Expression and Significance of Biomarker SENP1 in Acute Myeloid Leukemia Patients

Keywords
Prognosis, Biomarkers, Acute Myeloid Leukemia, SENP1

Abstract

Introduction
It aimed to investigate the expression of SUMO1/Sentrin specific peptidase 1 (SENPl) in patients with acute myeloid leukemia (AML) and its significance in the development of AML.

Material and methods
Patients with AML, hematological malignancies, and non-neoplastic hematological diseases were selected as the experimental subjects, and they were divided into initial diagnosis subjects (n=32), recurrence subjects (n=8), and remission subjects (n=22) according to their conditions. Meanwhile, patients with non-neoplastic hematological diseases were selected as the controls (n=30). The expression of SENP1 mRNA and protein in bone marrow samples of different groups were detected. The impacts of SENP1 silencing on cell multiplication and cycle were observed.

Results
The expression of SENP1 from low to high was the controls, the initial diagnosis subjects, the remission subjects, and the recurrence subjects. The incidence of adverse conditions was the lowest in the initial diagnosis subjects, followed by the remission subjects and the recurrence subjects. The proliferation of U937 decreased after SENP1 knockdown, suggesting that SENP1 has a major role in AML cell multiplication. In addition, the apoptosis rate of U937 was increased after SENP1 knockdown. The overall survival (OS) and disease-free survival (DFS) were ranked as: recurrence subjects < initial diagnosis subjects < remission subjects < controls.

Conclusions
This article preliminarily confirmed the high expression of biomarker SENPl in AML patients, and found that it was related to the clinical characteristics and prognosis of AML patients.

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Key words: Acute Myeloid Leukemia; SENP1; Biomarkers; Prognosis.

1. Introduction

AML, a hematological malignancy, is caused by the malignant proliferation of naive myeloid cells in the bone marrow [1-2]. It often leads to serious symptoms and complications in a short period of time [3]. Symptoms of AML include fatigue, anemia, easy bleeding and congestion, infection, fever, bone pain, etc. These symptoms are caused by the large accumulation of leukemia cells in the bone marrow, inhibiting normal hematopoietic function and resulting in abnormal numbers of red blood cells, platelets, and white blood cells in the blood.

Methods for the treatment of AML include chemotherapy, hematopoietic stem cell transplantation, and targeted therapy [4-6]. The medical community divides AML into eight types, but the prognosis evaluation is not good. WHO classifies AML into five categories, including AML of unknown lineage, not specially classified AML, treatment-related AML nuclear myelodysplastic syndrome, AML with multilineage dysplasia, and AML with recurrent genetic abnormalities [7]. Although the WHO classification method is more specific for the prognosis and treatment evaluation of AML with recurrent genetic abnormalities, there are still some patients who can’t be accurately evaluated for prognosis.

Some studies have shown that AML is associated with chromosomal and genetic abnormalities, but the pathogenesis is still unclear [8-9]. Studies have shown that protein post-translational modification is important in tumor development, among which

SUMOylation is an important way. Small ubiquitin-like modifier (SUMO) is a small ubiquitin-like modifier protein and represents an important form of post-translational modification. SUMOylation, the covalent attachment of SUMO to target proteins, is involved in the regulation of various cellular processes, including gene transcription, DNA repair, cell cycle progression, and apoptosis. SENP1 (SUMO1/Sentrin Specific Peptidase 1) is a specific deSUMOylating enzyme that catalyzes the removal of SUMO modifications from substrate proteins. By modulating the dynamic balance between SUMOylation and deSUMOylation, SENP1 plays a critical role in tumor initiation and progression. SENP1 can catalyze the removal of many proteins from SUMOylation, which is related to the development of varieties of tumors [10]. SENP1 acts in the development of B, T, and red blood cells, suggesting that the differentiation and development of hematopoietic system cells is closely related to SENP1 [11-12]. Chemotherapy is the main treatment, through the use of drugs to kill leukemia cells. Hematopoietic stem cell transplantation is a method of transplanting healthy blood stem cells into a patient's body in order to restore normal hematopoietic function. Targeted therapy is the use of specific drugs or antibodies to treat specific molecules in leukemia cells [13]. Protein abnormalities act in the prognosis and biological characteristics of AML, among which protein post-translational modification is the most delicate regulation. Common post-translational modifications of proteins in eukaryotic cells include SUMOylation, methylation, acetylation, phosphorylation, and ubiquitination.

At present, studies on the expression and significance of SENP1 in AML patients are

relatively limited. Therefore, this article aimed to further explore the level of SENP1 in people with AML and evaluate its relationship with the prognosis.

2. Methods and materials

2.1 Subjects

Between October 2020 and April 2023, patients diagnosed with AML in the Department of Hematology of Xianyang Central Hospital were selected for the study. These included newly diagnosed patients, those in complete remission, and those with relapsed AML. Patients with other types of hematologic malignancies (including acute lymphoblastic leukemia, chronic myelogenous leukemia, myelodysplastic syndrome, and multiple myeloma) as well as non-malignant hematologic diseases were included as the experimental group. The inclusion criteria for patients with other hematologic malignancies were as follows: diagnosis confirmed by morphology, immunology, cytogenetics, and molecular biology (MICM), and no concurrent systemic malignancies. They were grouped: initial diagnosis subjects (n=32), recurrence subjects (n=8), and remission subjects (n=22), in addition, at the same time, the tumor blood disease patients as controls (n = 30). Bone marrow samples were collected from all subjects, including 2mL bone marrow cells or 2mL peripheral blood mononuclear cells (PBMC) samples (which can be used to replace the bone marrow samples when the peripheral blood leukemia cell count of patients is >80%) from newly diagnosed AML patients before initial chemotherapy. 2mL bone marrow blood samples were collected from the controls.

Inclusion criteria: all enrolled patients met the diagnostic and treatment criteria for hematologic diseases. Informed written consent was obtained from all participants. This study was approved by the Ethics Committee of Xianyang Central Hospital (Approval No. 2022-IRB-63) and was conducted in accordance with the principles of the *Declaration of Helsinki*. Exclusion criteria: patients with other malignant tumors; people unwilling to cooperate with the experiment.

2.2 Acute monocytic leukemia cell lines

The cell line U937 is a commonly adopted laboratory model to study the pathogenesis and treatment of acute monocytic leukemia. The U937 strain was originally derived from a peripheral blood sample of an acute monocytic leukemia patient. These cells had characteristics of monocytes and could proliferate indefinitely under culture conditions. U937 cell line has been widely adopted in basic and clinical research on monocyte differentiation, apoptosis, immune regulation, and so on. U937 was saved by department of hematology laboratory, the Second Affiliated Hospital of Xi'an Jiaotong University.

2.3 T-PCR

Monocytes in bone marrow blood of the experimental group and the controls were isolated, the total RNA was extracted, using reverse transcription kit (TakaRa) for synthesis of cDNA and the Bio-Rad PCR amplification. After the end of the reaction, the expression of SENP1 was calculated by $2^{-\Delta\Delta Ct}$. The specific operation steps are displayed in Table 1.

Table 1. Operation steps for PCR amplification of DNA

Serial number	Steps
1	DNA template preparation: Extracting DNA from the sample
2	PCR reaction system preparation: Determining the required composition of the PCR reaction system according to the experimental design
3	Primer design: upstream 5'-AAGATTCCCAGACTCCAACTCCCA-3'; downstream 5'-TGAATGTTCCCGCTCCTGCAAT-3'
4	Setting the PCR reaction conditions: Determining the appropriate PCR reaction conditions according to the length and sequence characteristics of the target DNA
5	PCR amplification: The PCR reaction system was added to a PCR tube or microplate and put into a PCR instrument for amplification
6	PCR cycle: A PCR reaction typically consists of three main steps: denaturation, annealing, and extension.
7	PCR cycles: according to the required number of target DNA amplification, appropriate PCR cycles were set.
8	At end of PCR reaction: After completing the PCR reaction, stopping heating and keeping the reaction system standing at low temperature to prevent the production of

non-specific amplification products.

9 Analysis of PCR products: The PCR products were analyzed and detected by gel electrophoresis, real-time PCR, or other methods.

2.4 Western blot

Protein immunoblotting (Western blot) is a commonly adopted experimental technique to detect the presence and expressions of specific proteins in samples [14-15]. Mononuclear cells were isolated from the bone marrow blood of the experimental group and the controls. The cells were lysed by RIPA Lysis Buffer (RIPA) to extract protein, and the loading samples were prepared. The protein was analyzed by Quantiy One gel analysis software, the absorbance of each protein band was detected, and the protein expression was calculated. Each protein sample was tested in triplicate and the average value was taken.

2.5 Cell culture

Cell culture refers to the process of removing cells from the *in vivo* or *in vitro* environment and providing suitable conditions in an appropriate medium so that they can continue to grow and reproduce *in vitro* [16-17]. U937 was cultured in a sterile mixture of fetal bovine serum and RPMI-1640 medium in a ratio of 1:9. The culture conditions were 37°C and 5% CO_2 under saturated humidity. The growth of U937 was observed regularly. When the cells were in log phase, they were adopted for experimental study.

2.6 Cell transfection

Cell transfection refers to the process of introducing foreign genes or other biological molecules into cells, which plays a major role in biological research [18-19]. It can be adopted in gene function research, gene therapy, drug screening, and other fields. U937 in log phase was digested with trypsin. Cells in a plate having 6 wells at 1×105, regular training was carried out for 24 h, alignment around 75%, according to the Lipofectamine2000 introduction, transfection was carried out, and it was grouped: sh-NC, sh-SENP1. The silencing rate of sh-SENP1 was determined by RT-qPCR 48 h after transfection.

2.7 Cell cycle detected by flow cytometry

U937 was divided into sh-NC and sh-SENP1 groups. The cells in log phase were inoculated in a plate with 6 wells (5×10^4). After the cells adhered to the wall, the medium was discarded, and trypsin was added, tapping the Petri dish to wash it thoroughly, digesting and collecting the single cell suspension. After that, they were washed twice with 1mL of precooled PBS, supplemented with fluorescein stain PI and ribonuclease, and the cells were incubated at 37°C in the dark for half an hour. The distribution in G0/G1, S, and G2/M cycles was determined by flow cytometry. All experiments were repeated for 3 times to ensure the accuracy and reliability of the results.

2.8 Cell counting kit-8(CCK-8) assay to detect cell multiplication

CCK-8 is a commonly adopted cell multiplication and cytotoxicity assay. The method uses WST-8, a water-soluble MTT (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) was adopted as an indicator to determine the metabolic activity of cells through the reduction reaction [20-21]. Through 48 h of transfection, the cells were subjected digestion and resuspension into a plate having 96 wells at 5000 cells, 100 μL of medium was applied, and then it was routinely cultured overnight. Next, the cells were treated according to the steps in the CCK-8 kit instructions. At 24, 48, 72, and 96 h after inoculation, 10μL of CCK-8 assay solution was applied. Incubation was carried out for 4 h. The absorbance at 450nm was measured, and the growth curve was plotted. Every time the experiment was repeated three times, in order to ensure the accuracy. By comparing cell viability in the sh-NC and sh-SENP1, the impact of SENP1 gene on U937 proliferation and survival could be evaluated.

2.9 Statistical methods

Statistical analyses were performed using *SPSS version 21.0*. Continuous variables were expressed as mean ± standard deviation ($\bar{x} \pm s$). Comparisons between two groups were conducted using the independent samples t-test, while comparisons among multiple groups were performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. Kaplan–Meier method was employed to construct overall survival (OS) and disease-free survival (DFS) curves, and differences between groups were

assessed using the log-rank test. A two-tailed *P*-value < 0.05 was considered statistically significant. Sample size and power analysis: Due to the clinical difficulty in recruiting patients with relapsed AML, a prospective power analysis was not performed. A retrospective power calculation using *G*Power* indicated that, assuming an effect size (Cohen's d) of 0.8 and a significance level of α = 0.05 (two-tailed), the statistical power for detecting differences in SENP1 expression between the newly diagnosed group (n = 32) and the relapsed group (n = 8) was approximately 65%. The sample sizes for survival analysis were generally sufficient to meet the requirements for Kaplan–Meier curve estimation.

3. Results

3.1 General information

The general data of the subjects are displayed in Table 2. There was not obviously different in gender, age, body mass index (BMI) of the initial diagnosis subjects, the recurrence subjects, the remission subjects, and the controls.

Table 2. Contrast of general information of the subjects

Groups	Number of people	Gender (male/female)	Age (years)	BMI (kg/m^2)
Initial diagnosis subjects	32	14/18	66.4±5.7	23.5±4.7
Recurrence subjects	8	4/4	65.3±4.8	17.8±5.8

Remission subjects	22	12/10	67.7±6.3	18.5±6.3
Controls	30	16/14	65.8±7.54	24.3±5.5

3.2 Expression of SENP1

In Figure 1, the SENP1 expression was lowest in the control group and highest in the relapse group. Compared to the control group, the 95% confidence interval for SENP1 expression in the newly diagnosed group was (1.98-2.70), which was significantly higher ($P < 0.05$). The 95% confidence interval for SENP1 expression in the relapse group was (4.35-5.89), with a highly significant difference compared to the control group ($P < 0.001$).

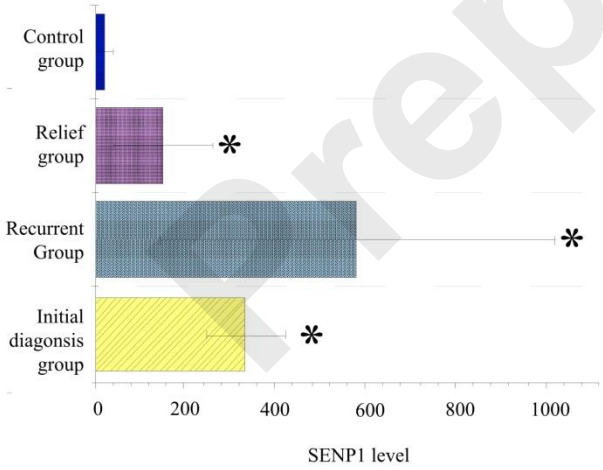


Figure 1. Contrast of RT-PCR results of SENP1 expression of patients

Note: * represents as against controls, $P < 0.05$

Figure 2 presents the Western blot bands of SENP1 for each patient group. The band intensity of SENP1 in the control group was the lowest, while the intensity in the relapse

group was the highest. The band intensities in the initial diagnosis and remission groups were intermediate.

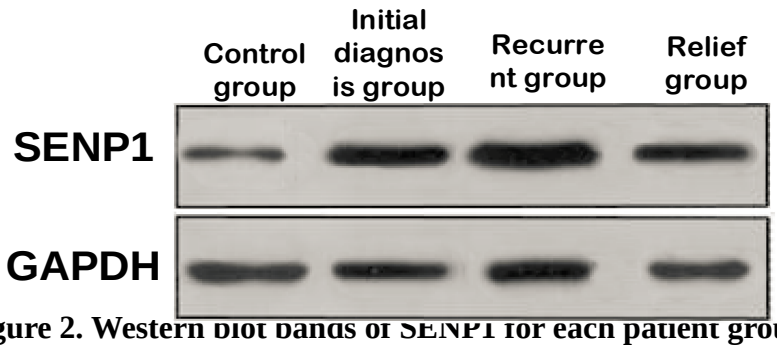


Figure 2. Western blot bands of SENP1 for each patient group

3.3 Contrast of poor prognosis

The 95% confidence interval for the incidence of adverse prognosis in the relapse group was 56.8%–93.2%, which was significantly higher than that of the newly diagnosed group (16.3%–45.9%) and the remission group (32.5%–67.5%). Chi-square test demonstrated that the differences among the groups were highly significant ($\chi^2 = 18.72$, $P < 0.001$).

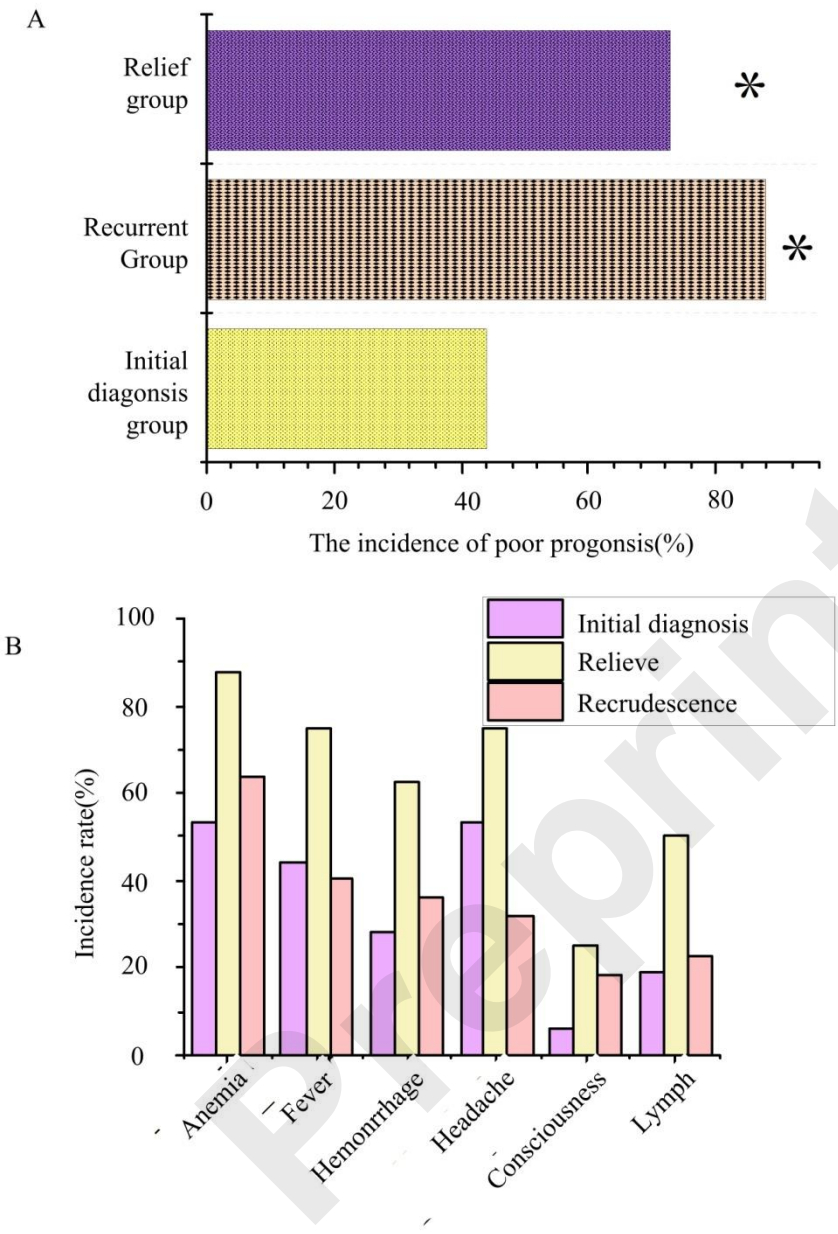


Figure 3. Contrast of poor prognosis

A: Contrast of the incidence of poor prognosis

B: The specific incidence of adverse conditions

Note: * represents recurrence subjects, remission subjects versus initial diagnosis subjects,

P <0.05

3.4 U937 transfection results

After transfection in sh-SENP1 group, the SENP1 expression level in U937 line decreased by about 70% as against sh-NC group, which indicated that the transfected cell line was a stable cell line with knockdown of SENP1 (Figure 4).

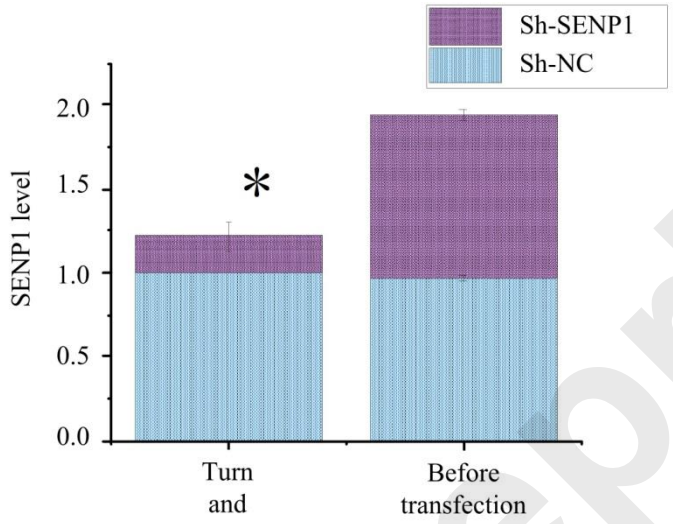


Figure 4. Results of U937 transfection

Note: * means sh-SENP1 versus sh-NC, P <0.05

3.5 Effect of SENP1 on AML cell multiplication

Under the condition of normal serum culture, constructed good stable transfection cell line was adopted to explore the impact of SENP1 on AML cells proliferation. The results suggested that SENP1 knockdown in U937 visibly reduced cell multiplication, suggesting that SENP1 plays a major role in promoting AML cell multiplication (Figure 5).

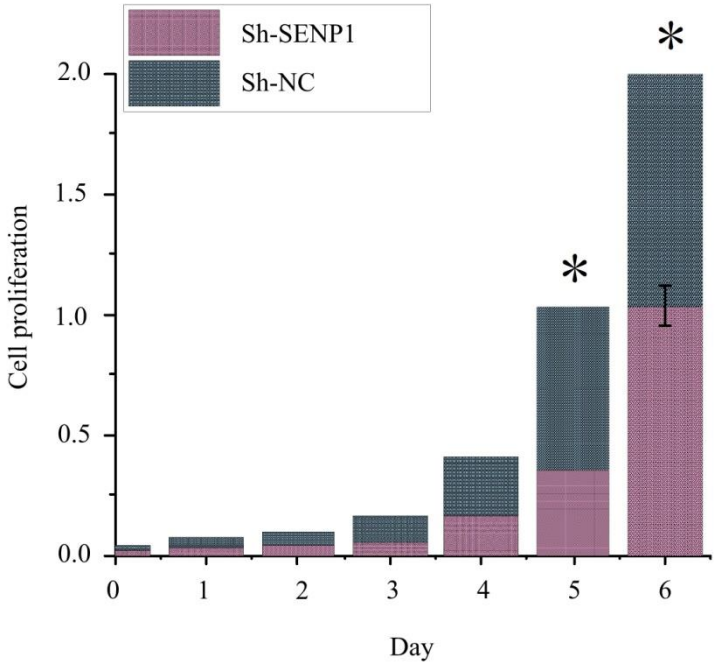


Figure 5. Contrast of cell multiplication ability between sh-SENP1 and sh-NC groups after knocking down SENP1

Note: * means sh-SENP1 versus sh-NC, P <0.05

3.6 SENP1 on AML apoptosis

The impact of SENP1 on spontaneous apoptosis of AML cells was investigated using the constructed stable transfected cell lines under normal culture conditions. The cells were collected, and the difference in the incidence of apoptosis between the sh-SENP1 and the sh-NC groups was detected adopting Annexin V/PI method. It suggested that the incidence of apoptosis in U937 was visibly increased after knocking down SENP1 (Figure 6).

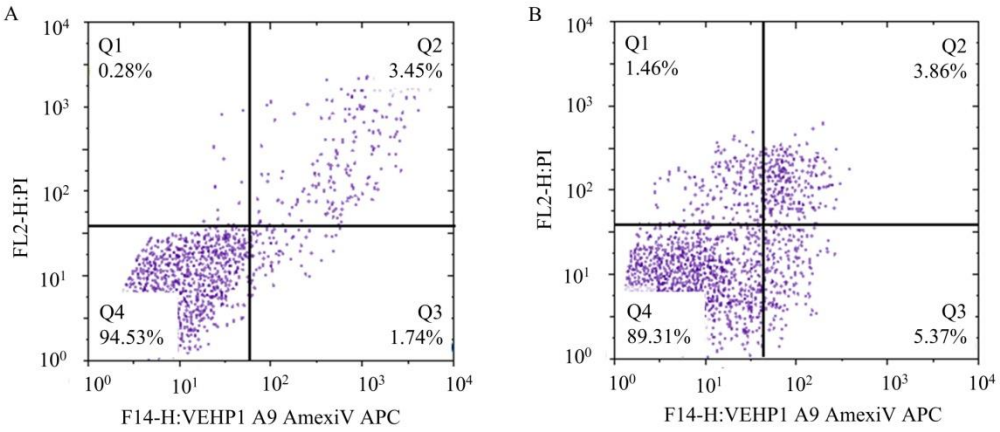


Figure 6. Spontaneous apoptosis rates in sh-SENP1 and sh-NC groups

A: spontaneous apoptosis rate in sh-NC; B: spontaneous apoptosis rate in sh-SENP1

Note: Q1, Q2, Q3, and Q4 represent G0/G1, S, G2, and M phases, respectively

3.7 SENP1 relationship with DFS and OS in AML

Kaplan–Meier analysis (Figure 7) demonstrated that the 1-year OS rate 95% confidence interval was 85.2%–98.8% in the control group and 18.2%–56.8% in the relapse group, with a significant difference between the survival curves (log-rank test, $P < 0.001$). The 95% confidence intervals for the OS rates in the newly diagnosed and remission groups were 68.3%–87.9% and 72.5%–91.3%, respectively, with a significant difference between these groups ($P = 0.021$).

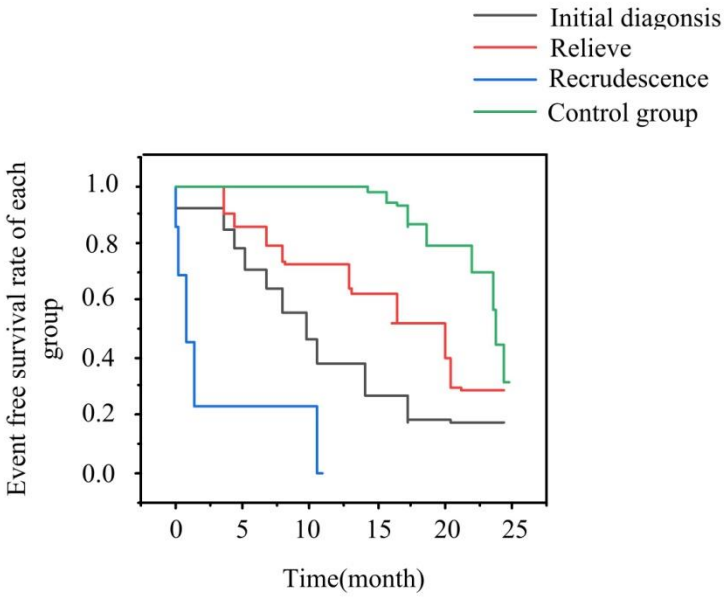


Figure 7. Relationship between SENP1 and OS and DFS in AML

4. Discussion

AML is a common hematological malignancy with an incidence of approximately 4 per 100,000 and is more common in middle-aged and elderly men. Many immature myeloid precursor cells appear in patients, leading to impaired hematopoietic function, resulting in symptoms such as infection, bleeding and anemia, and eventually leading to death [22]. With the increase of elderly population in China, the incidence of AML is increasing year by year, posing a threat to people's life and health. Studies have shown that SENP1 acts in tumor development, and scholars have achieved certain results [23-24]. Studies have found a high expression of SENP1 in AML patients' tissues. This is consistent with the results of previous studies, suggesting that SENP1 may act in the occurrence and progression of AML. SENP1 is an important ubiquitin-like modifying enzyme involved in

the regulation of a variety of cellular processes, including gene transcription, DNA repair, and apoptosis. The high expression of SENP1 may be related to the abnormal proliferation, anti-apoptosis, and decreased DNA damage repair ability of AML cells.

Research shows that SENP1 in AML plays a major role. Experiments have found that highly expressed SENP1 is associated with poor prognosis of people with AML. Specifically, highly expressed SENP1 was associated with shorter OS and DFS in people with AML. This may be because the highly expressed SENP1 promotes the abilities of AML cells, while inhibiting apoptosis and immune response. As a deubiquitylation enzyme, SENP1 can regulate the activity of varieties of signaling pathways, containing apoptosis, DNA repair, etc. Thus, the highly expressed SENP1 may promote AML cells and suppress apoptosis [25]. It suggested that the proliferation rate of AML cells was visibly decreased after SENPI knockdown. Through cell multiplication assay and cycle analysis, it was found that SENPI knockdown prolonged the G1 phase of AML cells, thereby limiting the cell multiplication. In addition, SENPI knockdown could also promote apoptosis of AML cells, which was verified by the incidence of apoptosis in sh-SENP1 and sh-NC groups detected by Annexin V/PI method. Because the SENP1 as an important role in the occurrence and development of AML, inhibition of SENP1 could become a new treatment strategy. It has been shown that SENP1 inhibitors can suppress the proliferation and induce apoptosis of AML cells [26]. Therefore, further studies can explore the potential of SENP1 inhibitors as therapeutic agents for AML and evaluate their safety and efficacy in the clinic. Secondly, the relationship between SENP1 expression and clinical characteristics of AML

patients was further explored. It was found that high SENP1 expression was associated with poor prognostic indicators, including lower OS and DFS. This suggests that the high expression of SENP1 may be closely related to the poor prognosis of AML patients, suggesting that SENP1 may serve as a potential prognostic marker. This finding holds potential clinical translational value. From a prognostic assessment perspective, measuring SENP1 expression levels in bone marrow samples may serve as an independent prognostic indicator—patients with high SENP1 expression at initial diagnosis may require intensified induction chemotherapy regimens, while SENP1 expression levels in relapsed patients could be utilized to monitor disease progression. In terms of therapeutic strategy, as a deSUMOylating enzyme, SENP1 represents a promising target for AML treatment. The development of SENP1 inhibitors may offer a novel targeted therapy approach by specifically suppressing SENP1 activity to arrest the AML cell cycle and induce apoptosis, particularly in relapsed or refractory cases. Notably, the inhibitory effect of SENP1 silencing on U937 cell proliferation observed in this study provides preliminary evidence supporting preclinical drug development.

In contrast to other experiments [27-29], this article aimed to explore SENP1 expressions and significance in AML patients. Although there have been many studies on SENP1 in other tumors, the role and significance of SENP1 in AML have not been fully explored. Therefore, this article fills this knowledge gap and is meaningful for in-depth understanding of AML. Secondly, this article explored the relationship between SENP1 expressions and clinical characteristics of AML patients. By analyzing the correlation

between high expression of SENP1 and poor prognostic indicators in AML patients, such as lower OS and DFS, this article provides the potential value of SENP1 as a prognostic marker. However, there are some limitations, and the sample size of this article is relatively small. Because AML is a rare disease and the number of patients is limited, the sample size may be limited. The reliability of the statistical analysis and the reliability of the results may be affected. Larger studies and multi-center collaborative studies can be helpful to increase the subjects and improve the credibility of results. This article mainly depends on the tissue samples of SENP1 expression, the lack of analysis of patients with other sources of biological specimens. SENP1 expressions in blood samples may better reflect the overall condition and disease progression of AML patients. Therefore, future studies could use blood samples as research subjects to further validate and extend these results; the mechanism of SENP1 expression was not studied in detail in this article. Understanding the regulatory mechanism of SENP1 may help better understand its role and significance in AML. Exploring the upstream regulators and downstream targets of SENP1, as well as possible signaling pathways and molecular mechanisms, will help reveal the functional mechanism of SENP1 in AML. Although this article discussed the significance of SENP1 in AML and potential therapeutic targets, but more experimental and clinical research are needed to verify the results. Especially for the clinical application of SENP1 inhibitors, more in-depth studies are needed, including the evaluation of drug safety, tolerability, and efficacy. Current research on SENP1 in hematologic malignancies has largely focused on acute lymphoblastic leukemia (ALL) and multiple myeloma (MM), with relatively limited

exploration of its function in AML. Studies showed that in acute B-lymphoblastic leukemia cells, SENP1 promotes cell proliferation by activating the c-Myc signaling pathway, and its high expression is associated with chemotherapy resistance. In multiple myeloma, SENP1 regulates ribosomal protein function through deSUMOylation, thereby influencing tumor cell invasiveness [30-32]. In contrast to these studies, this research is the first to demonstrate in AML patients that high SENP1 expression is significantly associated with disease relapse and poor prognosis. Furthermore, SENP1 silencing inhibits AML cell proliferation by blocking the G1 phase of the cell cycle and inducing apoptosis. This finding not only broadens the scope of SENP1's role in hematologic malignancies but also provides new insights for AML prognostic evaluation and targeted therapy. It is important to note that the mechanism of SENP1 in different types of hematologic cancers may differ, suggesting that its function could be tumor-type specific, which warrants further validation in subsequent studies.

This study indicated that high SENP1 expression is significantly associated with disease relapse and poor prognosis in AML patients, suggesting potential clinical translational value. From a prognostic assessment perspective, measuring SENP1 expression levels in bone marrow samples may serve as an independent prognostic indicator—patients with high SENP1 expression at initial diagnosis may require more intensive induction chemotherapy regimens, while SENP1 expression levels in relapsed patients could be used to monitor disease progression. In terms of therapeutic strategies, as a deSUMOylating enzyme, the development of SENP1 inhibitors could offer a new

direction for targeted therapy in AML. By specifically inhibiting SENP1 activity, it may be possible to block the AML cell cycle and induce apoptosis, particularly in relapsed or refractory cases, thus holding potential clinical application. It is noteworthy that the inhibitory effect of SENP1 silencing on U937 cell proliferation in this study provides preliminary evidence for preclinical drug development. Future research should focus on in vivo experiments involving SENP1 inhibitors, such as AML mouse models, to verify their efficacy and safety. Additionally, exploring the relationship between SENP1 expression levels and drug sensitivity could help guide clinical treatment decisions. For example, developing a combination regimen of "chemotherapy + SENP1 inhibitors" for patients with high SENP1 expression may improve prognosis. Furthermore, the combined detection of SENP1 with other prognostic markers, such as NPM1 mutations and FLT3-ITD, could potentially create a more precise AML prognostic stratification system, offering a foundation for personalized treatment strategies.

5. Conclusion

The results suggested that in AML patients, the high expression of SENP1 was correlated with clinicopathological features, and SENP1 promoted the development of AML by promoting the proliferation of AML cells. Therefore, SENP1 may become a potential biomarker for the development of diagnosis and treatment strategies for AML patients. Further studies are needed to explore the specific mechanism of SENP1 in the development of AML and to find targeted therapies against SENP1 to improve the prognosis of AML patients.

This study has several limitations. First, the sample size is relatively small. Since AML is a rare disease, the study included only 32 newly diagnosed patients and 8 relapsed patients. Such a small sample size may affect the reliability of statistical analysis, and future studies should aim to increase the sample size or conduct multi-center collaborative research to further validate the results. Second, the type of biological specimens used is relatively limited. The study only analyzed SENP1 expression in bone marrow tissue, without validation in other biological specimens such as blood. Blood samples may provide a more comprehensive reflection of the overall disease progression and status. Third, the mechanistic investigation is not sufficiently in-depth. The study only observed the effects of SENP1 on cell proliferation and apoptosis, without exploring its upstream regulatory factors and downstream signaling pathways, such as the SENP1/c-Myc signaling axis. Future research should incorporate additional molecular biology techniques to further elucidate the specific mechanisms of action.

Reference

[1] Pelcovits A, Niroula R. Acute myeloid leukemia: a review. R I Med J 2020; 103(3):38-40.

[2] Montesinos P, Recher C, Vives S, et al. Ivosidenib and azacitidine in IDH1-mutated acute myeloid leukemia. N Engl J Med 2022; 386(16):1519-1531.

[3] Goyal S, Tisdale J, Schmidt M, et al. Acute myeloid leukemia case after gene therapy for sickle cell disease. N Engl J Med 2022; 386(2):138-147.

[4] Carter JL, Hege K, Yang J, Kalpage HA, Su YW, Edwards H, Het al. Targeting multiple signaling pathways: the new approach to acute myeloid leukemia therapy. Signal Transduct Target Ther 2020; 5(1):288(1-29).

[5] Cui XM, Gao W, He JJ, Zhang B. Screening and clinical testing of key differentially expressed genes in childhood acute myeloid leukemia. J Henan Univ (Med Ed) 2023; 42 (01):23-27.

[6] Niu SQ, Zhao LM. Metagenomic next-generation sequencing clinches the diagnosis of Legionella pneumonia in a patient with acute myeloid leukemia: A case report and literature review. Front Cell Infect Microbiol 2022; 924597.

[7] Mhina CJ, Cole AC., Adapa K, Bryant AL, Wood A, Richardson DR. Either go through chemotherapy or go through dying: A qualitative study of the experiences of treatment decision-making among older newly-diagnosed adults with acute myeloid leukemia. J Clinical Oncol 2022; 40(28_suppl):253-253.

[8] Sun HJ, Du CK, Li H, Liu M, Zhang H. Diagnostic significance of cytogenetic testing methods for acute leukemia in children with MLL gene abnormalities. J Lab Med 2022; 37(12):1196-1199.

[9] Liu LN, Li T, Ge HY, Chen ZG, Yu J, Zheng XQ. Association between MTHFR C677T gene polymorphism and liver dysfunction after high-dose methotrexate treatment in children with acute lymphoblastic leukemia. J Wenzhou Med Univ 2020; 50(09):753-757.

[10] Liang YQ, Duan T, Xu QQ. Research progress on the correlation between SENP1 and

malignant tumors. Chongqing Medical Journal 2023; 52(05):776-781.

[11] Qin X, Chen X, Liu J, Zeng Y, Guo L, Liu WJ. Paeoniflorin inhibits the SENP1/c-Myc signaling pathway and induces apoptosis and cell cycle arrest in acute B lymphoblastic leukemia cells. Chin J Traditional Chin Med 2022; 47(12):3312-3319.

[12] Song JP, Shu P, Ma JL, Wang B, Chen B. Expression of ribosomal protein S9 in multiple myeloma and its impact on its cellular biological function. J Chin Acad Med Sci 2019; 41(02):175-182.

[13] Li Y, Li X, Chen Z. Research progress of SHP2 and its allosteric inhibitors in tumor targeted therapy. Pharm Res 2023; 42(01):48-53,67.

[14] Qian X, Deng YQ, Zhong N, Li YX, Wang JH, Yan JH, et al. Study on the reversal of epileptic drug resistance by tetramethylpyrazine in combination with carbamazepine through modulation of P-Glycoprotein expression in rat brain microvessel endothelial cell. Cell Mol Biol (Noisy-le-Grand) 2023; 69(11):254-259.

[15] You M, Yang L. Study on the regulatory effect of Gong's brain on the spinal MAPKs signaling pathway in a bone cancer pain model rat. J Liaoning Univ Traditional Chin Med 2023; 25 (11):24-28.

[16] Wu J, Yang Z, Yang B, Huang H. The application of organoids in the research of skeletal diseases: current status and prospects. Studies in health technology and informatics 2023; 308597-604.

[17] Xie J, Qi J. In vitro culture and identification of PEX gene transfected human umbilical cord mesenchymal stem cells. Med. Inform 2023; 36 (10):88-92,97

[18] Yu WH, Kang XH. The effect of LINC01410 on the proliferation, invasion, and migration of lung cancer cell A549. Chin J Gerontol 2023; 43(21):5317-5321

[19] Xuan CR. The effect and mechanism of CKLF1 overexpression on the proliferation, migration, and invasion ability of renal cancer cells. J Nankai Univ (Natur Sci Ed) 2023; 56(04):35-39.

[20] Lin XT, Zhang J, Xie CM. An optimized protocol to detect protein ubiquitination and activation by ubiquitination assay in vivo and CCK-8 assay. STAR protoc 2023; 4(2):102199.

[21] Yang LQ, Wang L, Wu JT, Wang YQ. Circ_0000069 contributes to the growth, metastasis and glutamine metabolism in renal cell carcinoma (RCC) via regulating miR-125a-5p-dependent SLC1A5 expression. Transpl Immunol 2022; 77101764.

[22] Peng XR, Cheng J. Research progress on chimeric antigen receptor modified NK cells in the treatment of acute myeloid leukemia. Chin J Exp. Hematol. 2023;31(06):1905-1909.

[23] Zhang YR, Shen YJ, Wei WQ, Wang WH, Jiang DJ, Ren YZ, et al. Dysregulation of SIRT3 SUMOylation confers AML chemoresistance via controlling HES1-Dependent fatty acid oxidation. Int J Mol Sci 2022; 23(15):8282.

[24] Tao Y, Wei L, You H. Ferroptosis-related gene signature predicts the clinical outcome in pediatric acute myeloid leukemia patients and refines the 2017 ELN classification system. Front Mol Biosci 2022; 954524.

[25] Li DT, Ai H, Huang SJ. Research progress of SUMO modification in breast cancer. J

Qiqihar Med Coll 2023; 44(06):563-568.

[26] Xu CM, Wang CH, Ding YM. Research progress on the role of ubiquitin specific protease 1 in tumors. Chin J Pract Diagn Treat 2022; 36(07):753-756.

[27] Song KP, Shu P, Ma JL, Wang B Chen B. Ribosomal protein S9 expression in multiple myeloma and its effect on cell biological function. Zhongguo Yi Xue Ke Xue Yuan Xue Bao 2019; 41(2):175-182.

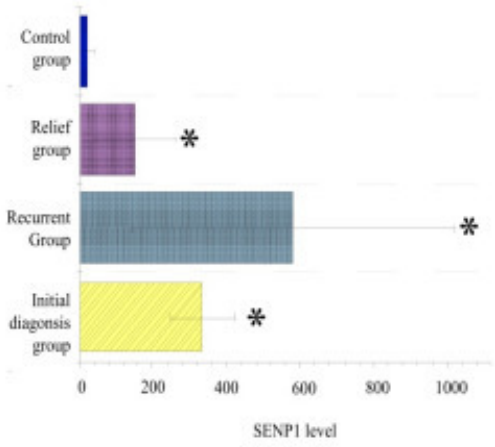
[28] Mrózek K. Prognostic importance of the fusion partners and measurable residual disease in patients with acute myeloid leukemia who harbor 11q23/KMT2A alterations. Transl Pediatr 2023; 12(10):1920-1925.

[29] Isidori A, Visani G, Ferrara F. Fms-like tyrosine kinase 3 positive acute myeloid leukemia. Curr Opin Oncol 2023; 35(6):589-593.

[30] Sun XX, Chen Y, Su Y, Wang X, Chauhan KM, Liang J, et al. SUMO protease SENP1 deSUMOylates and stabilizes c-Myc. Proc Natl Acad Sci U S A. 2018 Oct 23;115(43):10983-10988.

[31] Gao Q, Wang S, Zhang Z. E3 ubiquitin ligase SMURF2 prevents colorectal cancer by reducing the stability of the YY1 protein and inhibiting the SENP1/c-myc axis. Gene Ther. 2023 Feb;30(1-2):51-63.

[32] Xianjun F, Xirui X, Jie T, Huiwen M, Shaojun Z, Qiaoyun L, et al. Momordin Ic induces G0/1 phase arrest and apoptosis in colon cancer cells by suppressing SENP1/c-MYC signaling pathway. J Pharmacol Sci. 2021 Aug;146(4):249-258.


Control group
Relief group
Recurrent Group
Initial diagonsis group
0
200
400
600
800
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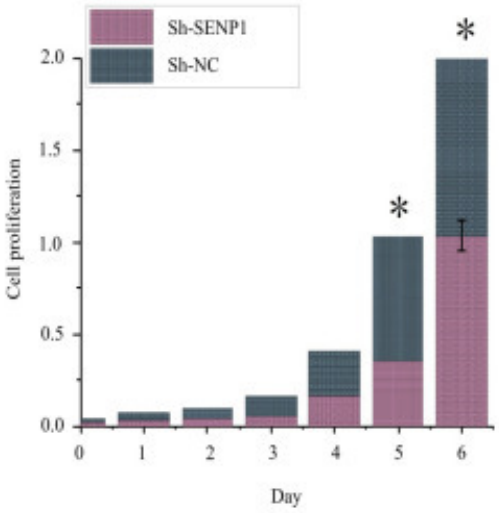
SENP1 level


Sh-SENP1
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Cell proliferation
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