SNHG14 IncRNA as a tumor suppressor in adult non-M₆ AML patients; The diagnostic biomarker

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Abstract

Acute myeloid leukemia (AML) is one of the most common adult blood malignancies that engages hematopoietic myeloid progenitors. A large number of studies have shown the involvement of long non-coding RNAs (lncRNAs) in AML pathogenesis. This study aimed to investigate the expression profile of lncRNA SNHG14 and its role in adult non-M AML pathogenesis, clinical features, and diagnosis. We compared the expression level of SNHG14 by qRT-PCR technique between bone marrow (BM) samples obtained from 50 adult non-M AML patients and 49 healthy controls. We also investigate the correlation between clinicopathological characteristics of AML patients with low and high SNHG14 expression. The expression level of SNHG14 was significantly decreased in BM tissues of adult non-M AML patients compared with healthy controls. Patients with low expression levels of SNHG14 were associated with poor overall survival (OS), while no correlation was observed between low expression levels of SNHG14 and relapse free survival (RFS). Thus, our findings suggest that SNHG14 expression has the potential to be a biomarker for diagnosing adult non-M AML patients. Put together, our findings suggest that IncRNA SNHG14 could be used as a potential diagnostic biomarker and may provide insights into novel therapeutic targets in adult non-M AML patients.

1. Introduction

Acute myeloid leukemia (AML) is the most prevalent type of leukemia in adults and is defined by uncontrolled proliferation, apoptosis, and differentiation of hematopoietic cells in the bone marrow (BM) and other hematopoietic organs such as the spleen, liver, and blood [1]. Despite the existence of numerous therapeutic options, the majority of AML patients relapse and fail to achieve remission, and the prognosis remains unfavorable. Therefore, it is crucial to identify novel biomarkers that can be used to improve the diagnosis and prognosis of AML as well as create more efficient monitoring and treatment options [2].

Long non-coding RNAs (lncRNAs) are defined as non-protein-coding transcripts with more than 200 nucleotides. Accumulating evidence suggests that lncRNAs regulate a wide range of physiological processes such as immunity, inflammation, cell proliferation, differentiation, and survival [3, 4]. Besides their participation in normal physiology, it has been shown that lncRNAs play essential functions in tumor growth, such as lung cancer [5], breast cancer [6], multiple myeloma [7], and AML [8, 9]. The IncRNA SBF2-AS1 was up-regulated in AML and knockdown of SBF2-AS1 inhibited cell proliferation and promoted apoptosis in AML cells [10]. In addition, ANRIL was found to increase malignant cell survival and cell glucose metabolism to accelerate AML progression via the AMPK/SIRT1 signaling pathway [11]. Therefore, these studies suggest that lncRNAs exert key roles in tumor growth and might be utilized as diagnostic markers in cancer.

The small nuclear protein RNA host gene 14 (SNHG14) belongs to a group of long non-coding RNAs that is located on chromosome 15q11.2 [12]. Recent studies have shown that SNHG14 exerts oncogenic functions through tumor proliferation, migration, invasion, and chemical resistance in different types of
cancers, including non-small cell lung [13], cervical [14], gastric [14], and leukemia [15]. Wang et al. assessed the expression level of SNHG14 in the BM samples of patients with childhood AML. SNHG14 was found to be overexpressed in AML patient samples compared to healthy controls, suggesting that it might act as a carcinogenic lncRNA in AML. They revealed that silencing SNHG14 decreased the viability and increased the apoptosis rate of AML cells by regulating miR-193b-3p/MCL1 axis [15]. In a similar manner, Gamaleldin et al. found that the lncRNA SNHG14 was overexpressed in AML patients, implying that it has a role in AML development and can be used as prognostic biomarkers that provide a promising therapeutic target and follow-up for AML patients [16].

However, the molecular mechanisms of SNHG14 in many tumors have been studied, there is not sufficient information on SNHG14 in adult AML patients. Therefore, we decided to investigate SNHG14 in adult non-M3 AML patients to explore new diagnostic and therapeutic targets for AML patients.

2. Materials and Methods

2.1 Patients and specimens

Upon approval of the Medical Ethics Committee of Tarbiat Modares University, we recruited 50 diagnosed non-M AML patients (mean age ± SD, 41.27 ± 15.98 years; age range, 14–76 years; male: female ratio, 27:23) from Hematology/Oncology and Stem Cell Transplantation Research Center, Shariati Hospital, Tehran, Iran between June 2020 and June 2021. A total of 49 patient (mean age ± SD, 40 ± 12.32 years; age range, 20–74 years; male: female ratio, 20:29) with normal bone marrow morphology and without any malignancy history were participated as the control group. The clinical characteristics of adult non-m3 AML patients listed in Table I. All study subjects signed an informed consent.

AML was diagnosed according to World Health Organization (WHO) criteria and Frenche America British (FAB) classification [17, 18]. We excluded patients with acute promyelocytic leukemia (M3) and those with history of treatment with chemotherapy. Non-M3 AML patients were managed in accordance with the treatment protocol as follow: they took standard induction chemotherapy consisting of daunorubicin 60mg/m² for 3 days and cytarabine 100 mg/m² for 7 days. Patients reached complete remission (CR) received at least one course of 5 + 2 consolidation chemotherapy regimen including 100 mg/m² cytarabine daily for 5 days plus 60 mg/m² daunorubicin daily for 2 days. Seven individuals experienced allogeneic hematopoietic stem cell transplantation (HSCT) following first remission.

BM samples were collected from non-M3 AML patients into tubes containing EDTA anticoagulant. BM mononuclear cells (MNC) were isolated by density gradient centrifugation on Ficoll-Paque (GE Healthcare, New York, USA), and were stored in TRizol at -80°C until use for RNA extraction.

2.2 Quantitative real-time polymerase chain reaction analysis (qRT-PCR)
Total RNA was isolated from the bone marrow samples using TRIzol Reagent (Invitrogen) according to the manufacturer's instruction. RNA quantity and quality were assessed using a Nano Drop spectrophotometer. Then, isolated RNA was reverse transcribed into complementary DNA (cDNA) using the cDNA Synthesis Kit (SMOBIO) according to the manufacturer's protocol. qPCR was conducted on StepOnePlus Real-Time PCR System (Applied Biosystems) 40 cycles using SYBR Green Master Mix (Ampliqon). The reaction program was set under the following conditions: initial denaturation at 95 °C for 15 min, then 40 cycles with denaturation at 95 °C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s. All of the reactions were run in triplicate and GAPDH was used as the reference gene. The primer sequences were designed by AlleleID6 as follows:

SNHG14 forward 5′-CGGACCCTCGCTATGATGATATGG-3′ and reverse 5′-CTGAAGCTGTGATGCTAGGATGC-3′; GAPDH forward 5′-ACAGCCTCAAGATCATCAGCAATG-3′; and reverse 5′-GCCATCACGCCACAGTTTCC-3′.

The $2^{-\Delta\Delta C_{\text{q}}}$ method was used to calculate mRNA expression levels of each gene.

### 2.3 Statistical analysis

Statistical analyses were performed by GraphPad Prism® software version 9.0 (GraphPad Software, USA). The normal distribution of data was checked with the Kolmogorov-Smirnov test. Data were reported as median ± standard deviation (SD). Mann-Whitney U test was used to compare two independent groups with non-normal distribution. Pearson chi square (X2) analysis was performed to compare the association between two categorical variables. A Receiver operating characteristic curve (ROC) and area under the ROC curve (AUC) were used to assess the value of lnRNA SNHG14 to discriminate non-M3 AML patients from healthy controls. Kaplan-Meier method was performed to assess the overall survival (OS) and relapse-free survival (RFS). Ns indicates no significant difference, while data with P values < 0.05 were considered to be statistically significant.

### 3. Results

#### 3.1 Characteristics of the study subjects

In the control group, the mean age was 40 ± 12.32 years, with 59% females and 41% males. In AML patients’ group, the mean age was 15.98 ± 41.27 with 46% females and 54% males. In terms of FAB classification, 4%, 18%, 20%, 36%, 18%, and 4% of patients were classified as M0, M1, M2, M4, M5, and M6. According to molecular genetics testing, 42% and 12% of AML patients demonstrated internal tandem duplications in FMS-like tyrosine kinase 3 (FLT3-ITD) and Nucleophosmin 1 (NPM1), respectively.

#### 3.2 Correlation between SNHG14 expression and clinical/laboratory characteristics in patients with non-M3 AML

For investigating the influence of SNHG14 expression on clinical/laboratory characteristics, patients were divided into two groups based on median level of SNHG14 expression: High expression, SNHG14
expression level $\geq$ median and low expression, SNHG14 expression level < median. The link between SNHG14 expression and clinical characteristics of patients is shown in Table 1. No significant association was found between the expression level of SNHG14 gene and other data including age, gender, WBC counts, Plt count, Hb level, BM blasts, FAB subclassification, NPM1 and FLT3 gene mutations, and the rates of CR after induction therapy (Table 1). We noticed that patients in SNHG14 low group had a higher frequency of poor cytogenetic (52%) than patients in SNHG14 high group (20%).

### 3.3 The expression profile of SNHG14 in adult non-M₃ AML patients

In order to evaluate the expression level of SNHG14 in BM samples collected from non-M3 AML patients and healthy controls, qRT-PCR was employed. The results indicate that SNHG14 gene expression was significantly decreased in patients compared with controls ($p \leq 0.0001$; Fig. 1a). Furthermore, the expression level of SNHG14 was assessed in CR (complete remission) and NR (nonresponse) patients. The results indicated that the low expression of SNHG14 in CR and NR patients was statistically significant compared with control, but no significant difference was reported between the CR and NR groups (Fig. 1b). We found no difference in the expression level of SNHG14 before and after treatment (Fig. 1c).

### 3.4 SNHG14 expression as a diagnostic marker of non-M₃ AML

ROC curve analysis revealed that SNHG14 could discriminate AML patients from non-malignant controls (AUC: 7220/0, 95% CI: 0.583–0.860, $p \leq 0.01$), with a sensitivity of 66% and a specificity of 66% at the best cut-off point (Fig. 2).

### 3.5 SNHG14 expression is associated with Overall Survival (OS) in adult non-M₃ AML

Survival analysis was conducted to assess the prognostic impact of SNHG14 expression in non-M3 AML patients. Patients with low SNHG14 expression levels were correlated with shorter OS ($p$ value = 0.0394; Fig. 3a). There was no significant difference between low SNHG14 expression and RFS pattern in adult non-M₃ AML patients ($p$ value = 0.1755; Fig. 3b).

### 4. Discussion

Uncontrolled proliferation and dysfunctional cell differentiation are hallmarks of leukemia. Therefore, any dysregulation in any IncRNA involved in gene expression control during the blood cells development, might lead to the occurrence and the progression of malignancies. Since their discovery, IncRNAs have been identified in various types of malignancies including leukemia. As scientists pushing the boundaries to reveal new insights, it has been revealed that IncRNA can be utilized as diagnostic and prognostic factors. Nevertheless, only limited number of IncRNAs have been directly involved in the tumorigenesis of
leukemia [19, 20]. For instance, ANRIL which is up-regulated in the BM samples collected from AML patients is negatively correlated with expression of miR-34a, a tumor suppressor markedly declined in individuals struggling with AML [21]. Another IncRNA involved in AML is LINC01018 which was found to be down-regulated in AML and that LINC01018-downexpression is related to AML pathogenesis via suppressing AML cell proliferation and promoting apoptosis, which is mediated by inhibiting miR-499a-5p and regulating PDCD4 expression. These observations offering LINC01018 can act as a molecular marker for the treatment of AML [22].

Nevertheless, the expression and role of SNHG14 in AML have not been clarified yet. Therefore, this study aimed to investigate the unknown role of IncRNA SNHG14 on clinical outcomes and diagnosis in adult non-M3 AML patients. Our result indicated that the IncRNA SNHG14 was down-regulated in AML patients compared to normal control groups (P = 0.0001), which may imply that SNHG14 plays a role in AML development and diagnosis. Our outcome is contrary to that of Gamaleldin et al., who found that SNHG14 was overexpressed in AML patients, indicating that it plays a role in AML development and prognosis [16]. Similarly, Wang et.al also reported that SNHG14 was considerably overexpressed in AML bone marrow tissue as compared to normal marrow tissue (P < 0.001) [15]. Our findings align with a study conducted by Wang et.al., which declares that SNHG14 functions as a tumor suppressor in glioma, significantly reducing cell invasion, compromising cell viability, and triggering apoptosis. In addition, prevail studies reported that SNHG14 might act as a sponge for miR-92a-3p and that there is a negative correlation between SNHG14 and miR-92a-3p expression [23]. Similarly, another study noted that the overexpression of miR-93-5p or ZBTB7A silencing can alleviate the suppressive effect of SNHG14 on the viability, migration, and invasion of endometrium carcinoma cells [24].

According to ROC analysis, SNHG14 transcription levels statistically distinguish non-M3 AML patients from healthy controls with 66% sensitivity and 66.67% specificity. We noticed that decreased expression levels of SNHG14 correlated with shorter OS, but there was no link between low expression of SNHG14 and RFS. Moreover, we demonstrated that the expression level of SNHG14 between complete remission and non-complete remission had no statistically significant difference (P = 0.9072), suggesting that SNHG14 might not be considered a predictor of remission and chemotherapy response. However, further study is needed to validate this claim in large cohort samples.

5. Conclusion

The present study demonstrated for the first time that the expression level of SNHG14 was significantly decreased in non-M3 AML bone marrow samples and that this decreased expression was associated with lower OS and poor cytogenetic outcomes. This suggests that SNHG14 acts as a tumor-suppressor and may be used as a diagnostic biomarker in non-M3 AML patients, offering a promising target for AML treatment.

Declarations
Authors’ contributions

Saba Seifpour, Amir Atashi, and Mina Soufi Zomorrod conceived and designed the experiments. Saba Seifpour, Amir Atashi, Mina Soufi Zomorrod performed the experiments and collected data. Sanaz Khaseb, Fatemeh Tavangar, and Mahdi Kohansal Vajari were involved in the acquisition, analysis, and interpretation of data. All authors coordinated the revision and manuscript preparation. All authors read and approved the final manuscript.

Data Availability

All of the data and materials are available.

Declaration of competing interest

None.

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References


**Table**

Table 1 is available in the Supplementary Files section.

**Figures**

![Figure 1](image-url)
(a) The SNHG14 expression in adult non-M AML patients was significantly lower than that in healthy controls. (b) Low expression of SNHG14 was observed in CR and NR patients compared with control, but expression levels showed no significant difference between the CR and NR groups. (c) There was no statistically significant difference in the expression level of SNHG14 before and after treatment. The 2-ΔΔCq method was used to analyze the relative mRNA expression level using GAPDH as an internal control. ns P >0.05, **P < 0.01, ***P < 0.001

Figure 2

Receiver operating characteristic (ROC) curve analysis of SNHG14 for discriminating adult non-M AML from healthy control.
Figure 3

Kaplan-Meier Survival Curves was Analyzed according to expression levels of SNHG14 in non-M3 AML patients. (a) Patients with low SNHG14 expression levels were correlated with shorter OS. (b) No correlation was observed between low expression levels of SNHG14 and RFS.

Supplementary Files

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- table.docx