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Correlation between IL-7 genomic protein methylation level and acute myeloid leukemia

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Abstract. – OBJECTIVE: To detect Interleukin-7 (IL-7) gene methylation status and transcription level in leukemia cells of peripheral blood of patients with Acute Myelocytic Leukemia (AML) and in the cell lines (HL-60, HL-60/ADM, SKM-1) of AML and myelodysplastic syndrome (MDS), and explore its relationship with the pathogenesis of AML.

PATIENTS AND METHODS: A total of 55 AML patients (AML group) and 30 healthy adults (Healthy group) from June 2015 to June 2018 were enrolled in this study. The genomic DNA of leukemia cells in peripheral blood was extracted. The methylation-specific PCR (MSP) method was used to detect the methylation rate of the IL-7 gene in peripheral blood of AML group and Health group. Meanwhile, the methylation level of the IL-7 gene leukemia cell lines HL-60/ADM, HL-60, and MV4-11 and SKM-1 were detected *in vitro*. At the same time, the expression level of IL-7 in peripheral blood was detected by Reverse Transcription-Polymerase Chain Reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA) kit.

RESULTS: The methylation rate of IL-7 gene in peripheral blood of the AML group and Healthy group was 72.7% (40/55) vs. 3.3% (1/30) ($p<0.01$); IL-7 gene methylation occurred in HL-60/ADM, HL-60, MV4-11 and SKM-1 cell lines. IL-7 gene methylation appears in peripheral blood leukemia cells and AML and MDS cell lines of AML patients.

CONCLUSIONS: The expression of IL-7 in peripheral blood of patients with AML is significantly decreased, suggesting that this phenomenon is related to the pathogenesis of AML.

Key Words:
Acute myelocytic leukemia, IL-7, Methylation.

Introduction

Acute Myelocytic Leukemia (AML) is a highly invasive hematological malignancy characterized by a massive proliferation of immature myeloid cells in the bone marrow and blood[1]. The pathogenesis of AML is closely related to the proliferation, differentiation and apoptosis of leukemia cells, and can be caused by different factors such as radiation, mutation and carcinogen[2,3]. The condition of AML is developing rapidly, and if it is not treated in time in the short term, it is very likely to be fatal[4]. Factors that have been reported to affect AML prognosis include age, gender, genetic mutations, complex karyotype abnormalities, history of the hematological disease, elevated white blood cell counts and history of radiotherapy and chemotherapy in other malignant tumors *in vivo*[5]. Although intensive chemotherapy has a significant effect on most patients with AML at the beginning of the disease, residual tumor cells in the body still cause recurrence of AML and poor prognosis[6]. In addition, current treatment of elderly AML patients and patients with relapsed and refractory AML remains challenging[7]. Therefore, understanding the molecular mechanism of AML can help improve its therapeutic effect and prolong the survival time of patients.

DNA methylation is an important epigenetic mechanism that regulates AML gene expression[8]. Studies[9,10] have shown that progression from myelodysplastic syndrome (MDS) to AML is associated with an abnormal increase in DNA methylation. For example, in AML, there is common methylation of p15 and E-cadherin[11]. In addition, in the AML cell line, secreted frizzled-related proteins (sFRP) sFRP1, sFRP12, sRFP13 and sFRP15 have a certain degree of methylation[12]. Survival analysis showed that GATA-binding protein 4 promoter methylation was significantly associated with shorter overall survival in children with AML[13]. Interleukin-7 (IL-7) is a T cell growth factor involved in late immune reconstitution, which can induce the expression of B_{7-1}

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and B_{7-2} in leukemia cells, thereby increasing the immunogenicity of leukemia cells[14]. However, the correlation between the methylation level of the IL-7 gene and the onset and prognosis of AML patients has not been reported yet.

In this work, the whole genome of peripheral blood leukemia cells and Myelodysplastic Syndrome (MDS) cell lines of AML patients and healthy people were collected, and IL-7 methylation was detected by methylation-specific PCR (MSP). At the same time, the mRNA and protein expression levels of serum IL-7 in AML patients were also detected.

Patients and Methods

Peripheral blood samples from 55 patients with AML who were admitted to the department of Hematology in Weifang People's Hospital from June 2015 to June 2018 were collected. The patients aged (54.23-71.89) years, 25 males and 30 females (AML group). The diagnostic criteria referred to the Chinese guidelines for the diagnosis and treatment of adult acute myeloid leukemia (non-acute promyelocytic leukemia) (2011 edition). The control group consisted of 30 healthy people in the physical examination center of the Weifang Medical University (Healthy group), aged (57.66-63.41) years, 15 males and 15 females (AML group). 4 ml of venous blood was taken, anticoagulated with sodium citrate (LI-COR Biosciences, Lincoln, NE, USA) and placed in a refrigerator at -20°C for storage. The study was approved by the Ethics Committee of the Weifang Medical University, and all of the participants signed the informed consent form.

Detection of IL-7 Expression in the Serum by ELISA

(1) 3 mL of the blood sample was collected; (2) standard solution was prepared in accordance with the instructions of the kit (Abcam, Cambridge, MA, USA); (3) standard solution and samples were added to each reaction well; (4) streptavidin-HRP was added for incubation; (5) washing and color was developed; (6) after adding the stop solution, the absorbance was detected by an ultraviolet spectrophotometer.

Detection of IL-7 Expression in the Serum by RT-PCR

(1) The total RNA of the peripheral blood was extracted by TRIzol method, then the concentration and purity of the extracted RNA was detected by UV spectrometer, samples with an A260/A280 was ready for use; (2) mRNA was synthesized into cDNA by reverse transcription, and stored in -80°C refrigerator; (3) Reverse Transcription-Polymerase Chain Reaction (RT-PCR) system: 10 ×Buffer 2.5 μl; cDNA 2 μl; forward primer (20 μ mol/ L) 0.25 μl; reverse primer (20 μmol/L) 0.25 μl; dNTPs (10 mmol/L) 0.5 μl; Taq (2×10^6 U/ L) 0.5 μl; ddH_2O 19 μl. The amplification systems for RT-PCR were identical. The reverse transcription kit (#4896866001) was purchased from Roche (Basel, Switzerland).

Sources of MDS Cell Line

4 MDS leukemia cell lines were adopted in this experiment: MV4-11 cell line (CCL-240, American Type Culture Collection (ATCC, Manassas, VA, USA); HL-60 (CRL-9591), HL-60/ADM cell line (004178, Tianjin Institute of Hematology); SKM-1 cell line (00145, Service Biotechnology, Wuhan, China).

MDS Cell Line Culture

Leukemia cells at a concentration of 1 $X10^6$/ ml were inoculated on 5 mL of medium containing 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA) double-antibody (Chloramphenicol and penicillin), in which the SKM-1 and HL-60/ADM cell lines were cultured in the Roswell Park Memorial Institute-1640 (RPMI-1640) medium, while MV4-11 and HL-60 were cultured in Iscove's Modified Dulbecco's Medium (IMDM; Gibco, Grand Island, NY, USA) medium. All 4 cell lines were cultured in an incubator at 37°C and 5% CO_2. After 2 days, the cells were passaged at a ratio of 1:2, subsequent experiment was performed using cells in the logarithmic growth phase.

Methylation-Specific Polymerase Chain Reaction (MSP)

EDTA anticoagulation blood 4 mL was collected from patients, genome DNA was extracted according to the instructions in the DNA extraction kit Service Biotechnology (Wuhan, China). 2 μmL was taken for electrophoresis in 1.5% agarose gel to detect the mass; at the same time, DNA concentration was detected by an ultraviolet spectrophotometer. 1000 ng DNA from the Healthy group and the AML group was collected for bisulfite pressure modification; the detailed procedure was in accordance with the kit instruction

Table I. Methylation-related primer sequence.

Name	Sequence	
Methylation-specific primers	Upstream	5'-ACGACTGATCGTAGCTGATGCTAG-3'
	Downstream	5'-TGCTGATCGTAGCTAGCTGATGCTGA-3'
Non-methylation-specific primers	Upstream	5'-CGATCGCGGTAGCTGATCGATGCCCC-3'
	Downstream	5'-ACGATCGATGCGGGGCATGCTGATGCTAG-3'

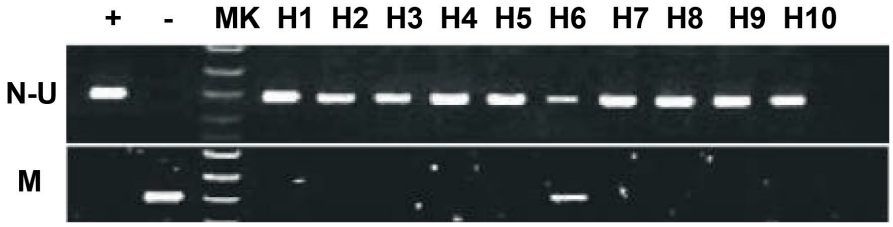

Figure 1. IL-7 methylation status in peripheral blood of healthy people. H1-H10 represents 10 healthy people, M represents methylation, and methylation of H6 is observed.

(Shanghai Kanglang Biotechnology Co., Ltd., Shanghai, China); the modified DNA was stored for later use. PCR reaction system: 200 ng of DNA modified with bisulfite, primers 10 μmol/L 1 μL, Premix Ex Taq HS 25 μL, ddH_2O top up to 50 μL; reaction conditions are as follows: 1) Pre-denaturation at 95°C for 10 min; 2) 94°C denaturation 0.5 min; 3) 59°C annealing 0.5 min; 4) 72°C extension 30 s, a total of 35 cycles; 5) 72°C extension 10 min; methylated and unmethylated primer sequences as shown in Table I. Subsequently, agarose gel electrophoresis was performed.

Statistical Analysis

All data were analyzed using SPSS 22.0 (SPSS IBM, Armonk, NY, USA). The enumeration data were expressed as frequency and percentage, and the measurement data were expressed as mean ± standard deviation. $p<0.05$ was considered statistically significant.

Results

IL-7 Methylation Status in Peripheral Blood of Healthy People

First, we examined the methylation levels of the peripheral blood whole DNA in the Healthy group of 30 healthy people. The results showed that only one sample showed incomplete methylation of IL-7, indicating that the incidence of IL-7 methylation in the normal population is approximately 3.3% (Figure 1).

IL-7 Methylation Status in Peripheral Blood of Patients With AML

Subsequently, we examined the incidence of IL-7 methylation in peripheral blood of patients with AML. The results showed (Figure 2) that a total of 40 patients in 55 patients developed methylation of IL-7, and the incidence of methylation was approximately 72.7% in the AML group (Figure 2).

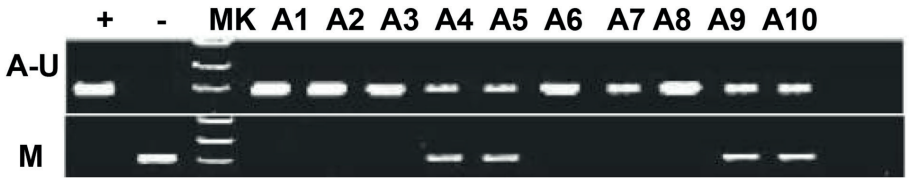

Figure 2. IL-7 methylation status in peripheral blood of patients with AML. A1-A10 represents 10 patients with AML, M represents methylation, and methylation occurs in A4/5/9/10.

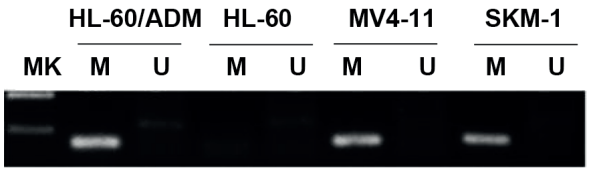

Figure 3. IL-7 methylation status in MDS leukemia cell line. M represents methylation.

IL-7 Methylation Status in MDS Leukemia Cell Lines

We also examined the methylation status of IL-7 in four leukemia cell lines, including HL-60/ADM, HL-60, MV4-11, and SKM-1. The results showed that all four cell lines showed IL-7 methylation (Figure 3).

Correlation Between IL-7 Methylation and Clinical Factors in Patients with AML

Furthermore, we analyzed the association of IL-7 methylation in peripheral blood with the clinicopathological features in patients with AML. The results showed that there was no significant correlation between IL-7 methylation and patient age and FAB typing ($p>0.05$; Table II). However, there was a statistically significant association with gender, recurrence and progression ($p<0.05$).

Expression of IL-7 mRNA in Serum of AML Patients

In addition, we detected the expression of IL-7 mRNA in AML patients and healthy people by RT-PCR. The results showed that the expression of IL-7 mRNA in the serum of the Healthy group was significantly higher than that of the AML group ($p<0.05$; Figure 4).

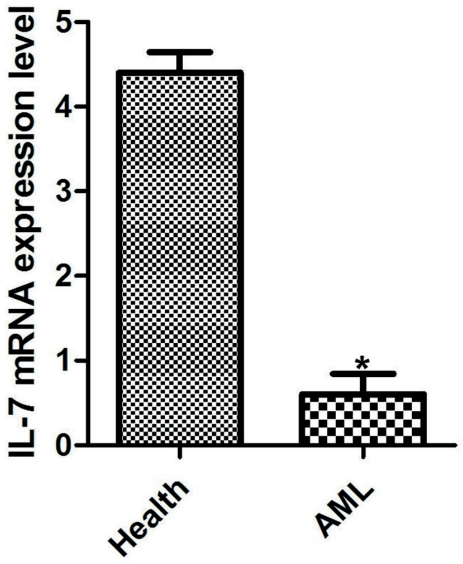

Figure 4. Expression of IL-7 mRNA in serum of AML patients. Health: healthy control group; AML: acute myeloid leukemia group. *Compared with the Healthy group, there is statistical difference, $p<0.05$.

Expression of IL-7 Protein in Serum of AML Patients

We used the enzyme-linked immunosorbent assay kits (ELISA; R&D Systems, Minneapo-

Table II. Correlation between IL-7 methylation and clinical factors in patients with AML

Item	Number of cases	IL-7 gene methylation positive		p
		Number of cases	%	
Age (years)				> 0.05
<60	32	24	75	
>60	23	16	69	
Gender				<0.05
Male	25	10	40	
Female	30	30	100	
FAB classification				> 0.05
M2	11	7	64	
M3	13	10	77	
M4	10	8	80	
M5	10	8	80	
M6	11	7	64	
Diagnostic staging				<0.05
Initially treated patient	40	38	95	
Refractory and relapsed	11	2	18	
Complete remission	4	0	0	

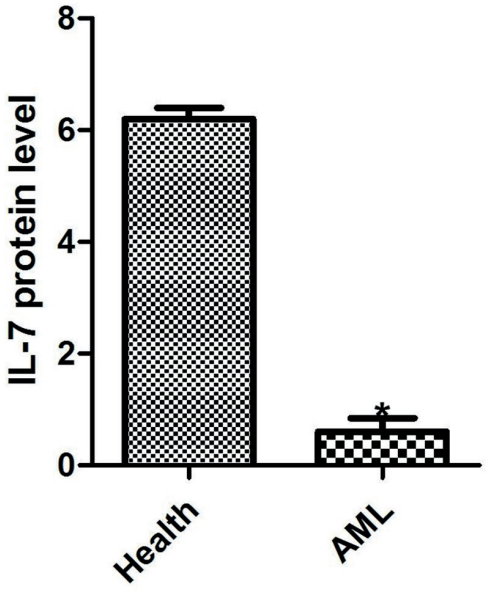

Figure 5. Expression of IL-7 protein in serum of AML patients. Health: healthy control group; AML: acute myeloid leukemia group. *Compared with the Healthy group, there is statistical difference, $p<0.05$.

lis, MN, USA) to detect the level of IL-7 protein expression in serum of each group. The results showed that IL-7 protein expression levels in serum of patients with AML were significantly increased ($p<0.05$; Figure 5).

Discussion

AML is one of the most common leukemia in adults, with poor prognosis and low survival. DNA methylation has been shown to play an important role in the development of AML[15]. The study found that more than 60% of human gene promoter regions have CpG islands. Once this position is methylated, it can cause epigenetic modification of genes, leading to abnormal expression of related proteins and ultimately affecting normal life activities[16]. There is extensive gene methylation in hematological malignancies. On the one hand, methylation of these genes can lead to abnormal proliferation, differentiation and apoptosis of tumor cells[17]. On the other hand, the epigenetic modification of these genes can affect the normal function of immune cells through methylation, breaking the body's original immune regulation network homeostasis, making tumor cells more susceptible to escape[18,19]. Zhang et al[20] used bioinformatics analysis to screen for closely related and abnormally methylated genes in AML, including MYF6, RPTOR, MMP10, SH3PXD2B, VHL and GABBR1. Therefore, to clarify the epigenetic pathogenesis of AML is of great significance for the early prevention and precise treatment of AML in the future.

Located on the short arm of human chromosome 8, IL-7 is an important immunoregulatory gene produced primarily by thymic stromal cells[21]. After specific binding by its receptor, IL-7 can rapidly initiate multiple signaling pathways in the cell, regulate the metabolism and cell cycle of target cells and, at the same time, promote lymphocyte proliferation and differentiation and maintain multiple immune functions such as antitumor[22]. Once IL-7 is expressed, it can cause hematological malignancies and autoimmune diseases[23]. Studies[24] have shown that IL-7 is important for the homeostasis and survival of memory T cells and $CD4^+CD8^+$. Also, IL-7 sustained signal-mediated co-stimulatory and anti-apoptotic effects are critical for the survival and proliferation of naive T cells[25]. In addition to the effects on lymphocytes, IL-7 has a certain regulatory effect on other immune cells. For example, IL-7 directly determines the fate of dendritic cells and mobilizes myeloid cells[26]. IL-7 also exerts a synergistic effect on the killing effect of NK cells[27]. This suggests that IL-7 may have an important impact on hematological malignancies, especially AML. In this work, the level of IL-7 methylation in peripheral blood of patients with AML and healthy subjects were examined. The results showed that the proportion of AML patients with IL-7 methylation was markedly higher than that of healthy controls and the occurrence of IL-7 methylation has a statistically significant correlation with the gender and diagnostic stage of AML patients. In addition, we cultured four leukemia cell lines HL-60/ADM, HL-60, MV4-11, and SKM-1 *in vitro*, and detected IL-7 methylation in four groups of cells. The incidence of methylation in these four groups of cells was 100%. Finally, by ELISA and RT-PCR, we detected the mRNA and protein expression levels of IL-7 in healthy people and AML patients. It was found that IL-7 expression in the AML group was significantly decreased at the mRNA level and protein level. Despite this, there are still some shortcomings in this study: 1) the sample size was relatively small; 2) the level of IL-7 methylation in bone marrow cells was not detected. 3) The pathogenesis of AML and acute lymphoblastic leukemia is different. Whether the level of IL-7 methylation is related to acute

lymphoblastic leukemia still needs further experiments to verify.

Conclusions

We showed, for the first time, that IL-7 is down-regulated in peripheral blood of AML patients, which may be related to its abnormal methylation. Therefore, the methylation level of IL-7 is expected to become a new target for the diagnosis and treatment of AML.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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