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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] $\times 10^9$/L versus 7.34 [2.38, 26.63] $\times 10^9$/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)

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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/m^2 (for some patients >60 years, cytarabine at 1–1.5 g/m^2) 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 M_1 or M_2. Patients with *CEBPA* mutations had a higher percentage of NK (89.80%, 44/49) than those without (52.21%, 130/249; $\chi^2=23.808$, $P<0.001$). 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; $\chi^2=6.185$, $P=0.013$). 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 1 Clinical characteristics of patients with and without *CEBPA* mutations

	***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	$\chi^2=0.041$	0.840
Female	27	135		
FAB classification				
M_1	3	7	$\chi^2=16.732$	0.005
M_2	35	113		
APL	0	46		
M_4	10	64		
M_5	8	46		
M_6	3	8		
Cytogenetics				
Normal	44	130	$\chi^2=23.808$	<0.001
Abnormal	5	119		
Genetic mutations				
NPM1 mutations (%)	6.78 (4/59)	16.43 (47/286)	$\chi^2=3.618$	0.057
FLT3-ITD (%)	10.17 (6/59)	14.34 (41/286)	$\chi^2=0.721$	0.396
c-kit mutations (%)	1.75 (1/57)	5.28 (15/284)	$\chi^2=1.321$	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)	$u=0.970$	0.332
CD34 (%)	1.00 (1.00, 1.00)	20.07 (0.00, 49.84)	$u=5.524$	<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 ($\times10^9$/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	$t=4.156$	<0.001
Platelet ($\times10^9$/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.

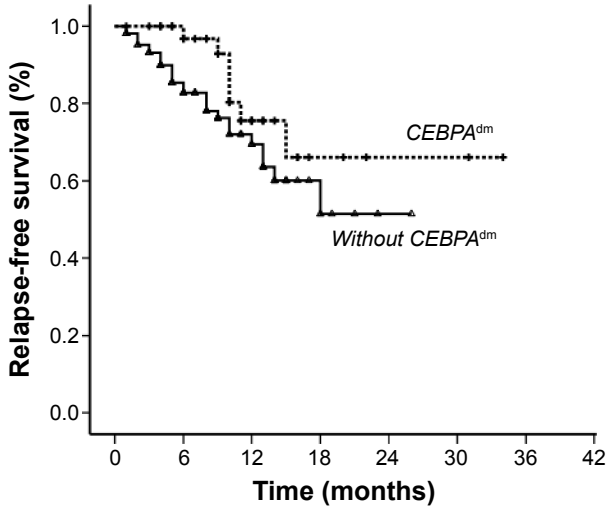

Figure 1 Relapse-free survival in patients with and without *CEBPA* mutations.
Abbreviation: *CEBPA*^{dm}, double mutation *CEBPA*.

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 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.

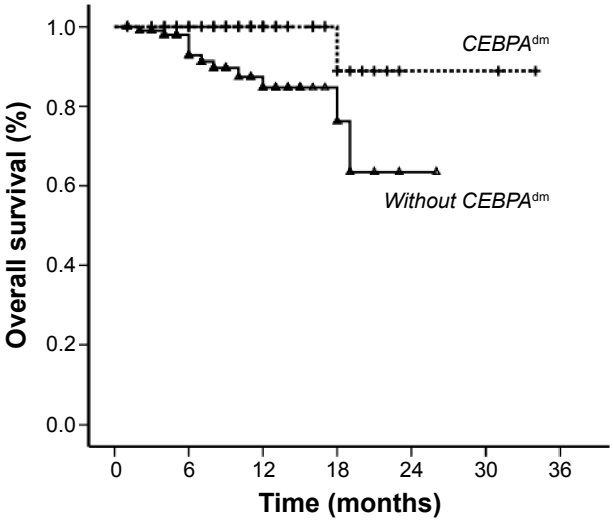

Figure 2 Overall survival in patients with and without *CEBPA* mutations.
Abbreviation: *CEBPA*^{dm}, double mutation *CEBPA*.

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 al[12] 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 M_1 and M_2 (66.10% of the mutated patients were M_1 or M_2).[2,4] The correlation between M_1 and M_2 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 al[14] reported that positive rates (the cutoff value for positive result was defined as ≥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 Germany[2] supported the observation in this study, but another German study[3] 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.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Fröhling S, Schlenk RF, Stolze I, et al. *CEBPA* mutations in younger adults with acute myeloid leukemia and normal cytogenetics: prognostic relevance and analysis of cooperating mutations. *J Clin Oncol*. 2004; 22(4):624–633.
2. Dufour A, Schneider F, Metzeler KH, et al. Acute myeloid leukemia with biallelic *CEBPA* gene mutations and normal karyotype represents a distinct genetic entity associated with a favorable clinical outcome. *J Clin Oncol*. 2010;28(4):570–577.
3. Marcucci G, Maharry K, Radmacher MD, et al. Prognostic significance of, and gene and microRNA expression signatures associated with, *CEBPA* mutations in cytogenetically normal acute myeloid leukemia with high-risk molecular features: a Cancer and Leukemia Group B Study. *J Clin Oncol*. 2008;26(31):5078–5087.
4. Green CL, Koo KK, Hills RK, Burnett AK, Linch DC, Gale RE. Prognostic significance of CEBPA mutations in a large cohort of younger adult patients with acute myeloid leukemia: impact of double *CEBPA* mutations and the interaction with *FLT3* and *NPM1* mutations. *J Clin Oncol*. 2010;28(16):2739–2747.
5. Pabst T, Eyholzer M, Fos J, Mueller BM. Heterogeneity within AML with *CEBPA* mutations; only *CEBPA* double mutations, but not single *CEBPA* mutations are associated with favorable prognosis. *Br J Cancer*. 2009;100(18):1343–1346.
6. Wouters BJ, Löwenberg B, Erpelinck-Verschueren CA, van Putten WL, Valk PJ, Delwel R. Double *CEBPA* mutations, but not single *CEBPA* mutations, define a subgroup of acute myeloid leukemia with a distinctive gene expression profile that is uniquely associated with a favorable outcome. *Blood*. 2009;113(13):3088–3091.
7. Renneville A, Boissel N, Gachard N, et al. The favorable impact of *CEBPA* mutations in patients with acute myeloid leukemia is only observed in the absence of associated cytogenetic abnormalities and *FLT3* internal duplication. *Blood*. 2009;113(21):5090–5093.
8. Taskesen E, Bullinger L, Corbacioglu A, et al. Prognostic impact, concurrent genetic mutations, and gene expression features of AML with *CEBPA* mutations in a cohort of 1182 cytogenetically normal AML patients: further evidence for *CEBPA* double mutant AML as a distinctive disease entity. *Blood*. 2011;117(8):2469–2475.
9. Shaffer LG, Slovak ML, Campbell LJ, editors. *ISCN 2009: An International System for Human Cytogenetic Nomenclature (2009)*. Basel: Karger; 2009.
10. Rulina AV, Spirin PV, Prassolov VS. Activated leukemic oncogenes AML1-ETO and c-kit: role in development of acute myeloid leukemia and current approaches for their inhibition. *Biochemistry (Mosc)*. 2010; 75(13):1650–1666.
11. Ruan GR, Niu JH, Li LD, et al. DNA fragment length analysis for screening of *CEBPA* mutations in acute myeloid leukaemia. *Clin J Clin*. 2011;5(1):64–69.
12. Shen Y, Zhu YM, Fan X, et al. Gene mutation patterns and their prognostic impact in a cohort of 1185 patients with acute myeloid leukemia. *Blood*. 2011;118(20):5593–5603.
13. Ruan GR, Jiang B, Niu JH, et al. Detection of *CEBPA* gene mutations in acute myeloid leukaemia and its clinic significance. *Clin J Clin*. 2011; 5(17):4987–4991.
14. Lin LI, Chen CY, Lin DT, et al. Characterization of *CEBPA* mutations in acute myeloid leukemia: most patients with *CEBPA* mutations have biallelic mutations and show a distinct immunophenotype of the leukemic cells. *Clin Cancer Res*. 2005;11(4):1372–1379.