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ORIGINAL ARTICLE

Expression of NAP1L5 in Patients with First Diagnosis of Acute Myeloid Leukemia and its Clinical Significance

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SUMMARY

Background: **The objective of this study was to assess the expression levels of NAP1L5 in individuals diagnosed with acute myeloid leukemia (AML) and to investigate its clinical and prognostic significance in those with primary AML.**
Methods: **Between June 2021 and June 2023, the Department of Hematology at the First Affiliated Hospital of Bengbu Medical University collected 100 bone marrow specimens from primary AML patients and 30 from individuals with idiopathic thrombocytopenic purpura (ITP) for this medical research study. The researchers gathered comprehensive clinical data from patients diagnosed with acute myeloid leukemia (AML). Quantitative real-time polymerase chain reaction (qRT-PCR) was utilized to measure the mRNA levels of NAP1L5 in both the experimental and the control group. The investigation involved analyzing the association between NAP1L5 expression related to AML, clinical features, and overall prognosis. The study employed the Kaplan-Meier method to evaluate the impact of NAP1L5 expression on the overall survival time (OS) in AML patients, while the Cox hazard regression model was used to identify significant prognostic factors.**
Results: **NAP1L5 was found to be significantly more expressed in individuals with AML compared to those with ITP. Patients diagnosed with AML and exhibiting elevated NAP1L5 expression levels correlated with older age, ineffective response to chemotherapy, and a more unfavorable prognosis compared to those with lower NAP1L5 expression. Additionally, the group with high expression demonstrated a noticeably decreased overall survival (OS), compared to the group with low expression. Furthermore, a significant association was observed between the elevated NAP1L5 expression levels and an unfavorable clinical outcome in patients diagnosed with AML.**
Conclusions: **The initial exploration of the elevated expression of NAP1L5 in AML and its clinical significance offers potential targets for AML treatment and prognostic evaluation.**
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KEYWORDS

NAP1L5, AML, clinical significance, prognosis

INTRODUCTION

Acute myeloid leukemia (AML) represents the most common form of acute leukemia in adults, characterized by the uncontrolled proliferation of aberrant myeloid cells within the hematopoietic system [1]. Despite advancements in AML treatment, involving the use of demethylating agents and targeted therapies, the overall clinical prognosis remains unsatisfactory and highly

variable. This variability is influenced by several factors, including the patient's age, health status, clinical attributes, and the unique genetic characteristics of the leukemia itself [2-4]. Consequently, there is an urgent need for the development of novel therapeutic agents, molecular targets, and diagnostic markers to improve treatment outcomes and the prognosis for individuals diagnosed with AML.

The NAP1 family comprises six isoforms, with NAP1L1 and NAP1L4 being widely expressed in various tissues, while the remaining isoforms are predominantly found in brain tissues [5,6]. NAP1L5, a member of this family, plays a crucial role in the structural support of DNA replication and repair mechanisms. It influences the transcription of the Herc3 gene and is involved in processes such as cell division, cellular ageing, and tumor initiation, as documented in various studies [5,7,8]. Some research has indicated a potential association between the aberrant expression or mutation of NAP1L5 and the development and progression of certain tumors. Furthermore, NAP1L5 is implicated in the regulation of genes during hematopoiesis, leukemogenesis, and development [9,10]. However, the role of NAP1L5 in acute myeloid leukemia (AML) has been inadequately explored. Therefore, the aim of this research is to examine the expression of NAP1L5 in patients diagnosed with AML and to investigate its correlation with clinical characteristics and prognosis. This study seeks to provide valuable insights for the management and prognostic assessment of AML.

MATERIALS AND METHODS

Participants

From June 2021 to June 2023, the Department of Hematology at the First Affiliated Hospital of Bengbu Medical University collected bone marrow samples from a cohort of 130 patients. This cohort comprised 100 individuals initially diagnosed with acute myeloid leukemia (AML) and 30 individuals diagnosed with immune thrombocytopenic purpura (ITP). All participants had undergone at least one round of chemotherapy and myeloid cytology to evaluate the efficacy of the treatment. In line with the 2021 National Comprehensive Cancer Network (NCCN) guidelines for AML [11], comprehensive clinical data were collated for the AML patients, including information on gender, age, blood cell counts, French-American-British (FAB) classification, chemotherapy regimen, effectiveness evaluation, chromosomal karyotypes, mutations, and prognosis classification. Active surveillance was conducted for both surviving and deceased patients, with no loss to follow-up, until December 31, 2023, for survivors and until the date of death for those who had passed away. The duration of overall survival (OS) was calculated from the initial diagnosis to the follow-up cut-off date or the occurrence of death. The research protocol was endorsed by the Ethics Committee of the First Affiliated Hospital of Bengbu Medical University, and informed consent was obtained from all patients.

Apparatus and reagents

The TRIzol reagent, M-MuLV first strand cDNA synthesis kit, 2X SG fast qPCR premix (Low Rox) kit, and the primers for the study were supplied by Shanghai Bioengineering Co., Ltd. An applied biosystems PCR instrument was procured from Thermo Fisher Scientific.

RNA extraction methods

To prepare the bone marrow-derived blood sample for analysis, the sample tube was maintained at 4°C until processed. Erythrocytes were lysed by using a solution comprising NH4Cl, NaHCO3, EDTA-Na2, and distilled water. Following adjustment of the solution's pH to 7.4, the lysed cells were thoroughly washed. After 2 - 3 centrifugation cycles, the individual cells were defrosted, collected, and stored at -80°C. After isolation, the cells were centrifuged, and 200 μL of TRIzol reagent was added. Then, 200 μL of chloroform was introduced, and the purity and concentration of the total RNA were determined by using a SMA1000 Ultra Micro spectrophotometer, after RNA extraction with 100 μL of isopropanol. The objective was to achieve an OD260/280 ratio between 1.8 and 2.0 and a total RNA concentration ranging from 30 to 70 ng/μL.

qRT-PCR

cDNA synthesis was performed by using the M-MuLV first strand cDNA synthesis kit from Shanghai Bioengineering Co., Ltd., following the manufacturer's protocol. The qRT-PCR reaction mix included 2X SG fast qPCR master mix (Low Rox), forward and reverse primers, sample cDNA, DNase/RNase-free buffer, and PCR-grade water. The SYBR green fluorescent dye method, in accordance with the 2X SG fast qPCR premix (Low Rox) kit's instructions from Shanghai Bioengineering Co., was utilized for qPCR. Amplification conditions were set to 94°C for 10 minutes, followed by 40 cycles of 94°C for 20 seconds, 60°C for 20 seconds, and 72°C for 20 seconds. The relative expression levels of the NAP1L5 gene were determined by using the $2^{-\Delta\Delta Ct}$ method, with GAPDH serving as the internal control. Additionally, the primer sequences for each target gene are detailed in Table 1.

Mutation detection methods

Bone marrow specimens from patients were cryopreserved and promptly transported to Jinyu Medical Laboratory for analysis. Employing second-generation sequencing, the laboratory conducted whole-exome sequencing on 30 AML-related genes, including single-nucleotide variants and small insertions/deletions, achieving an average sequencing depth of 2,000 ×. This process utilized the Illumina HiSeq X Ten Sequencer, encompassing steps such as DNA extraction, whole-genome library preparation targeting specific genes with the Rapid DNA lib prep kit for Illumina from Wuhan

Aiportech, targeted next-generation sequencing (NGS) by using the Illumina HiSeq X Ten Sequencing Instrument, and the subsequent analysis of the results.

Chromosomal examination methods

Bone marrow specimens were forwarded to Hefei Jinyu Medical Laboratory for chromosomal analysis via cell culture G-banding technique. The procedure involved culturing bone marrow cells for 24 hours, followed by collection and routine preparation of the cells. Prepared chromosome specimens were then baked and subjected to trypsin enzyme solution for digestion. Specimens were stained by using Giemsa staining solution, rinsed with tap water, dried, and subsequently examined. Microscopic examination was performed under a high magnification eyepiece to identify clear chromosomal bands, indicating a suitable specimen.

Statistical analysis

The statistical package SPSS 26 was employed for a comprehensive analysis by using both statistical and analytical methodologies. Data visualization was achieved by using GraphPad Prism 9.5. Quantitative data between the groups were compared by using the independent Student's *t*-test. To explore the relationship between clinical characteristics of AML patients and NAP1L5 expression levels, patients were stratified into two categories based on the median value of NAP1L5 relative expression: high expression and low expression group. The study compared the clinical characteristics of these groups by using either the chi-squared test or Fisher's exact probability method. Overall survival (OS) was evaluated by using the Kaplan-Meier method, with intergroup differences assessed via the Log-rank test. Variables with $p < 0.1$ in univariate analysis were selected for inclusion in the multivariate Cox regression model. It is noteworthy that all tests were two-sided, with a p-value < 0.05 considered statistically significant.

RESULTS

Detection of NAP1L5 expression by qRT-PCR

The study utilized qRT-PCR to detect NAP1L5 expressions in patients diagnosed with either ITP or AML. The results, illustrated in Figure 1, demonstrated significantly higher NAP1L5 expression levels in the AML group compared to the ITP group, with statistical analysis confirming this significant difference ($t = 3.656$, $p < 0.001$).

Relationship of the NAP1L5 expression levels with clinical characteristics

The investigation of the correlation between NAP1L5 expression levels and various clinical characteristics of AML patients at initial diagnosis (presented in Table 2) revealed a significant association. A higher prevalence of patients aged ≥ 60 years was observed in the NAP1L5 high-expression group, compared to the low-expression group ($p = 0.016$). However, no statistically significant differences were noted between the two groups in terms of gender, number of bone marrow primordial cells at initial diagnosis, white blood cell counts, hemoglobin levels, platelet counts, and FAB classification ($p > 0.05$).

Relationship between the NAP1L5 expression levels with chemotherapy regimen and efficacy

Patients with AML under the age of 60 were treated with an idarubicin + cytarabine (IA) regimen, while those aged 50 years or older with poor physical status (ECOG > 2 points) received a combination regimen of hypomethylating agents (HMA) ± cytarabine-containing (Ara-C) regimens. These HMA regimens included decitabine (D) and azacitidine (A), alongside Ara-C-containing regimens such as D + HAG (Homoharringtonine + Ara-C + granulocyte-stimulating factor), A + HAG, D + Ara-C, and A + Ara-C. All patients aged ≥ 60 years were treated with a combination regimen of HMA ± Ara-C. Among the 100 enrolled patients, 28 received the IA regimen and 72 received the HMA ± Ara-C regimen. According to the efficacy assessment criteria, there were 40 complete remissions (CR), 26 partial remissions (PR), and 34 non-remissions (NR). Analysis of the chemotherapy regimens and efficacy across different NAP1L5 expression groups revealed (Table 3) a significantly higher number of NR cases in the high-expression group compared to the low-expression group, with a statistically significant difference ($p < 0.01$); however, there was no statistically significant difference in the chemotherapy regimens ($p > 0.05$).

Relationship between the NAP1L5 expression levels with karyotype, gene mutation, and risk grouping

Among the enrolled AML patients, 58 had normal karyotypes and 42 had abnormal karyotypes, including numerical and structural abnormalities. A total of 88 cases exhibited gene mutations, with 64 cases having mutations in more than three genes. Prognostic risk stratification, according to AML diagnostic and treatment guidelines, included 8 cases in the good prognosis group, 43 in the intermediate prognosis group, and 49 in the poor prognosis group. The results showed (Table 4) that there were significantly more patients with a poor prognosis in the high NAP1L5 expression group than in the low expression group, with a statistically significant difference ($p = 0.034$); while no statistically significant difference was observed in chromosomal karyotypes and gene mutations between patients with different NAP1L5 expression levels ($p > 0.05$).

Relationship of the NAP1L5 expression levels with OS

The analysis included 100 patients diagnosed with acute myeloid leukemia (AML), with their survival rate evaluated by using the Kaplan-Meier technique. The results, as depicted in Figure 2, demonstrated a significant dis-

Table 1. Sequences in PCR.

Genes	Sequences
NAP1L5	Forward: 5'-GCCGAGGACGAGGTAATGG-3'
	Reverse: 5'-CATTTCACGGAATTGGGCAAG-3'
GAPDH	Forward: 5'-GGGAGCCAAAAGGGTCAT-3'
	Reverse: 5'-GAGTCCTTCCACGATACCAA-3'

Table 2. Relationship between the NAP1L5 expression levels and clinical characteristics of AML patients.

Items	NAP1L5 expression levels		F/χ^2	p-value
	Low (n = 50)	High (n = 50)		
Gender			0.360	0.548
Male	26	23		
Female	24	27		
Age (years)			5.769	0.016
≥ 60	20	32		
< 60	30	18		
BM blast cells (%)			1.099	0.487 [a]
≥ 30	47	44		
< 30	3	6		
WBC count ($\times 10^9$/L)			2.564	0.109
≥ 10	22	30		
< 10	28	20		
HB count (g/L)			0.170	0.680
≥ 70	32	30		
< 70	18	20		
PLT count ($\times 10^9$/L)			2.102	0.147
≥ 30	35	28		
< 30	15	22		
FAB classification			0.805	0.848
M1	9	10		
M2	19	15		
M4	7	9		
M5	15	16		

[a] - indicates that the Fisher's exact test was used.

Table 3. Relationship of the NAP1L5 expression levels with chemotherapy regimen and efficacy in AML patients.

Items	NAP1L5 expression levels		F/χ^2	p-value
	Low (n = 50)	High (n = 50)		
Curative effect of chemotherapy			27.920	<0.01
CR	32	8		
PR	4	22		
NR	14	20		
Chemotherapy regimen			1.786	0.181
IA	17	11		
HMA ± Ara-C	33	39		

Table 4. Relationship of the NAP1L5 expression levels with karyotype, gene mutation, and risk grouping in AML patients.

Items	NAP1L5 expression levels		F/χ^2	p-value
	Low (n = 50)	High (n = 50)		
Karyotype			**0.164**	**0.685**
Normal	**28**	**30**		
Abnormal	**22**	**20**		
Gene mutation			**3.808**	**0.936** [a]
CEBPA	**8**	**8**		
ASXL1	**9**	**3**		
IDH1/2	**10**	**10**		
RUNX1	**7**	**7**		
TP53	**3**	**4**		
FLT3-ITD	**12**	**13**		
K/N-RAS	**11**	**11**		
DNMT3A	**17**	**18**		
TET2	**7**	**8**		
NPM1	**10**	**13**		
Risk grouping			**6.723**	**0.034** [a]
Favorable	**5**	**3**		
Intermediate	**27**	**16**		
Poor	**18**	**31**		

[a] **- indicates that the Fisher's exact test was used.**

Table 5. Multifactorial analysis of the independent risk factors affecting the prognosis of patients with AML.

Items	HR (95% CI)	p-value
NAP1L5 expression levels (low expression)	**0.444 (0.259 - 0.759)**	**0.003**
Age (≥ 60 year)	**2.278 (1.232 - 4.212)**	**0.009**
Curative effect of chemotherapy (CR/PR)	**0.174 (0.085 - 0.354)**	**< 0.001**
Karyotype (normal)	**0.564 (0.344 - 0.924)**	**0.023**

parity in the overall survival (OS) between individuals with high NAP1L5 expression levels and those with low expression. The group with elevated expression exhibited significantly reduced OS ($p < 0.0001$), underscoring the importance of NAP1L5 in the prognostic assessments of AML patients. These findings suggest that elevated NAP1L5 levels could serve as a predictive marker for adverse outcomes in patients with AML.

Multifactorial analysis

The survival status of AML patients served as the dependent variable, with various clinical characteristics considered as independent variables in a univariate Cox regression model. Factors with $p < 0.1$ in the univariate analysis were subsequently analyzed in a multifactorial Cox regression model. This analysis identified NAP1L5 expression level, age, therapeutic efficacy, and chromosomal karyotype as independent risk factors, significantly influencing the prognosis of AML patients ($p < 0.05$) (Table 5).

DISCUSSION

Over recent years, investigations of the pathogenic mechanisms underpinning acute myeloid leukemia (AML) and the progressive refinement of molecular diagnostic techniques have yielded considerable advances and a deeper understanding of this markedly heterogeneous condition [4,12]. The treatment paradigm

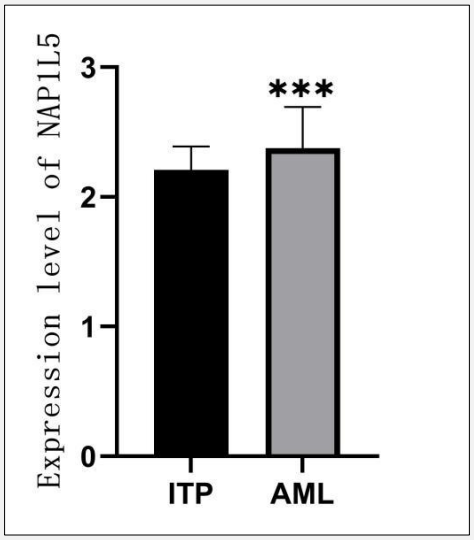

Figure 1. The expression of NAP1L5 detected by qRT-PCR in AML and ITP patients compared with the ITP group.

***** - p < 0.001.**

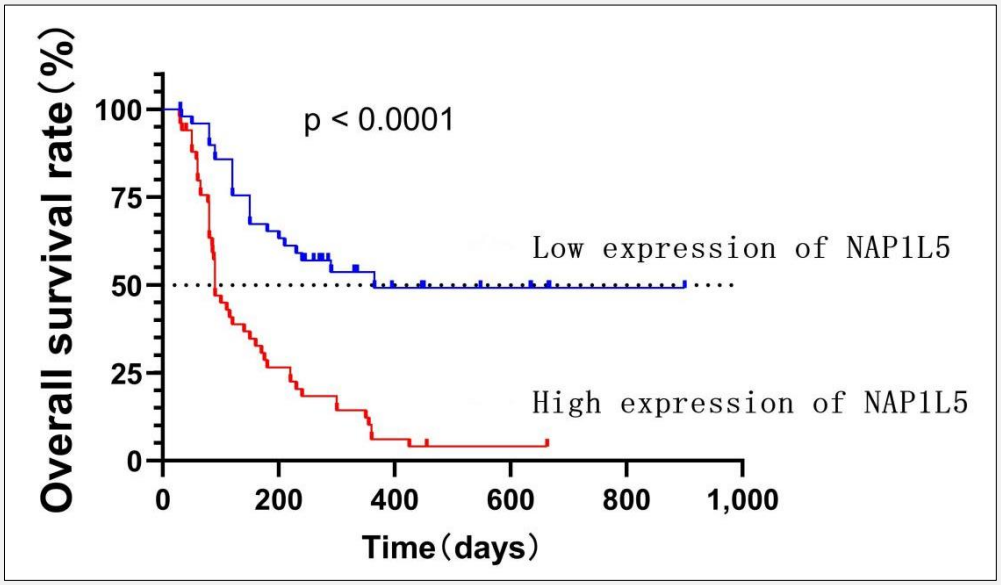

Figure 2. Kaplan-Meier curve analysis of OS in AML patients with different NAP1L5 expression levels.

for AML has evolved from traditional single-agent chemotherapy to personalized and targeted therapies, that account for patient-specific characteristics and disease attributes at the cytogenetic and molecular levels [13, 14]. This transition encompasses the adoption of inhibitors targeting FLT3, IDH1/2, TP53, and BCL-2, which have markedly improved patient survival rates and their quality of life [15-19]. However, the limited insight into the role of molecular markers in AML, coupled with an increasing prevalence of drug-resistant patient cohorts, presents significant challenges in clinical practice [20]. The imperative to identify additional biomarkers and develop novel targeted therapeutics for AML patients is critical to augmenting treatment strategies and prognostic outcomes.

The NAP1L5 gene, located on chromosome 4q22.1, was initially identified as a paternally imprinted gene in murine models [21]. Previous research has elucidated the pivotal role of aberrant NAP1L5 expression in the pathogenesis of various diseases. For instance, NAP1L5 has been shown to induce cardiomyocyte hypertrophy by enhancing translation in a mechanistic target of rapamycin (mTOR)-independent manner, through the direct regulation of nucleosome assembly, ribosome biogenesis, and thus increasing cellular protein synthesis rates [22]. Additionally, NAP1L5 is implicated in the promotion of pancreatic ductal adenocarcinoma (PDAC) development by activating the tumor suppressor PHLPP1, which leads to the ubiquitination and degradation of the AKT/mTOR signaling pathway [23]. Furthermore, differential expression of NAP1L5 in ovarian cancer has been associated with patient prognosis [24], while its low expression in hepatocellular carcinoma correlates with a poor prognosis. In the latter, NAP1L5 inhibits cell proliferation, migration, invasion, and induces apoptosis through the regulation of the PI3K/AKT/mTOR signaling pathway by MYH9 [25]. Additionally, NAP1L5 is involved in neurodegenerative diseases, including Alzheimer's disease, where it regulates AD-like pathological processes and exerts neuroprotective effects through the inhibition of the GSK3B/Wnt/β-Catenin signaling pathway [26]. Therefore, further research into NAP1L5 is anticipated to unveil its mechanistic roles and potential as a therapeutic target across various diseases, offering valuable theoretical and practical insights for disease prevention and treatment. Notably, the role of NAP1L5 in AML remains unreported in the existing literature. This study aims to elucidate the expression of NAP1L5 in AML by using qRT-PCR and to analyze its correlation with the clinical characteristics and prognosis of AML patients. Through this research, we aspire to determine whether NAP1L5 could serve as a potential biomarker and therapeutic target for AML. The findings of this study elucidate that NAP1L5 is significantly overexpressed in acute myeloid leukemia (AML), as determined by quantitative real-time PCR (qRT-PCR), suggesting a role of NAP1L5 in the etiology of AML. Following this, AML patients were stratified, based on the median expression level of NAP1L5, into groups with high and low expression. Amongst patients aged ≥ 60 years in the high NAP1L5 expression cohort, a notably higher incidence of non-remission (NR) and poor prognosis was observed in comparison to those in the low NAP1L5 expression group. This indicates a correlation between the level of NAP1L5 expression and age-associated AML characteristics, with elevated NAP1L5 expression being potentially linked to a reduced therapeutic efficacy, drug resistance, and unfavorable outcomes. Multivariate analysis corroborated that NAP1L5 expression level, patient age, therapeutic response, and karyotypic abnormalities constitute independent prognostic factors for AML, positing NAP1L5 as a viable prognostic marker.

Nonetheless, it is imperative to recognize the constraints of this investigation. The insights garnered herein offer a preliminary comprehension of NAP1L5's involvement in AML pathogenesis, yet the precise mechanism through which NAP1L5 operates remains to be elucidated, warranting further investigation through cellular and animal models in future studies. Moreover, the sample size of this study is relatively modest, and the range of cases covered is restricted. For instance, owing to the comparatively rare occurrence of M0/M6/M7 subtypes within the cohort, patients with these subtypes were excluded, and none of the participants had received allogeneic hematopoietic stem cell transplantation therapy. To surmount these limitations, forthcoming research should aim to augment the sample size and incorporate multicentric data, thereby including patients across various subtypes and enhancing the robustness of the findings.

CONCLUSION

This research endeavored to ascertain the significance of NAP1L5 in individuals diagnosed with AML, revealing that NAP1L5 holds promise as a novel biomarker for predicting AML outcomes. Furthermore, this study proposes that NAP1L5 may offer fresh insights into identifying potential therapeutic targets for AML management.

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Declaration of Interest:
The authors have no conflicts of interest to declare.

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