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ORIGINAL RESEARCH

CEBPA mutations in patients with de novo acute myeloid leukemia: data analysis in a Chinese population

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Background: This study was aimed to explore the clinical characteristics and prognoses of acute myeloid leukemia (AML) patients with *CEBPA* mutations.

Patients and methods: Three hundred and forty-five patients with de novo AML were retrospectively analyzed with regard to *CEBPA* mutations, clinical characteristics, therapeutic responses, and long-term outcomes.

Results: *CEBPA* mutations were detected in 59 patients (17.10%), with 47 cases harboring double mutations and 12 cases harboring single mutations. In those with a normal karyotype (NK), 44 cases (25.29%) were detected with *CEBPA* mutations. The following characteristics were observed in *CEBPA*-mutated patients: most (66.10%) of them were M_1 or M_2 ; they presented with higher peripheral white blood cell counts (23.71 [12.6, 60.02] ×10%/L versus 7.34 [2.38, 26.63] ×10%/L; *u*=4.944, *P*<0.001) and higher hemoglobin levels (89.64±23.05 g/L versus 75.65±23.65 g/L; *t*=4.156, *P*<0.001) than those observed in patients without the mutation; and the expression of CD7 and HLA-DR was higher, whereas that of CD34 and CD56 was lower in patients with the mutation than in those without the mutation. Compared with those without the mutation, patients with *CEBPA* mutations had a superior complete remission rate (75.0% versus 56.54%; χ^2 =6.185, *P*=0.013) and superior overall survival (*P*=0.034).

Conclusion: The frequency of *CEBPA* mutations may be higher in Chinese patients with AML than has been reported in populations of western countries, and the presence of *CEBPA* mutations is an indication of favorable prognoses for these patients.

Keywords: acute myeloid leukemia, *CEBPA* mutations, immunophenotype, complete remission, long-term prognoses

Introduction

Genetic mutations can provide important information for the prognoses of patients with acute myeloid leukemia (AML). *CEBPA* is a leucine zipper transcription factor with a pivotal role in myeloid differentiation. Mutations in *CEBPA* have been described in ~5%–14% of patients with AML.^{1–8} They can occur across the whole gene, but cluster in two main hotspots: N-terminal out-of-frame insertions/deletions cause translation of a 30 kDa protein, from an internal ATG start site, that lacks transactivation domain 1 and has a dominant negative effect over the full-length p42 protein, and C-terminal mutations are generally in-frame insertions/deletions, in the DNA-binding or leucine zipper domains, that disrupt binding to DNA or dimerization.² Patients who have AML with *CEBPA* mutations can be separated into two subgroups, namely, those with a single mutation *CEBPA* (*CEBPA*sm) and those with a double mutation *CEBPA* (*CEBPA*^{dm}).⁸ AML patients with mutated *CEBPA* have better overall survival (OS)

OncoTargets and Therapy 2016:9 3399-3403

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© 2016 Su et al. This work is published and licensed by Dove Medical Press Limited. The full terms of this license are available at https://www.dovepress.com/terms.php hereby accept the Terms. Non-commercial uses of the work are permitted without any further permission from Dove Medical Press Limited, provided the work is properly attributed. For permission for commercial use of this work, please see paragraphs 4.2 and 5 of our Terms (https://www.dovepress.com/terms.php). and relapse-free survival (RFS) and tend to possess a higher complete remission (CR) rate than those without *CEBPA* mutations.^{1–8} However, recent data have suggested that the good prognoses may be limited to patients with *CEBPA*^{dm} and may not extend to those with *CEBPA*sm.^{2,4–6,8} *CEBPA* mutations were adopted as important indicators for AML, in both the National Comprehensive Cancer Network guideline and the European Leukemia Net classification. AML with mutated *CEBPA* has been designated as a provisional disease entity in the category "AML with recurrent genetic abnormalities" in the current World Health Organization classification of AML.

Although *CEBPA* mutations have been studied for many years in AML, there were limited data about its prevalence and prognostic significance in Chinese patients with AML. In this study, we retrospectively analyzed *CEBPA* mutations in 345 patients with de novo AML in our clinical center.

Patients and methods Patients and treatment

From August 1, 2011, to May 30, 2015, 345 patients with de novo AML (including 183 males and 162 females), aged 3-80 years (median age: 44 years), and who were residents of the northeast region of the People's Republic of China, including the Jilin, Heilongjiang, and Liaoning provinces, were enrolled in this study. The patients were categorized into French-American-British (FAB) subtypes based on morphological diagnoses. Acute promyelocytic leukemia (APL) patients were treated with arsenic trioxide and all-trans retinoic acid for induction therapy. Darubicin + cytarabine and mitoxantrone + cytarabine regimens were consolidated for the subsequent therapy. Non-APL patients were treated with the standard "3+7" regimen for initial induction therapy (darubicin/idarubicin + cytarabine). In some elderly patients, a cytarabine + aclarubicin + granulocyte-colony stimulating factor (G-CSF) regimen was administered. Response was assessed by bone marrow aspiration performed on days 14 and 28. The first consolidation therapy was generally the same as that used to achieve CR. Four courses of high-dose cytarabine at 2–3 g/m² (for some patients >60 years, cytarabine at 1–1.5 g/ m²) were administered for consolidation therapy. High-risk patients, and those with a matched sibling, were treated with hematopoietic stem cell transplantation (HSCT). All the participating patients gave written informed consent prior to enrollment in the study, and this study was approved by the ethics committee of the First Hospital of Jilin University and conducted in accordance with the Declaration of Helsinki.

Cytogenetic, molecular mutation, and surface marker analyses

Standard culturing and banding techniques were used to analyze the chromosome karyotype, and the clonal abnormalities were defined and described according to the International System for Human Cytogenetic Nomenclature.⁹ Mutational statuses of *NPM1*, *FLT3*-ITD, *c-kit*, and *CEBPA* were analyzed, and polymerase chain reactions were performed as previously described.^{2,4,10} The expressions of CD34, CD33, HLA-DR, CD11c, CD13, CD14, CD15, CD123, CD7, CD56, and other surface markers were analyzed by flow cytometry.

Statistics

Chi-square test, independent sample *t*-test, or Mann–Whitney *U*-test, as appropriate for the type of data being analyzed, were used to assess the statistical significance of the difference between the two groups. Kaplan–Meier method was employed for survival analysis, and log-rank test was used to compare differential survival between groups. OS was defined as the time from day 1 of induction to death, HSCT, or last contact. RFS was the time from CR to relapse, death, HSCT, or last contact. *P*<0.05 was considered significant. SPSS software (Version 16.0; SPSS Inc., Chicago, IL, USA) was used to calculate statistically significant differences.

Results

FAB classification and cytogenetics

The most common subtype in the present cohort was M_2 (42.90%, n=148), followed by M_4 (21.45%, n=74), M_5 (15.65%, n=54), and APL (13.33%, n=46). The frequency of other subtypes was <5% (M_1 : 2.90% [10/345] and M_6 : 3.19% [11/345]). Successful cytogenetic analyses were achieved in 298 (86.38%) patients, among whom 174 (58.39%) were considered cytogenetically normal.

Molecular mutations

Of the 345 patients, 59 (17.10%) were detected as *CEBPA* mutants, in which 47 cases were *CEBPA*^{dm} and 12 were *CEBPA*sm. The frequency of *CEBPA* mutations was 25.29% (44/174) in those with a normal karyotype (NK). The incidence rates of *NPM1* and *FLT3*-ITD mutations were 14.78% (51/345) and 13.62% (47/345), respectively. In those with an NK, the frequencies were 25.29% (44/174) and 18.39% (32/174) for *NPM1* and *FLT3*-ITD mutations, respectively. Sixteen patients (4.69%, 16/345) with *c-kit* mutations were detected.

Clinical characteristics of patients with CEBPA mutations

Clinical characteristics of patients with or without CEBPA mutations are listed in Table 1. There was no significant difference in age or sex between patients with or without CEBPA mutations (P > 0.05). Of the 59 cases with CEBPA mutations, 39 (66.10%) were M₁ or M₂. Patients with CEBPA mutations had a higher percentage of NK (89.80%, 44/49) than those without (52.21%, 130/249; χ^2 =23.808, P<0.001). Although the frequencies of NPM1, FLT3-ITD, and c-kit were lower in patients with CEBPA mutations than those without, no significant difference was detected for any such mutation (each P > 0.05). Compared with those without mutations, expression of CD7 and HLA-DR was higher, whereas that of CD34 and CD56 was lower in patients with CEBPA mutations (each P < 0.05). CEBPA-mutated patients presented with higher white blood cell counts and hemoglobin levels than those without such mutations (P < 0.05 for each hemocytological analysis). Platelet counts of patients with CEBPA mutations tended to be lower than of those without CEBPA mutations; however, there was no significant difference (P=0.179).

Therapeutic response and outcomes

Of the 299 patients with non-APL, 39 did not choose chemotherapy and the remaining 260 were administered one course of chemotherapy, with 13 patients not being subsequently evaluated for this study. One-hundred and fifty cases achieved CR, a rate of 60.73% (150/247). Forty-one cases achieved partial remission, and the overall response rate was 77.33% (191/247). The CR rate was higher in patients with *CEBPA* mutations (75.0%, 42/56) than in those without *CEBPA* mutations (56.54%, 108/191; χ^2 =6.185, *P*=0.013). Two patients with *CEBPA* mutations and eight without *CEBPA* mutations received HSCT. The follow-up time ranged from 1 month to 34 months (median: 8 months). At the time of analyses, 34 patients (12.8%) relapsed and 13 (10.6%) had died. Two-year RFS was 66.1% in patients

Table	Ľ.	Clinical	characteristics	of	natients	with	and	without	CERPA	mutations
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	CEBPA mutations	No CEBPA mutation	Statistical value	P-value
Age, median (year) (range)	41 (11-80)	44 (3–80)	t=1.575	0.116
Sex				
Male	32	151	χ²=0.041	0.840
Female	27	135		
FAB classification				
M	3	7	χ ² =16.732	0.005
M ₂	35	113		
APL	0	46		
M₄	10	64		
M _s	8	46		
M ₆	3	8		
Cytogenetics				
Normal	44	130	χ ² =23.808	<0.001
Abnormal	5	119		
Genetic mutations				
NPM1 mutations (%)	6.78 (4/59)	16.43 (47/286)	χ ² =3.618	0.057
FLT3-ITD (%)	10.17 (6/59)	14.34 (41/286)	χ²=0.72 Ι	0.396
c-kit mutations (%)	1.75 (1/57)	5.28 (15/284)	χ ² =1.321	0.250
Surface molecules				
CD7 (%)	30.50 (0.00, 60.58)	0.00 (0.00, 0.00)	u=8.362	<0.001
CD15 (%)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	<i>u</i> =0.970	0.332
CD34 (%)	1.00 (1.00, 1.00)	20.07 (0.00, 49.84)	<i>u</i> =5.524	<0.001
CD56 (%)	0.00 (0.00, 0.00)	0.00 (0.00, 54.05)	u=3.652	<0.001
HLA-DR (%)	53.64 (43.43, 0.00)	28.42 (0.00, 61.31)	u=4.268	< 0.001
Peripheral blood cells				
WBC (×10 ⁹ /L)	23.71 (12.60, 60.02)	7.34 (2.38, 26.63)	u=4.944	< 0.001
Hemoglobin (g/L)	89.64±23.05	75.65±23.65	<i>t</i> =4.156	<0.001
Platelet (×10%L)	39.02±37.67	54.39±85.84	t=1.347	0.179
Marrow blasts (%)	50.10±32.37	54.20±31.68	t=0.901	0.368

Abbreviations: FAB, French-American-British; APL, acute promyelocytic leukemia; WBC, white blood cell.



Figure I Relapse-free survival in patients with and without *CEBPA* mutations. **Abbreviation:** *CEBPA*^{dm}, double mutation *CEBPA*.

with *CEBPA*^{dm}, which was higher than in those without such mutations (51.5%), but no significant difference was detected (*P*=0.145; Figure 1). Patients with *CEBPA*^{dm} had superior OS compared with those without *CEBPA*^{dm} (2-year OS: 88.9% versus 63.5%; *P*=0.034; Figure 2).

Discussion

AML is a heterogeneous disease. Cytogenetics and molecular markers play very important roles in diagnoses, treatment selections, and prognoses. However, ~60% of our AML patients had NK, and their prognoses can be further stratified according to molecular mutations; *CEBPA* mutations were such molecular markers for prognoses. However, there are limited data about the prevalence and prognostic significance of *CEBPA* mutations in AML patients from a Chinese



Figure 2 Overall survival in patients with and without *CEBPA* mutations. **Abbreviation:** *CEBPA*^{dm}, double mutation *CEBPA*.

population. No study has been performed to investigate the immunophenotype of Chinese AML patients with *CEBPA* mutations. In this study, we analyzed the clinical characteristics, therapeutic responses, and long-term outcome details in a consecutive cohort of Chinese patients.

The frequency of CEBPA mutations was 17.10% in this study, which was higher than that reported in populations from Switzerland (8.48%),⁵ the Netherlands (6.90%),⁶ the UK (7.0%),⁴ or France (8.0%),⁷ but was approximately the same as that reported in the scientific literature on a Chinese population (20.6%).¹¹ Shen et al¹² reported that the occurrence rate of CEBPA mutations was 12.2% in Chinese patients with AML. However, the proportion of APL was much higher in their cohort (32.7%) than in the present study (13.33%) or in other published reports. In this and previous studies on APL patients,^{12,13} the incidence rates of CEBPA mutations or NKs in Chinese patients were 25.29% and 22.0%-26.1%, respectively, which were higher than those reported in patients from the western world (10%-18%).^{1-3,8} Hence, the prevalence of CEBPA mutations may be higher in Chinese patients with AML than in their European counterparts, and further research is needed to validate.

Consistent with previous studies, CEBPA mutations were linked to morphologies M₁ and M₂ (66.10% of the mutated patients were M₁ or M₂).^{2,4} The correlation between M₁ and M₂ FAB subtypes and CEBPA mutations observed in this study and previous studies supports the critical role of the CEBPA gene in the intermediate stages of granulocytic differentiation. This also might be the case in patients with CEBPA mutations presenting with lower platelet counts, although no statistical significance to that effect was calculated in this study. We found that CEBPA-mutated patients presented with higher peripheral white blood cell counts, which was not previously observed in non-Chinese patients, but is consistent with one study on a population from the People's Republic of China.¹² This may be due to the following reasons: 1) higher CEBPA mutations were observed in this study and Shen et al's study and 2) the frequency of NPM1 mutations, which was associated with higher peripheral leukocyte counts, was higher in patients with CEBPA mutations in this study (6.78% versus 0.0%–3.3% in previous studies^{2,8}).

In the present study, we also analyzed the immunophenotype of leukemia cells from AML patients. Lin et al¹⁴ reported that positive rates (the cutoff value for positive result was defined as \geq 20% cells) of CD7, CD34, CD15, and HLA-DR were significantly higher in patients with *CEBPA* mutations. We used the percentages of cells with clusters of differentiation markers as our immunophenotype criterion and found that the expression levels of CD7 and HLA-DR increased, whereas those of CD34 and CD56 decreased. There was some controversy for CD34 expression in *CEBPA*-mutated patients. One study from Germany² supported the observation in this study, but another German study³ reported contrasting results. One relevant consideration is that the patients in these two German studies were those with NK.^{2,3}

We also observed that *CEBPA*-mutated patients have higher CR rates similar to those of previous studies.^{8,12} Although we did not find a significant difference for RFS between patients with and without *CEBPA*^{dm}, both this study and previous studies indicate that *CEBPA*^{dm} patients had better OS compared with those without the mutation. We did not evaluate the influence of single or double *CEBPA* mutations on prognoses, owing to the small number of patients with the single mutation.

FLT3-ITD is an indicator of unfavorable prognoses in patients with AML. In this study, six patients with *CEBPA* mutations had the *FLT3*-ITD mutation. Three patients refused further treatment after induction therapy, including one with CR and two with NR, owing to personal reasons. The remaining three patients showed continued CR after four cycles of high-dose cytarabine consolidation, and they received maintenance therapy with biological cellular immune therapy or decitabine.

Conclusion

Both this study and previous studies suggest a higher prevalence of *CEBPA* mutations in AML patients from Chinese population than that in AML patients from populations of western countries, and *CEBPA*^{dm} had a favorable impact on prognoses in AML patients.

Acknowledgments

The authors thank the Department of Hematology and Oncology of The First Hospital, Bethune Medical College of Jilin University, for their assistance in this work. This work was supported by the Fifth Youth Developmental Foundation of The First Hospital of Jilin University (no JDYY52014004).

Disclosure

The authors report no conflicts of interest in this work.

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