

The prognostic relevance and expression of progranulin in adult patients with acute myeloid leukemia

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Abstract

Progranulin (PGRN) is a secreted protein that can regulate cell cycle progression, cell motility, and tumorigenesis. The PGRN expression in hematological malignancies is limited to multiple myeloma, but its expression and survival prognostic role in acute myeloid leukemia (AML) is still controversial.

To evaluate the PGRN expression and estimate its survival prognostic role in AML patients.

In this study, all patients were divided into three groups, which included 38 newly diagnosed adult AML patients, 33 complete remissions (CR-AML) patients, and 60 healthy control (HC) patients. The endpoints were relapse-free survival (RFS) and overall survival (OS). We investigated plasma PGRN levels by using enzyme-linked immunosorbent assay.

Plasma PGRN levels in AML patients were higher than that in CR-AML and HC groups. After two chemo cycles, 16 patients had complete remission (CR). The level of plasma PGRN in non-CR patients compared to CR patients was obviously different (median 44.19 vs 21.10 ng/mL) ($P = .025$). In non-M3 (French–American–British classification) patients, 70% (21/30) patients relapsed in 1 year and 80% (24/80) patients died in the observed time. Using the value (median 19.95) as a “cut-off” value, we have divided non-M3 patients into low- and high-PGRN expression groups. High-PGRN expression patients had a poorer RFS with a median of 5.4 months (95% CI 3.7–7.1) and low-PGRN expression patients had a good RFS with a median of 8.9 months (95% CI 6.3–11.5; $P = .027$). In the survival analyses, high-PGRN expression of AML patients had shorter OS than low-PGRN expression of AML patients (6.2 vs 20.5 months, $P = .008$).

PGRN is overexpressed in AML, which is a convenient and independent prognostic marker that is measured easily in AML patients.

Abbreviations: AML = acute myeloid leukemia, CR = complete remission, ELISA = enzyme-linked immunosorbent assay, FAB = French–American–British, HC = healthy control, OS = overall survival, PD = progress disease, PGRN = progranulin, RFS = relapse-free survival.

Keywords: acute myeloid leukemia, prediction, prognostic biomarker, progranulin

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1. Introduction

Acute myeloid leukemia (AML) is a malignant proliferative monoclonal hematological disease with rapid progression and poor prognosis. AML is the most common acute leukemia in adults, and its incidence is three to five in every 100 thousand in the United States.^[1] Patients who did not receive effective treatment had a high-mortality rate within 6 months.^[2] The classic treatment range is cytarabine-based chemotherapy, targeted drug therapy and hematopoietic stem cell transplantation, the 5-year overall survival (OS) rate for patients is still around 30%.^[3] In addition, although chemotherapy and targeted drug therapy can prolong the survival of AML patients, there are still some patients who cannot obtain a good prognosis, still have drug resistance and relapse, or even death.

Progranulin (PGRN), a precursor of granule protein, is a newly discovered autocrine growth factor.^[4] It is widely distributed^[5] and plays an important role in the development of the embryo, wound repair, inflammation, tumor formation, and development.^[4] High expression of PGRN in solid tumors frequently has a poor prognosis. The overexpression of PGRN has been detected in various types of tumors, such as breast cancer, ovarian cancer, prostate cancer, bladder cancer, biliary tract cancers, and liver cancer.^[6,7] Also, the overexpression of PGRN has been investigated in chronic lymphocytic leukemia.^[6] Cuevas et al reported that the high expression of PGRN was associated with

cancer development and progression.^[8] The expression of PGRN in endometrial cancer, leiomyosarcoma, and glioma was positively correlated with the histological grade of the tumors.^[10–12] In cervical and hepatocellular carcinomas, high expression of PGRN may lead to increased migration and invasion of cancer cells. In drug therapy, PGRN overexpression was associated with poor response to treatment in patients with advanced biliary tract carcinoma (BTC)^[9] and high expression of PGRN can reduce glioma sensitivity to temozolomide.^[13] However, there is limited literature about the incidence of PGRN expression in AML, and the prognostic roles of PGRN in AML patients remain elusive. Therefore, the aim of this study is to evaluate the PGRN expression in patients with AML and estimate the prognostic role of PGRN.

2. Materials and methods

2.1. Patients and control subjects

This study included 38 newly diagnosed adult AML patients (AML), 33 AML patients with complete clinical and laboratory remission (CR-AML), and 60 healthy controls (HC), who were enrolled from Taizhou Hospital of Zhejiang Province during January 2016 to December 2017. Patients who had other malignancies were not the subjects of this study. Plasma samples were centrifuged at 600 G for 6 minutes within 4 hours after blood collection and stored at -80°C until analysis. All AML patients were diagnosed by bone marrow morphology, histochemical staining, and flow cytometry. The disease classification was according to (French–American–British) FAB criteria.^[11] The study protocol was approved by the Ethics Committee of the Taizhou Hospital and all patients' written informed consent was provided in accordance with the Declaration of Helsinki. The first blood sample collection was from the patients enrolled from hospital and the second blood collection was from patients after receiving two chemotherapy cycles. For the patients who had received prior therapy, the date of sample collection was defined as the starting point for the follow-up period.

2.2. Measurement of PGRN levels

Our study was performed using enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN) to assess the PGRN levels in the plasma of 38 adult AML patients, 33 CR-AML patients, and 60 HC. The protocol of the ELISA is described in the previous study.^[14]

2.3. Treatments and evaluation

The treatment of AML consists mainly of remission induction and post-remission therapy guided by NCCN Clinical Practice Guidelines in Oncology: Acute Myeloid Leukemia. In terms of induction therapy, patients received a combination of anthracycline for 1 to 3 days and standard-dose cytarabine for 1 to 7 days. After two cycles of treatment, patients were judged to be complete remission (CR) or not. One-year relapse-free survival (RFS) for patients who were calculated from the first day of therapy to relapse or death in 1 year and OS was calculated from the first day of therapy to death or last visit. Patients with the type of M3 had targeted drug therapy and achieved relatively good clinical efficacy and prognosis. In this study, RFS and OS were observed in patients who were not M3 (non-M3).

Table 1

Characteristics of patients in different groups.

Group	AML (N=38)	CR-AML (N=33)	HC (N=60)
Sex			
Male (%)	26 (68.4)	18 (54.5)	40 (66.7)
Female (%)	12 (31.6)	15 (45.5)	20 (33.3)
Age			
Median, range	53 (16–77)	44 (16–68)	50 (22–70)
FAB (%)			
M0	3 (7.9)	0	–
M1	2 (5.3)	0	–
M2	10 (26.3)	9 (27.3)	–
M3	8 (21.1)	13 (39.4)	–
M4	5 (13.1)	1 (3.0)	–
M5	10 (26.3)	10 (30.3)	–

AML=acute myeloid leukemia, CR=complete remission, FAB=French–American–British, HC=healthy control.

2.4. Statistical analysis

All data were described by the median statistical description. Statistical comparison was using Mann–Whitney *U* test. Survival curves were performed by Kaplan–Meier, and RFS and OS were compared with the Log-rank test. A two-sided *P* value <0.05 was considered statistically significant. All statistical analysis was using SPSS version 22.0 (IBM, NY) and GraphPad Prism 6.0 (San Diego, CA).

3. Results

3.1. Patients baseline characteristics

Thirty-eight newly diagnosed patients were included in this study, 26 patients (60%) were male, and the median age was 53 years (range 16–77). Among AML patients, eight patients were M3 type and the 30 patients were non-M3 type. All patients' characteristics are summarized in Table 1.

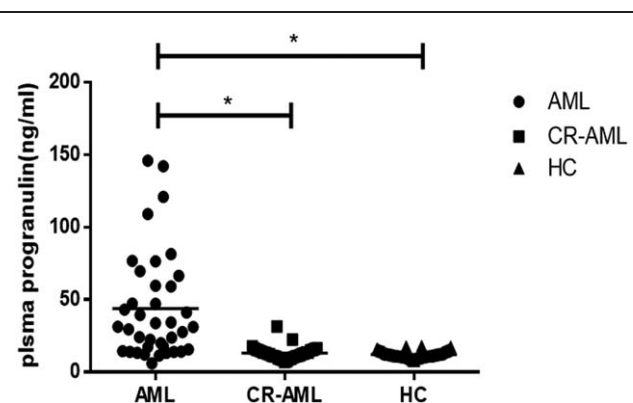


Figure 1. The PGRN plasma levels detected by ELISA in AML group, CR-AML group and HC group. ELISA analysis of plasma samples collected from AML patients (N=38), CR-AML group (N=33) and HC (N=60) reveals statistically significant differences in PGRN concentrations (Mann–Whitney *U*-test). **P* < .05. AML=acute myeloid leukemia, CR=complete remission, ELISA=enzyme-linked immunosorbent assay, HC=healthy control, PGRN=progranulin.

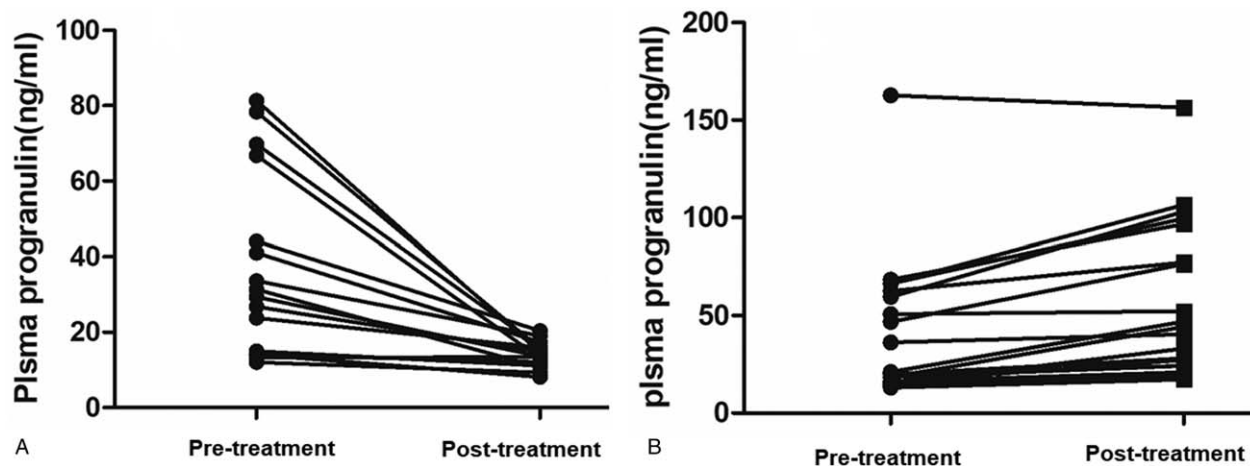


Figure 2. The PGRN plasma levels changes before and after treatment in AML group. (a) The PGRN plasma levels of 16 CR patients decreased between pre- and post-treatment; (b) The PGRN plasma levels of 22 non-CR patients increased softly between pre- and post-treatment. AML=acute myeloid leukemia, CR=complete remission, PGRN=progranulin.

3.2. PGRN concentrations in AML patients and control

The median expression plasma level of PGRN in AML patients was 31.2 ng/mL (range 5.9–146.0 ng/mL), which was significantly higher than that in CR-AML group (median 12.5 ng/mL, range 6.8–22.5 ng/mL, $P < .001$) and HC group (median 11.4 ng/mL, range 7.6–17.9 ng/mL, $P < .001$). While the difference between the CR-AML group and the HC group was not statistically significant ($P > 0.05$) (Fig. 1).

3.3. Treatment and response

The CR rate in AML patients was only 42.1% (16/38) after two chemotherapy cycles. The PGRN plasma level in non-CR patients (22/38) (median 44.19 ng/mL, range 11.37–145.98 ng/mL) was much higher than that in CR patients (median 21.10 ng/mL, range 5.88–76.77 ng/mL) ($P = .025$). Of all 38 patients, the 16 CR and 22 non-CR patients’ inspection reports were collected completely. We acquired the 16 CR patients and 22 non-CR patients’ results, which showed that the levels of PGRN in 16 CR patients were declined obviously and the bone marrow performance of them was relieved (Fig. 2a). In contrast, the levels of PGRN in 22 non-CR patients suggested a subtle increase and their bone marrow performance was not relieved (Fig. 2b). The possible reason for PGRN increase was that high expression of PGRN was associated with the cancer development and progression and the dynamic changes of PGRN levels may show the chemotherapy response rate to the AML patients that high expression of PGRN patients had low chemotherapy response rate.

3.4. PGRN expression according to clinical features

The plasma PGRN concentration in AML patients whose proportion of immature cells in bone marrow is more than 60% than that in those proportion <60% (Median, 47.04 vs 17.26 ng/mL, $P = .001$). The results of peripheral blood were similar to those of bone marrow. The level of plasma PGRN expression in AML patients was also correlated with WBC counts in peripheral blood, but not related to sex, age, platelet count, and FAB type (Table 2).

3.5. The correlation between outcome and the PGRN expression

Among 38 patients, M3 patients (8/38) had a better outcome, and there was only one patient died of heart disease, the other seven were alive with no disease progression. In non-M3 patients, 21 patients relapsed in 1 year (Table 3), and the relapsed patients showed higher media PGRN levels compared to non-relapsed patients (39.25 vs 17.26 ng/mL, $P = .020$). Using the median value of 19.55 ng/mL as a “cut-off” value, we divided non-M3 patients (N=30) into low (<19.55 ng/mL) and high (>19.55 ng/mL) PGRN expressing groups. Patients with high expression of PGRN had a poorer 1-year RFS (median, 5.4 months) (95% CI

Table 2
PGRN expression of AML patients according to clinical features (N=38).

Characteristics	N (%)	PGRN (median, range, ng/mL)	P value
Sex			.769
Male	26 (68.4)	31.52 (22.6–42.5)	
Female	12 (31.6)	31.20 (14.7–42.5)	
Age			.498
<50	16 (42.1)	17.89 (12.4–23.8)	
≥50	22 (57.9)	34.23 (19.9–50.7)	
Bone marrow blasts (%)			.001*
<60	12 (31.6)	19.86 (11.1–24.3)	
≥60	26 (68.4)	43.25 (27.1–49.5)	
Peripheral blood blast (%)			.001*
<60	13 (34.2)	17.26 (18.8.1–33.5)	
≥60	25 (65.8)	47.04 (28.1–53.5)	
WBC count			.045*
<50 × 10 ⁹ /L	29 (76.3)	24.13 (20.1–43.5)	
≥50 × 10 ⁹ /L	9 (23.7)	47.29 (33.1–53.5)	
Platelet count			.815
<50 × 10 ⁹ /L	20 (52.6)	32.50 (24.1–39.5)	
≥50 × 10 ⁹ /L	18 (47.4)	25.87 (20.1–33.2)	
French–American–British			.739
M3	8 (21.1)	31.08 (27.1–40.5)	
Non-M3	30 (78.9)	29.38 (20.0–33.1)	

AML=acute myeloid leukemia, PGRN=progranulin.
*Significant difference.

Table 3
The relevance with PGRN and prognostic in non-M3 patients (N = 30).

Characteristics	N (%)	PGRN (median, range, ng/mL)	P value
Prognostic grade [#]			.576
Good	6 (20.0)	29.34 (18.9–30.9)	
Moderate-risk	6 (20.0)	39.34 (20.1–43.5)	
High-risk	7 (35.0)	57.52 (40.3–145.92)	
1-Year relapse			.020*
Yes	21 (70.0)	39.35 (22.1–43.9)	
No	9 (30.0)	17.26 (14.1–25.3)	
Survival			.325
Yes	6 (20.0)	19.95 (16.1–33.6)	
No	24 (80.0)	32.78 (18.7–41.5)	

PGRN = progranulin.

* Significant difference.

[#] The prognostic grade detections included 31 pairs of fusion genes, NPM1, FLT3-ITD, c-KIT, CEBPA genes and chromosome karyotype, and so on. 11 patients did not have these tests.

3.7–7.1) than low expression with a median of 8.9 months (95% CI 6.3–11.5; $P = .027$) (Fig. 3a).

The survival rate in the observation period was 20% (6/30) in non-M3 patients, and the OS was 8.9 months (95% CI 4.3–13.5). The influence of PGRN expression level on the OS was significant, with PGRN high-expressing group patients having OS of only 6.2 months (95% CI 2.8–9.6), compared to PGRN low-expressing group with OS of 20.5 months (95% CI 5.5–24.5; $P = .008$) (Fig. 3b).

4. Discussion

In our study, we evaluated plasma PGRN expression in AML patients by ELISA. Our results indicated that the overexpression of PGRN was a common occurrence in AML patients, and the plasma level of PGRN in AML was significantly higher than that in CR-AML patients and HCs. Previous data demonstrated the same results in some solid tumors.^[5–12] We found that the expression of PGRN is related to the proportion of immature cells in peripheral blood and bone marrow, the proportion of

immature cells which was more than 60%, the expression of PGRN in plasma increased significantly. This result is similar in solid tumors, in which the expression of PGRN is related to the histological grading.^[9,10] In breast cancer, the overexpression of PGRN is more common in invasive ductal adenocarcinoma and is closely related to tumor grading, growth index, and P53 expression.^[15,16]

In our study, only 42.1% (16/38) patients completely remised after two cycles of chemotherapy, and the levels of plasma PGRN in CR patients were lower than that in non-CR patients. Such poor response to chemotherapy may be caused by chemotherapy resistance, but the reason was not clear yet. Some studies have reported that PGRN activates ERK/MAPK pathways, which promotes tumor proliferation in hormone-positive breast cancer without estrogen-dependent growth signals, such as estrogen-dependent pathways, which would make anticancer therapy by inhibiting the original pathway ineffective.^[17,18] Therefore, we speculate that high-plasma PGRN levels may have adverse effects on chemotherapy, and re-examination after treatment can reflect the patient's condition.

AML patients are prone to relapse after CR, which was difficult to achieve a good long-term prognosis except for M3. Since the M3 has targeted drug therapy, so those patients have relatively good clinical efficacy and prognosis.^[19] In our data, non-M3 patients relapsed 70% during the 1-year time and had an 80% mortality rate (24/30) during the research period. Non-M3 with high plasma of PGRN had poor RFS ($P = .026$) and OS ($P = .008$). Cytogenetics, fusion genes, and karyotype are the prognostic factors of AML patients,^[20] but there was no clear correlation between PGRN and these factors. This study suggests that the evaluation of plasma PGRN expression may assist in predicting prognostic in non-M3 patients.

However, the limitations of this study are the small sample size and single-center research. We need multi-center study and large sample data to verify the prognostic biomarker. Nevertheless, the results of overexpression PGRN may have poor prognostic for AML patients.

In conclusion, PGRN is overexpressed in AML patients and patients with high-plasma PGRN levels have a poor response to

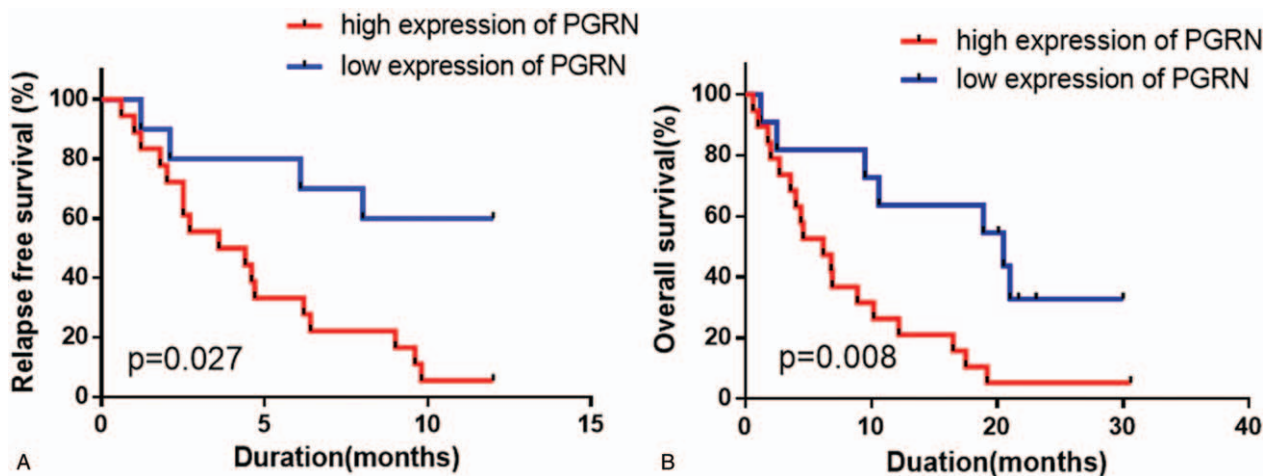


Figure 3. (a) One-year RFS of non-M3 AML patients according to PGRN expression. (b) Overall survival of non-M3 AML patients according to PGRN expression. Statistical comparisons between patients with high- (>19.55 ng/mL) and low-PGRN plasma levels (<19.55 ng/mL) were performed using the log-rank test. AML = acute myeloid leukemia, PGRN = progranulin, RFS = relapse-free survival.

chemotherapy. In terms of RFS and OS, the overexpression of PGRN in AML may indicate the poor prognostic in disease progression.

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Writing – review & editing: Shigao Huang.

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