 4. Survival outcome. Kaplan Meier curves showing EFS (A), OS (B) for AML patients comparing those patients who show high expression of UCA1 vs. those that show low expression of UCA1 at baseline.

the potential role of UCA1 in mature/healthy cells and the involvement of this lncRNA in pathways required for leukemogenesis.

The strength of our study lies in the analysis of UCA1 expression which was performed on a large number of samples from patients with AML, which is a first of its kind. The expression pattern observed in our study is consistent with those identified in the LAML and Leukemia MILE datasets. Moreover, we have extended our analysis to include cell lines with distinct molecular and cytogenetic abnormalities, revealing a comparable pattern of expression both *in vivo* and *in vitro*. The evolutionary conservation of the UCA1 sequence in primates suggests its potential functional significance. Our study, therefore, points to a potential tumor suppressive role of UCA1 and it would be interesting to delineate the functional mechanism of this lncRNA using detailed *in vitro* and *in vivo* work which is a limitation of this study. Expanding the cohort of acute myeloid leukemia (AML) patients under investigation would have augmented the robustness of our results. Further dissection of the data into subsets categorized by cytogenetic subgroups would have facilitated the identification of putative biomarkers associated with UCA1 expression. Our expression profiling analyses and preliminary *in vitro* investigations suggest a potential role for UCA1 as a tumor suppressor. The functional mechanisms underlying the involvement of UCA1 in AML pathogenesis warrant further detailed *in vitro* and *in vivo* studies. Additionally, the molecular and cellular consequences of UCA1 downregulation in AML require further elucidation through mechanistic illustrations.

In summary, UCA1 is a long non-coding RNA that is thought to have an oncogenic role in several cancers, however, we have shown that its expression is significantly lower in patients with AML compared to healthy individuals. This was

observed in data from two public data sets, our cohort of 69 patients, as well as in our cell lines. Additionally, the expression of UCA1 is high in patients who achieve remission similar to the expression levels in healthy individuals, and patients who relapsed had low expression of lncRNA UCA1, similar to *de novo* AML patients. This indicates that UCA1 is required for healthy and normal cells, and via mechanisms yet unknown, the levels in AML are downregulated. More *in vitro* and *in vivo* work is required to gauge the functional role of the lncRNA UCA1.

Acknowledgements

The authors acknowledge and are thankful for funding from DST-SERB (Department of Science and Technology - Science and Engineering Research Board), CSIR (Council of Scientific and Industrial Research; for Fellowship), Government of India, and the All India Institute of Medical Sciences, intramural research grant for this work.

Disclosure of conflict of interest

None.

Address correspondence to: Drs. Surender K Sharawat and Sameer Bakhshi, Department of Medical Oncology, Dr. B.R.A. IRCH, All India Institute of Medical Sciences, New Delhi 110029, India. E-mail: sksharawat@gmail.com (SKS); sambakh@hotmail.com (SB)

References

[1] Conneely SE and Stevens AM. Acute myeloid leukemia in children: emerging paradigms in genetics and new approaches to therapy. Curr Oncol Rep 2021; 23: 16.

[2] de Rooij JD, Zwaan CM and van den Heuvel-Eibrink M. Pediatric AML: from biology to clinical management. J Clin Med 2015; 4: 127-149.

[3] Elgarten CW and Aplenc R. Pediatric acute myeloid leukemia: updates on biology, risk stratification, and therapy. Curr Opin Pediatr 2020; 32: 57-66.

[4] Bhat AA, Younes SN, Raza SS, Zarif L, Nisar S, Ahmed I, Mir R, Kumar S, Sharawat SK, Hashem S, Elfaki I, Kulinski M, Kuttikrishnan S, Prabhu KS, Khan AQ, Yadav SK, El-Rifai W, Zargar MA, Zayed H, Haris M and Uddin S. Role of non-coding RNA networks in leukemia progression, metastasis and drug resistance. Mol Cancer 2020; 19: 57.

[5] Qian Y, Shi L and Luo Z. Long non-coding RNAs in cancer: implications for diagnosis, prognosis, and therapy. Front Med (Lausanne) 2020; 7: 612393.

[6] Wang XS, Zhang Z, Wang HC, Cai JL, Xu QW, Li MQ, Chen YC, Qian XP, Lu TJ, Yu LZ, Zhang Y, Xin DQ, Na YQ and Chen WF. Rapid identification of UCA1 as a very sensitive and specific unique marker for human bladder carcinoma. Clin Cancer Res 2006; 12: 4851-4858.

[7] Wang F, Li X, Xie X, Zhao L and Chen W. UCA1, a non-protein-coding RNA up-regulated in bladder carcinoma and embryo, influencing cell growth and promoting invasion. FEBS Lett 2008; 582: 1919-1927.

[8] Li J, Wang M and Chen X. Long non-coding RNA UCA1 modulates cell proliferation and apoptosis by regulating miR-296-3p/Myc axis in acute myeloid leukemia. Cell Cycle 2020; 19: 1454-1465.

[9] Li JJ, Chen XF, Wang M, Zhang PP, Zhang F and Zhang JJ. Long non-coding RNA UCA1 promotes autophagy by targeting miR-96-5p in acute myeloid leukaemia. Clin Exp Pharmacol Physiol 2020; 47: 877-885.

[10] Liang Y, Li E, Zhang H, Zhang L, Tang Y and Wanyan Y. Silencing of lncRNA UCA1 curbs proliferation and accelerates apoptosis by repressing SIRT1 signals by targeting miR-204 in pediatric AML. J Biochem Mol Toxicol 2020; 34: e22435.

[11] Wang J, Uddin MN, Hao JP, Chen R, Xiang YX, Xiong DQ and Wu Y. Identification of potential novel prognosis-related genes through transcriptome sequencing, bioinformatics analysis, and clinical validation in acute myeloid leukemia. Front Genet 2021; 12: 723001.

[12] Zhang Y, Liu Y and Xu X. Knockdown of LncRNA-UCA1 suppresses chemoresistance of pediatric AML by inhibiting glycolysis through the microRNA-125a/hexokinase 2 pathway. J Cell Biochem 2018; 119: 6296-6308.

[13] Chaudhary S, Ganguly S, Palanichamy JK, Singh A, Bakhshi R, Jain A, Chopra A and Bakhshi S. PGC1A driven enhanced mitochondrial DNA copy number predicts outcome in pediatric acute myeloid leukemia. Mitochondrion 2021; 58: 246-254.

[14] Schmittgen TD and Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc 2008; 3: 1101-1108.

[15] Ghafouri-Fard S and Taheri M. UCA1 long non-coding RNA: an update on its roles in malignant behavior of cancers. Biomed Pharmacother 2019; 120: 109459.

[16] Li J, Li Z, Bai X, Chen X, Wang M, Wu Y and Wu H. LncRNA UCA1 promotes the progression of AML by upregulating the expression of CXCR4 and CYP1B1 by affecting the stability of METTL14. J Oncol 2022; 2022: 2756986.