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Research Article

DOI:10.2478/rrlm-2022-0026

Expressions of TGF-β1 and VEGF in patients with acute myeloid leukemia and associations with prognosis

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Abstract

***Background**: To study the expressions of transforming growth factor-β1 (TGF-β1) and vascular endothelial growth factor (VEGF) in patients with acute myeloid leukemia (AML) and their values for prognosis. **Methods**: A total of 120 AML patients treated from January 2015 to December 2018 were selected. Bone marrow mononuclear cells were isolated. The expressions of TGF-β1 and VEGF were detected by RT-PCR, and their associations with clinical characteristics were analyzed. The overall survival (OS) and disease-free survival (DFS) were assessed using the Kaplan-Meier method. The risk factors for prognosis were analyzed through the Cox proportional hazards model. **Results**: The AML group had significantly lower relative expression of TGF-β1 and higher relative expression of VEGF than those of the control group (P<0.05). TGF-β1 and VEGF levels were significantly correlated with white blood cell count, hemoglobin, platelets, and peripheral blood juvenile cells (P<0.05). TGF-β1 level was higher and VEGF level was lower in the patients with complete response than those in the patients with partial response and no response (P<0.05). Both OS and DFS of the patients with high TGF-β1 expression were better than those of the patients with low TGF-β1 expression, while they were also superior among the patients with low VEGF expression (P<0.05). Platelets, TGF-β1 and VEGF were independent influencing factors for OS, and white blood cells, TGF-β1 and VEGF were independent influencing factors for DFS (P<0.05). **Conclusions**: AML patients have decreased expression of TGF-β1 and increased expression of VEGF, and such changes are closely associated with the prognosis of AML.*

***Keywords**: TGF-β1, VEGF, acute myeloid leukemia, prognosis*

Received: 26th November 2021; Accepted: 6th June 2022; Published: 14th June 2022

Introduction

Acute myeloid leukemia (AML) is a hematological disease caused by the malignant cloning of myeloid hematopoietic stem cells, which can lead to hematopoiesis inhibition (1). The patients primarily suffer from infection, anemia and systemic fever, and even hepatomegaly, splenomegaly and lymphadenectasis in severe cases. The pathogenesis of AML remains unclear, probably

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being caused by viral infection, radiation damage, chemical and drug toxicity, and genetic factors (2,3). Currently, the symptoms of 80% of AML patients can be relieved after treatment, but a considerable number of patients are still prone to recurrence, deterioration, or even death. Therefore, it is of great clinical significance to further enhance the efficacy on AML patients. Transforming growth factor-β1 (*TGF-β1*) is a polypeptide growth factor with obvious regulatory effects on cell growth, development, and metabolism (4). Vascular endothelial growth factor (*VEGF*) is a crucial factor promoting angiogenesis *in vivo*, and can facilitate the proliferation, differentiation, and infiltration of tumor cells (5). In the present study, the expression levels of *TGF-β1* and *VEGF* in AML patients were detected, and their associations with the prognosis of AML patients were analyzed, aiming to provide references for treatment and prognostic evaluation.

Materials and methods

General data

This study has been approved by the ethics committee of our hospital (approval No. PHD201501003, 4th January 2015), and written informed consent has been obtained from all patients. A total of 120 AML patients treated in our hospital from January 2015 to December 2018 were selected as the subjects, including 67 males and 53 females aged (73.1±3.6) years old. They all met the diagnostic criteria for AML in *Blood Disease Diagnosis and Efficacy Criteria* (6). All patients were diagnosed by the morphology, immunology, cytogenetics and molecular biology classification, including 30 cases of M1 subtype, 60 cases of M2 subtype and 30 cases of M5 subtype. At the same time, 40 healthy people receiving physical examination were enrolled as controls. There were 24 males and 16 females in the control group, with an average age of (73.4±3.5) years old. The two groups had similar demographic variables (e.g. age and gender) (P>0.05). Inclusion criteria: 1) Patients with complete clinical data and aged 15-70 years old, 2) those diagnosed as AML by cell morphology, immunology and immunohistochemical staining, and 3) those diagnosed as AML for the first time. Exclusion criteria: 1) Patients undergoing AML treatment in other hospitals, 2) those complicated with severe diseases, such as liver cancer, lung cancer and vital organ failure, or 3) those who were allergic to the drugs used in treatment or had poor tolerance.

Isolation of peripheral blood mononuclear cells (PBMCs)

After anesthesia with 2% xylocaine, 2 mL of bone marrow fluids were extracted with a puncture needle, added the same volume of lymphocyte separation medium Ficoll (Beijing Solarbio Science & Technology Co., Ltd., China), and centrifuged at 300 ×g for 25 min. Then, the milk white liquid between the upper and middle layers was the PBMC layer. PBMCs were transferred into another centrifuge tube with a capillary pipette, washed with Hanks' solution and stored at -80°C for later use.

Detection of TGF-β1 and VEGF levels by reverse transcription-polymerase chain reaction (RT-PCR)

PBMCs were lysed with TRIzol reagent (Invitrogen, USA) and centrifuged, from which total RNA was extracted, and its mass was detected with NanoDrop spectrophotometer (Thermo Scientific, USA). RNA was synthesized into cDNA by CFX96 Touch RT-PCR system (Bio-Rad, USA) using 4 μL of 5 × Prime Script Buffer, 1 μL of Prime Script RT Enzyme Mix, 1 μL of RT Prime Mix and 10 μL of RNase-free H_2O (Takara Biotechnology (Dalian) Co., Ltd., China) (water bath at 37°C for 5 min and at 85°C for 10 s). The reaction product was stored at -80°C

for later use. The expression levels of *TGF-β1* and *VEGF* in patients were determined using RT-PCR under the following conditions: 94°C for 5 min, 30 cycles × (94°C for 30 s, 56°C for 30 s, 72°C for 30 s), and extension at 72°C for 10 min, with 3 replicates for each sample. The relative expression of target gene was assessed by $2^{-\Delta\Delta CT}$, with U6 as the internal reference. The primer sequences are shown in **Table 1**.

Criteria for treatment outcomes of AML

AML patients received corresponding induction, consolidation or supportive therapy, and all chemotherapy regimens were conducted according to the "Chinese guidelines for diagnosis and treatment of adult acute myeloid leukemia (not APL) (2017 Edition)" (7). Complete response (CR): The symptoms of AML patients disappeared, the neutrophil count was $\geq 1.5\times10^9$/L, the platelet count $\geq 100\times10^9$/L, and the bone marrow blast count was ≤5%. Partial response (PR): The symptoms of AML patients partially disappeared, and some indices such as neutrophil, platelet and bone marrow blast counts met the CR criteria. No response (NR): The symptoms, or bone marrow and blood indices of AML patients were not relieved.

Statistical analysis

All data were statistically analyzed by SPSS 26.0 software. The measurement data were expressed as mean ± standard deviation, and intergroup comparisons were performed by the t test. The count data were represented as percent and subjected to the χ^2 test. The overall survival (OS) and disease-free survival (DFS) of patients were assessed using the Kaplan-Meier method, and the risk factors for prognosis were analyzed through the Cox proportional hazards model. $P<0.05$ was considered statistically significant.

Results

TGF-β1 and VEGF levels in AML patients and healthy subjects

The AML group had significantly lower relative expression level of *TGF-β1* ($t=47.745$, $P=0.000$) and higher relative expression level of *VEGF* than those of the control group ($t=50.891$, $P=0.000$) (**Figure 1**).

Correlations between levels of TGF-β1 and VEGF and clinical characteristics of AML patients

The levels of *TGF-β1* and *VEGF* were significantly correlated with the white blood cell count, hemoglobin, platelets and peripheral blood juvenile cells in AML patients ($P<0.05$), but they had no correlations with gender, age, *NPM1*, *FLT3* or lactate dehydrogenase ($P>0.05$) (**Table 2**).

Levels of TGF-β1 and VEGF in AML patients with different treatment outcomes

The level of *TGF-β1* in the AML patients with CR was higher than those in the patients with PR and NR ($P<0.05$), while higher in the AML patients with PR than that in the patients with NR ($P<0.05$). The level of *VEGF* in the AML

Table 1. Primer sequences

Gene	Sequence
TGF-β1	Forward: 5'-GAGAAGAACYGCTGCGTGCGG-3'
	Reverse: 5'-GCGTGTCCAGGCTCCAAATGT-3'
VEGF	Forward: 5'-GCTACAAACGCGTACGCTGAT-3'
	Reverse: 5'-GCGCGCGTGCGGCCGCTTTTT-3'
U6	Forward: 5'-GATGACCTTGCCCACAGCCT-3'
	Reverse: 5'-ATCTCTGCCCCCTCTGCTGA-3'

TGF-β1: Transforming growth factor-β1; *VEGF*: vascular endothelial growth factor.

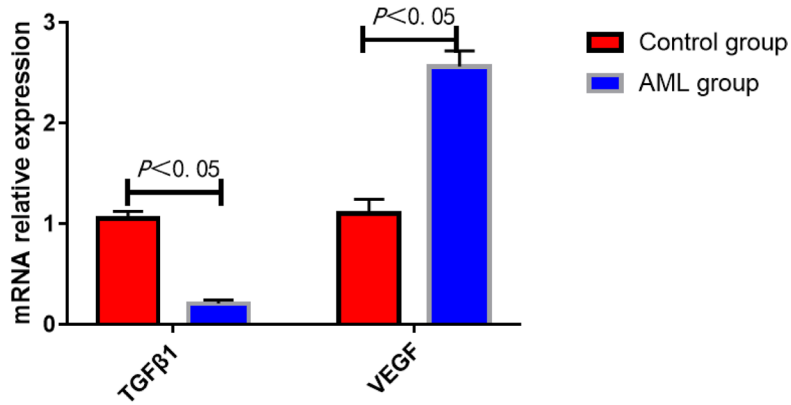

Fig. 1. TGF-β1 and VEGF levels in AML patients and healthy subjects. AML: Acute myeloid leukemia; TGF-β1: transforming growth factor-β1; VEGF: vascular endothelial growth factor.

patients with CR was lower than those in the patients with PR and NR (P<0.05), while lower in the AML patients with PR than that in the patients with NR (P<0.05) (**Figure 2**).

Table 2. Correlation between levels of *TGF-β1* and *VEGF* and clinical characteristics of AML patients

Clinical factor	TGF-β1	P	VEGF	P
Sex		0.169		0.661
Male (n=81)	0.19±0.04		2.59±0.11	
Female (n=39)	0.20±0.03		2.60±0.13	
Age (year)		1.000		0.675
<60 (n=79)	0.20±0.02		2.58±0.12	
≥60 (n=41)	0.20±0.03		2.59±0.13	
WBC ($\times10^9$/L)		0.000		0.031
<10 (n=55)	0.24±0.03		2.61±0.13	
≥10 (n=65)	0.11±0.02		2.58±0.12	
Hb (g/L)		0.000		0.000
<80 (n=60)	0.12±0.03		2.11±0.12	
≥80 (n=60)	0.23±0.02		2.88±0.13	
LDH (U/L)		0.051		0.211
<250 (n=44)	0.21±0.02		2.62±0.15	
≥250 (n=76)	0.18±0.03		2.57±0.11	
Plt ($\times10^9$/L)		0.000		0.000
<50 (n=42)	0.22±0.02		2.18±0.11	
≥50 (n=78)	0.12±0.03		2.92±0.15	
Peripheral blasts (%)		0.000		0.000
<80 (n=65)	0.23±0.04		2.12±0.11	
≥80 (n=55)	0.11±0.01		2.86±0.16	
NPM1		0.242		0.185
Positive (n=51)	0.21±0.04		2.61±0.11	
Negative (n=69)	0.20±0.05		2.58±0.13	
FLT3		0.218		0.079
Positive (n=29)	0.21±0.03		2.62±0.14	
Negative (n=91)	0.20±0.04		2.57±0.13	

AML: Acute myeloid leukemia; *FLT3*: Fms-like tyrosine kinase 3; Hb: hemoglobin; LDH: lactate dehydrogenase; *NPM1*: nucleophosmin 1; Plt: platelet; *TGF-β1*: transforming growth factor-β1; *VEGF*: vascular endothelial growth factor; WBC: white blood cell count.

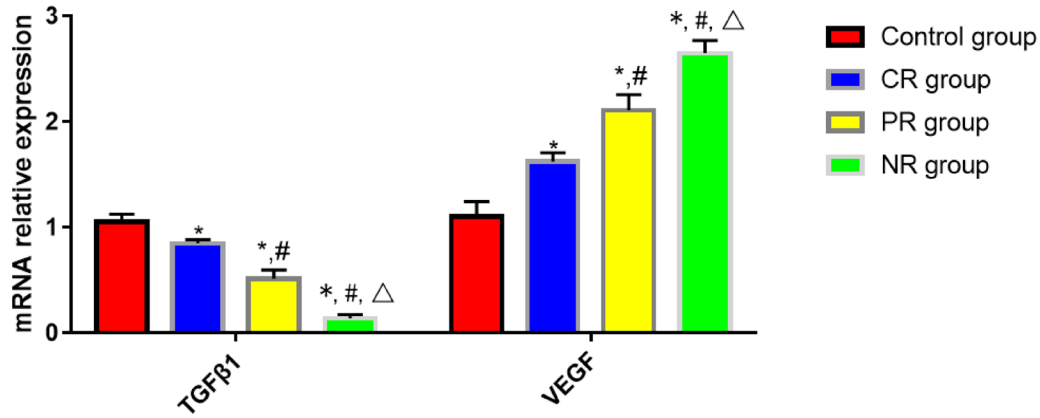

Fig. 2. Levels of TGF-β1 and VEGF in AML patients with different treatment outcomes. *P<0.05, compared with control group; #P<0.05, compared with CR group; ΔP<0.05, compared with PR group. AML: Acute myeloid leukemia; CR: complete response; PR: partial response; TGF-β1: transforming growth factor-β1; VEGF: vascular endothelial growth factor.

Influencing factors for prognosis of AML patients

After correction using age and gender, the results of Kaplan-Meier survival analysis showed that both OS and DFS of the AML patients with high *TGF-β1* expression were better than those of the patients with low *TGF-β1* expression (P<0.05), while also superior among the AML patients with low *VEGF* expression than those among the patients with high *VEGF* expression (P<0.05). OS and DFS of *NPM1*-positive patients were significantly better than those of *NPM1*-negative patients (P<0.05), and OS and DFS of *FLT3*-negative patients were better than those of *FLT3*-positive patients (P<0.05). It was found by multivariate Cox regression analysis that platelets, *TGF-β1,* and *VEGF* were independent influencing factors for OS (P<0.05), and white blood cells, *NPM1*, *FLT3*, *TGF-β1* and *VEGF* were independent influencing factors for DFS (P<0.05) (**Table 3** and **Figure 3**).

Discussion

AML is a hematological disease characterized by hematopoietic stem cell dysfunction, which can lead to fatigue, purpura, bacterial infection and fever in patients (8). Leukemia cells can infiltrate organs or tissues such as the liver, spleen and lymph nodes, greatly harming the health

Table 3. Multivariate analysis results of influencing prognostic factors for AML patients

Parameter	OS			DFS		
	HR	95%CI	P	HR	95%CI	P
WBC	0.232	0.232-1.232	0.109	0.483	0.108-0.792	0.038
Plt	0.483	0.278-0.982	0.014	0.782	0.293-1.092	0.076
TGF-β1	0.762	0.483-0.913	0.019	0.564	0.413-0.886	0.012
VEGF	1.782	1.342-2.983	0.005	1.543	1.154-2.456	0.009
NPM1	0.784	0.562-0.941	0.024	0.627	0.559-0.947	0.019
FLT3	1.451	1.123-2.965	0.023	1.462	1.297-3.023	0.016

AML: Acute myeloid leukemia; CI: confidence interval; DFS: Disease-free survival; *FLT3*: Fms-like tyrosine kinase 3; HR: hazard ratio; *NPM1*: nucleophosmin 1; OS: overall survival; Plt: platelet; *TGF-β1*: transforming growth factor-β1; *VEGF*: vascular endothelial growth factor; WBC: white blood cell count.

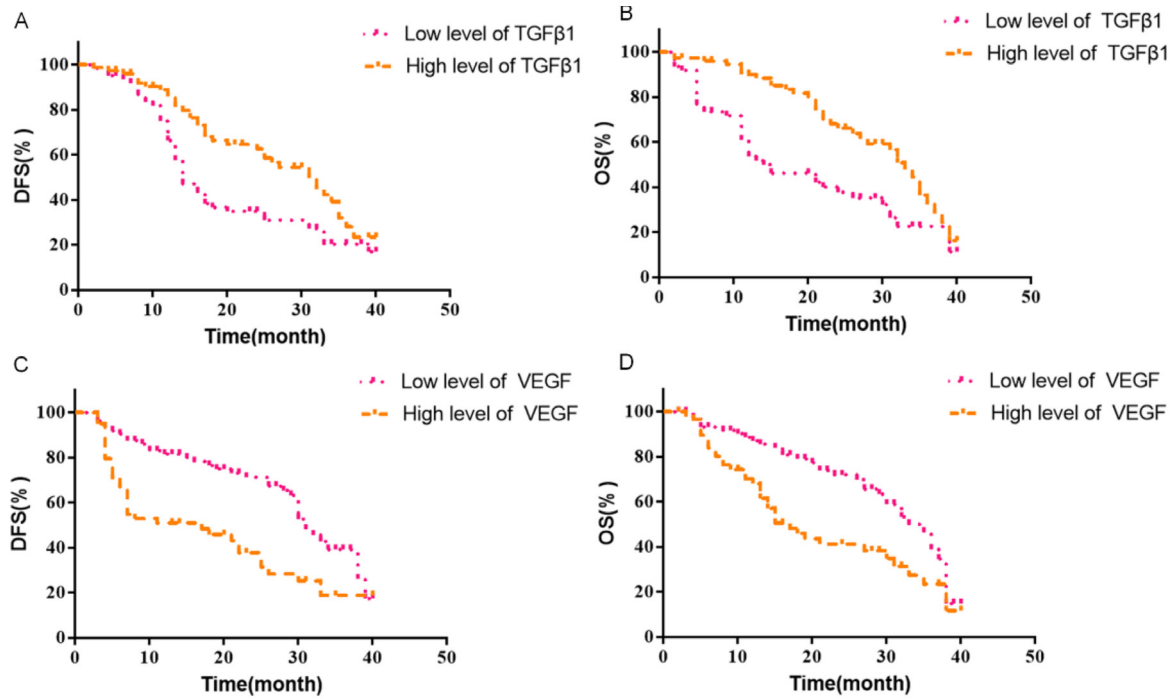

Fig. 3. Relationship between OS, DFS rates and expression of TGF-β1, VEGF analyzed by Kaplan-Meier survival curves. A and C: Correlation of DFS rate with TGF-β1 and VEGF; B and D: correlation of OS rate with TGF-β1 and VEGF. DFS: Disease-free survival; OS: overall survival; TGF-β1: transforming growth factor-β1; VEGF: vascular endothelial growth factor.

of patients (9,10). Due to complex pathogenesis, AML easily relapses, or the symptoms cannot be readily relieved. Therefore, it is of great significance to further improve the treatment outcomes of AML patients. The expressions of *TGF-β1* and *VEGF* are abnormal in skin cancer cells, which are closely related to cell proliferation and migration (11). The levels of *TGF-β1* in lung and intestinal cancer tissues are significantly lower than that in normal tissues, being correlated with the prognosis of patients (12,13). Zhou *et al*. found that the expression of *TGF-β1* declined, while the expression of *VEGF* rose in AML cells, which had a correlation with the malignancy grade (14). Therefore, this study aimed to explore the changes in *TGF-β1* and *VEGF* levels in AML patients.

TGF-β1 has a wide range of biological effects, participating in physiological processes such as embryo growth and development, cell proliferation and differentiation, angiogenesis, extracellular matrix secretion and immunoregulation. The expression of *TGF-β1* in AML cells cultured *in vitro* is significantly lower than that in normal cells (15,16). The level of *VEGF* is associated with the occurrence of lung cancer and liver cancer, as a key factor facilitating the growth of tumor cells (17,18). In this study, the relative expression levels of *TGF-β1* and *VEGF* in AML patients had significant differences from those in control group, indicating that the levels were closely related to the occurrence of AML. The level of *TGF-β1* in the culture supernatant of primary AML cells significantly declines compared with that in normal cells, being consistent with the results in this study (19,20). Herein, the levels of *TGF-β1* and *VEGF* were significantly correlated with the white blood

cell count, hemoglobin, platelets and peripheral blood juvenile cells in AML patients, but they had no correlations with gender, age or lactate dehydrogenase. Possibly, the abnormal levels of *TGF-β1* and *VEGF* can trigger the uncontrolled proliferation of primitive hematopoietic cells in the bone marrow, and the malignant proliferation of leukemia cells, thereby aggravating AML. Moreover, the AML patients with better prognosis had higher *TGF-β1* level and lower *VEGF* level than those of the patients with worse prognosis. Hence, the levels of *TGF-β1* and *VEGF* can work as reference indices for the prognostic prediction of AML patients. Besides, the level of serum *TGF-β1* in AML patients significantly declines, and returns to normal after reaching CR by treatment, being in accordance with the findings in this study (21). The results of multivariate Cox regression analysis revealed that *TGF-β1* and *VEGF* were the influencing factors for OS and DFS of AML patients. Probably, abnormally expressed *TGF-β1* and *VEGF* led to the proliferation of leukemia cells, resulting in poor prognosis. In addition, AML patients with high *VEGF* expression level are prone to AML relapse, suggesting that such level is a risk factor for poor prognosis, being consistent with the results in this study (22). Therefore, detecting the levels of *TGF-β1* and *VEGF* in AML patients is of great clinical significance. *NPM1* and *FLT3* are also influencing factors for OS and DFS of AML patients. The patients with *NPM1* mutation alone have longer survival and lower recurrence rate, as a good prognostic factor (23). Additionally, *FLT3*-mutated AML patients often have poor response to chemotherapy, high recurrence rate after treatment and short survival, as a poor prognostic factor (24).

In conclusion, AML patients have decreased expression of *TGF-β1* and increased expression of *VEGF*, being closely associated with the prognosis. The severity of AML can be assessed based on the levels of *TGF-β1* and *VEGF*, which can provide references for clinical treatment. However, this is a single-center study with a small sample size, so the results may be biased. Multicenter studies with larger sample sizes are in need to further verify the results of this study.

Abbreviations

AML: Acute myeloid leukemia; CI: confidence interval; CR: complete response; DFS: Disease-free survival; *FLT3*: Fms-like tyrosine kinase 3; HR: hazard ratio; *NPM1*: nucleophosmin 1; NR: no response; OS: overall survival; Plt: platelet; PBMC: peripheral blood mononuclear cell; PR: partial response; RT-PCR: reverse transcription-polymerase chain reaction; *TGF-β1*: transforming growth factor-β1; *VEGF*: vascular endothelial growth factor; WBC: white blood cell count.

Acknowledgments

This study was not financially supported.

Authors' contributions

YX and XZ designed this study and prepared this manuscript; XY collected and analyzed clinical data. All authors have approved the submission and publication of this manuscript.

Conflict of interest

The authors declare no conflict of interest.

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