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# Long noncoding RNA SNHG5 is up-regulated and serves as a potential prognostic biomarker in acute myeloid leukemia

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**Abstract. – OBJECTIVE: Growing evidence has demonstrated that the dysregulation of long non-coding RNAs (lncRNAs) may act as an important role in human tumorigenesis. Our present study aimed to explore the expression pattern and prognostic value of a newly discovered lncRNA small nucleolar RNA host gene 5 (SNHG5) in acute myeloid leukemia (AML).**

**PATIENTS AND METHODS: The expression of SNHG5 was determined using Real-time reverse transcription-polymerase chain reaction (qRT-PCR) in bone marrow and plasma obtained from AML patients and healthy controls. The correlation between SNHG5 expression and clinical features were statistically analyzed. The association between SNHG5 expression and overall survival was estimated by the Kaplan-Meier method. Univariate and multivariate analyses were performed to analyze the prognostic significance of SNHG5 expression.**

**RESULTS: SNHG5 expression levels were consistently higher in the bone marrow and plasma of AML patients than those in the healthy controls ($p<0.01$). Furthermore, SNHG5 upregulation more frequently occurred in AML patients with advanced FAB classification ($p<0.005$) and unfavorable cytogenetics ($p=0.001$). In addition, the data of Kaplan-Meier method revealed that overall patient survival for those with high plasma SNHG5 expression was significantly shorter than those patients with low SNHG5 expression ($p<0.0070$). Importantly, univariate and multivariate Cox regression analysis identified increased SNHG5 expression as an independent factor predicting poor prognosis for AML patients.**

**CONCLUSIONS: Our findings provide evidence that plasma SNHG5 is an independent biomarker for patients with AML, suggesting the potential role of SNHG5 as a highly specific and sensitive biomarker.**

Key Words

LncRNA SNHG5, Acute myeloid leukemia, Prognosis.

## Introduction

Acute myeloid leukemia (AML) is a heterogeneous disease of hematopoietic stem cells characterized by proliferation and maturation arrest of myeloid blasts in bone marrow and blood[1,2]. Leukemia results from the accumulation of genetic and epigenetic alterations during the multistep process of tumorigenesis[3]. Without treatment, AML quickly becomes fatal, and historically, it has always been associated with a poor prognosis[4]. During the past decades, growing advances have been performed to enhance the diagnostic and therapeutic strategies and to improve the clinical prognosis of patients with AML. However, approximately 40% of patients with AML achieve long-term survival[5-7]. Thus, it is urgent to identify new predictive markers and therapeutic targets for AML.

Long non-coding RNAs (LncRNAs), non-protein coding transcripts longer than 200 nucleotides, have been identified as gene expression regulators[8]. With the advanced study methods, lncRNAs have recently undergone a rapid expansion of research and discovery[9]. Recent research[10,11] demonstrated that lncRNAs served as important roles in a wide range of biological processes, such as transcriptional and posttranscriptional regulation, cell growth and activity regulation of protein. In addition, the rapid development of human genomics has highlighted the important role of lncRNAs in diverse biological processes of cancer[12]. For instance, Lu et al[13] reported that lncRNA NKILA suppressed migration and invasion of non-small cell lung cancer via NF-κB/Snail pathway. Zhang et al[14] found that suppression of lncRNA NEAT1 could suppress proliferation and metastasis of breast cancer cells. Gao et al[15] found that high lncRNA



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HSP90AA1-IT1 expression was significantly associated with poor prognosis of glioma patients and its knockdown inhibited the migration and invasion of glioma cells by modulating targeting miR-885-5p-CDK2 pathway. Those results indicated that the deregulated of lncRNAs correlated with the progression and prognosis in various kinds of human cancers; however, the biological function and clinical significance of lncRNAs in AML remain largely unexplored.

Small nucleolar RNA host gene 5 (SNHG5), located on chromosome 6q15, has been reported to be strongly implicated in such processes as cell differentiation, cell proliferation, metastasis and aberrantly expressed in human cancer[16-18]. Importantly, He et al[19] firstly identified SNHG5 as a positive regulator and provided evidence that SNHG5 expression was significantly upregulated in chronic myeloid leukemia patients. However, to our best knowledge, whether SNHG5 expression was abnormal and its clinical significance in AML have not been reported. We firstly provided evidence that SNHG5 has the potential to be a prognostic biomarker of AML.

## Patients and Methods

### Patients and Tissue Samples

A total of 194 AML patients and 61 healthy volunteers from the Shandong Blood Center were enrolled in this study. None of these patients had previously been diagnosed with any type of malignancy. The patients were diagnosed with AML according to a morphologic assessment of the Wright-Giemsa stained smears of the bone marrow aspirates. There were 110 males and 84 females, with a medium age of 41.5 (range 17.3-66.2) years. Patients were uniformly treated according to the study protocols of the Dutch-Belgian Hematology-Oncology Cooperative Group. According to the French-American-British (FAB) classification, 30 patients had AML M1, 29 had M2, 33 had M3, 21 had M4, 20 had M5, 13 had M6 and 48 M7. The characteristics of the cervical cancer patients enrolled in the study are displayed in Table I. Written informed consent was obtained from each patient, and the present study was approved by the Ethics Committee of Shandong Blood Center.

**Table I.** Correlation between serum SNHG5 expression and different clinicopathological features in AML patients.

| Variable | Number | Serum SNHG5 expression | | *p*-value |
|---|---|---|---|---|
| | | High | Low | |
| ***Gender*** | | | | 0.137 |
| Male | 110 | 51 | 59 | |
| Female | 84 | 48 | 36 | |
| ***Age*** | | | | 0.900 |
| < 60 | 103 | 53 | 50 | |
| ≥ 60 | 91 | 46 | 45 | |
| ***WBC*** | | | | 0.088 |
| < 10 | 72 | 31 | 41 | |
| ≥ 10 | 122 | 68 | 54 | |
| ***Blast in BM*** | | | | 0.415 |
| < 50% | 73 | 40 | 33 | |
| ≥ 50% | 121 | 59 | 62 | |
| ***Extramedullary disease*** | | | | 0.607 |
| Absent | 124 | 65 | 59 | |
| Present | 70 | 34 | 36 | |
| ***FAB classification*** | | | | 0.005 |
| M1-M6 | 146 | 66 | 80 | |
| M7 | 48 | 33 | 15 | |
| ***Cytogenetics*** | | | | 0.001 |
| Favorable | 49 | 13 | 36 | |
| Intermediate | 107 | 59 | 48 | |
| Unfavorable | 38 | 27 | 11 | |

### Sample Collection

Blood was collected from AML patients and controls in sodium heparin tubes (Ruibang, Guangzhou, Guandong, China) and then collected in separating gel vacuum collection tubes and centrifuged at 3000×g for 10 min. The supernatant plasma was then carefully collected and stored at -80°C until to be used.

### Total RNA Extraction and Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted from sera and bone marrow samples using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RNA concentration was examined with a NanoDrop ND-2000 spectrophotometer (Life Technologies, Carlsbad, CA, USA). First-strand cDNA was generated with the Primer-Script™ one step RT-PCR kit (TaKaRa, Otsu, Shiga, Japan). Real-time polymerase chain reaction (RT-PCR) was performed using the SYBR Green Master Mixture (Roche, Haidian, Beijing, China) reagent in ABI 7500 Real-time PCR instrument, using the following protocol: 95°C for 3 min, 40 cycles of 95°C for 15 s, 60°C for 15 s and 72°C for 30 s. The expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used for normalization. The fold-change for target genes normalized by internal control was determined by the formula $2^{-\Delta\Delta Ct}$. The following primers were used: SNHG5: 5'-GAGCAG CTCTGAAGATGCAA-3' (forward) and 5'-TTTTAACCAAGCGATTTT CCA-3' (reverse), GAPDH: 5'- GTCAACGGATTTGGTCT-GTATT-3' (forward) and 5'-AGTCTTCTGGGT-GGCAGTGAT-3' (reverse).

### Statistical Analysis

All statistical analysis was performed using SPSS 17.0 software package (SPSS Inc., Chicago, IL, USA). Data are expressed as the mean ± standard deviation from at least three separate experiments. The differences between two groups were analyzed using the Student's *t*-test. The relationship between plasma SNHG5 expression and clinical characteristics of patients with AML was evaluated with the application of the $x^2$-test. Overall survival rates were calculated by the Kaplan-Meier method with the log-rank test applied for comparison. Significant variables in univariate models were further analyzed by multivariate Cox proportional hazards regression models to identify the independent prognostic values. $p$-value < 0.05 was considered as statistically significant.

## Results

### Up-Regulation of SNHG5 in Bone Marrow and Plasma of AML Patients

To evaluate the effect of SNHG5 on AML, the expression levels of SNHG5 in 194 AML patients and healthy controls were analyzed by qRT-PCR. As shown in Figure 1A, it was observed that the expression level of bone marrow SNHG5 was significantly higher in AML patients compared with healthy controls ($p$<0.01). In addition, we also found that SNHG5 levels in plasma from osteosarcoma patients were significantly higher than those in healthy controls ($p$<0.01). The data indicated a potential function of SNHG5 in AML.

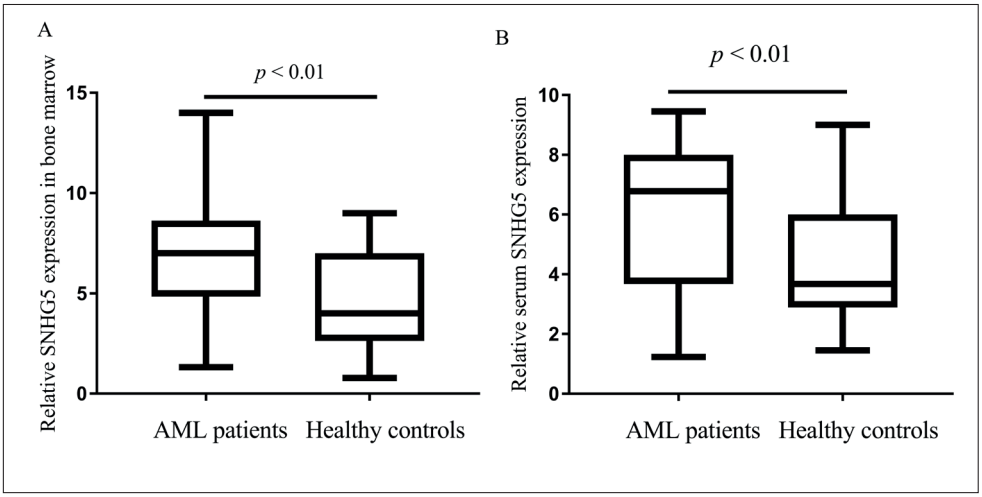


**Figure 1.** Expression levels of SNHG5 in human bone marrow and patients' plasma detected by qRT-PCR assay. **A**, The expression level of bone marrow SNHG5 was significantly higher in AML patients compared with healthy controls ($p$<0.01). **B**, Plasma SNHG5 expression level was significantly higher in AML patients compared with healthy controls ($p$< 0.01).

### The Correlation Between Plasma SNHG5 and Clinicopathological Features

In order to explore the clinical significance of SNHG5 in AML patients, we classified AML patients into two groups based on the median expression levels: AML patients who expressed SNHG5 at levels less than the median expression value were assigned to the low expression group (n=99), and those with expression above the median expression value were assigned to the high expression group (n=95). The correlation between the expression of plasma SNHG5 and clinical characteristics are listed in Table I. We found that the high expression group displayed more advanced FAB classification ($p$<0.005) and more unfavorable cytogenetics ($p$=0.001) when compared with the low expression group. However, there were no significant correlations between plasma SNHG5 expression and other clinicopathological factors of patients. Our results suggested that that overexpression of SNHG5 might be involved in AML progression.

### Impact of Plasma SNHG5 Expression on Prognosis of AML Patients

During the entire follow-up period, 104 of the 194 patients (53.6%) with AML died, and the median overall survival time of all patients was 40 months. Then, Kaplan-Meier analysis and log-rank test were used to evaluate the impact of SNHG5 expression on overall survival. Our results showed that patients with high SNHG5 expression had a significantly poorer prognosis than those with low SNHG5 expression ($p$<0.0070) (Figure 2). Furthermore, Cox regression analyses were conducted to evaluate the prognostic factors in 194 AML patients. The results of univariate analysis showed that SNHG5 overexpression was an unfavorable prognostic factor in AML patients ($p$=0.001, Table II), regardless of FAB classification and cytogenetic. Finally, the results of multivariate analysis revealed that SNHG5 expression was an independent prognostic indicator for overall survival (HR=3.532, 95% CI, 1.277-5.235; $p$=0.006) in patients with AML.

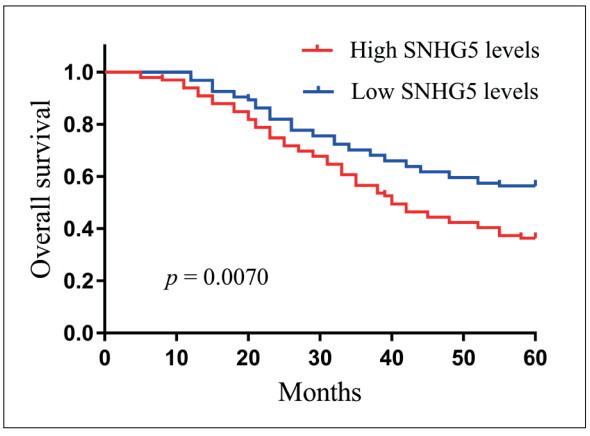


**Figure 2.** Kaplan-Meier curves for overall survival of 194 AML patients, divided according to plasma SNHG5 expression levels. High plasma SNHG5 expression was significantly associated with poor survival ($p$=0.0070, log-rank test).

## Discussion

Clinical outcome of AML remains unsatisfactory despite of the therapeutic advances in AML treatment recently[20]. Prognostic factor detection in AML is crucial to predict overall survival of patients and choose optimal therapeutic strategies[21]. Based on the World Health Organization (WHO) categorization of AML, cytogenetic and molecular analyses play a crucial role in determining the clinical prognosis of AML patients[22]. However, poor sensitivity and specificity greatly limited clinical applications of those methods. Therefore, the discovery of new biomarkers for prognosis of AML remains important. Recently, more and more studies reported that dysregulation of lncRNAs was significantly associated with overall survival of cancer patients, including AML. For instance, lncRNA MALAT-1, a well-studied lncRNA, was reported to be highly expressed in AML and to be closely associated with poor prognosis in AML patients[23]. LncRNA IRAIN, a newly identified lncRNA, was also considered to be an independent prognostic marker for overall survival of AML patients[24]. However, the related researches of lncRNAs function and clinical significance in AML are far from being fully elucidated.

**Table II.** Univariate analysis of overall survival in AML patients.

| Prognostic variables | Overall survival | | |
|---|---|---|---|
| | HR | 95% CI | *p*-value |
| Gender | 1.433 | 0.562-2.775 | 0.323 |
| Age | 1.678 | 0.671-2.556 | 0.219 |
| WBC | 2.123 | 0.778-2.672 | 0.137 |
| Blast in BM | 1.448 | 0.822-2.329 | 0.214 |
| Extramedullary disease | 2.553 | 0.568-3.342 | 0.127 |
| FAB classification | 3.778 | 1.326-5.663 | 0.005 |
| Cytogenetics | 4.231 | 1.667-8.834 | 0.001 |
| SNHG5 expression | 3.932 | 1.547-6.563 | 0.001 |

**Table III.** Multivariate analysis of overall survival in AML patients.

| Prognostic variables | Overall survival | | |
|---|---|---|---|
| | HR | 95% CI | *p*-value |
| FAB classification | 3.321 | 1.117-4.783 | 0.009 |
| Cytogenetics | 3.213 | 1.332-6.672 | 0.004 |
| SNHG5 expression | 3.532 | 1.277-5.235 | 0.006 |

LncRNAs have received growing attentions due to their critical regulatory effect in many pathophysiological processes[25]. As a newly identified lncRNA, abnormal expressions of SNHG5 have been reported to be associated with tumor growth, carcinogenesis, or metastasis in several tumors. For instance, Nkerorema et al[26] reported that depletion of SNHG5 induces cell cycle arrest and apoptosis *in vitro* and limits tumor outgrowth *in vivo* in colorectal cancer by counteracting STAU1-mediated mRNA destabilization. Ma et al[27] found that SNHG5 exerted its tumor-promotive role by promoting bladder cancer cell proliferation via targeting p27. However, Zhao et al[28] suggested that SNHG5 was lowly expressed in gastric cancer and its forced expression suppressed gastric cancer cell proliferation and metastasis *in vitro* and *in vivo* by trapping MTA2 in the cytosol. Those results revealed that SNHG5 play different roles according the types of cancers. He et al[19] showed that the expression levels of SNHG5 in chronic myeloid leukemia patients were significantly higher than that from healthy donors. Further experiments showed that deregulated SNHG5 was involved in imatinib resistance in chronic myeloid leukemia via acting as a ceRNA against miR-205-5p, suggesting that SNHG5 functioned as a tumor promoter in this disease. However, neither the expression pattern nor the clinical significance of SNHG5 have been elucidated in AML so far.

In this work, we firstly determined the expression levels of SNHG5 in AML patients and healthy controls and the results showed that SNHG5 expression was significantly higher in the bone marrow and plasma of patients with AML than that of normal controls. The results of our study, together with those reported previously, suggested that SNHG5 played a critical role in progression of many tumors. Then, we explored the correlation between plasma SNHG5 expression and clinical features of AML patients, finding that its high levels were significantly associated with FAB classification and cytogenetics. Subsequently, the results of Kaplan-Meier analysis showed that plasma SNHG5 expression was associated with poor clinical survival of patients with AML. Finally, univariate and multivariate analyses indicated high SNHG5 expression level to be an independent predictor of poor prognosis in AML patients. To be honest, our study just explored the clinical significance of SNHG5 in AML patients. The molecular mechanisms of SNHG5 that involved in AML need to be further studied.

## Conclusions

We provide evidence for the first time that SNHG5 expression was significantly up-regulated in AML, and that it may represent a potential biomarker of poor prognosis for AML patients. In the coming future, more large-scale and high-qualified investigations are needed to confirm our results.

### Conflict of interest

The authors declare no conflicts of interest.

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