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Integrative analysis of prognostic factors in Chinese core binding factor leukemia

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ABSTRACT

The characteristics of core binding factor (CBF) leukemia appear to differ between Chinese and Caucasian patients. In this study, we analyzed the biological and clinical characteristics of 76 Chinese CBF leukemia patients out of 425 newly diagnosed acute myeloid leukemia (AML) patients. The frequency of CBF AML was 17.9%. Patients harboring t(8;21) were predominant in CBF AML. The incidence of c-kit mutation in CBF AML was 28.9%. The N822K mutation appeared to be more prevalent in Chinese CBF AML patients. Multivariate analysis showed that c-kit mutation and high white blood cell count could negatively impact overall survival (OS) (HR = 2.74 and 6.24, $P = 0.007$ and 0.022, respectively) but did not affect relapse-free survival (RFS). Kaplan–Meier analysis showed a significant difference in both OS and RFS between wild-type and mutated c-kit patients. Although we had included recently reported prognostic indicators in our analysis, our results demonstrated that only c-kit mutation and high white blood cell count had prognostic impact on Chinese CBF AML patients.

1. Introduction

The chromosome translocation t(8;21) and the inversion (inv)16/t(16;16) are categorized as core binding factor (CBF) acute myeloid leukemia (AML). The alterations generate fusion proteins, AML1-ETO and CBFβ-MYH11, that disrupt the α and β subunits of the CBF complex, respectively, and result in impaired hematopoietic differentiation [1,2]. CBF AML is classified as a specific AML subtype because of not only its characteristic molecular origin but also its relatively favorable outcome compared to other non-acute promyelocytic leukemia AML subtypes [3,4]. The overall 5-year survival rate in CBF AML is over 50% when treated with high-dose cytarabine-based chemotherapy; therefore, currently, chemotherapy is the first-line treatment option for CBF AML [5,6].

Importantly, non-Caucasian populations have been regarded as one of the adverse prognostic factors in CBF AML [7]. However, until now, most reports on non-Caucasian CBF AML have been performed in the African–American population, while the prognosis of Chinese CBF AML patients remains controversial. For example, the 2-year survival of Chinese CBF AML patients in different medical centers ranges from 36.2% to 74.8% [8–10]. These studies have raised the concern of whether Chinese CBF AML has a distinct disease pattern.

Several factors are considered to have a prognostic impact on CBF AML. The most well-established risk factor is mutation of the c-kit gene [11,12]. The major forms of c-kit mutation in CBF AML are point mutations in exon 17 or small deletions/insertions in exon 8. These mutations give rise to constitutively activated receptor tyrosine kinase or hyperactivation, respectively, and are associated with the adverse prognosis of CBF AML [13–15]. In addition, aging (60 years or older) and the high expression of an alternative splice isoform, AML1-ETO9a, were documented as unfavorable prognostic indicators [10,16]. In recent years, there have been significant advances in defining genetic markers as risk stratification methods to distinguish subgroups of AML [17]. Despite the rapid advances in CBF AML therapy and risk stratification, there are limited integrative data of Chinese CBF AML available. In this study, we examined clinical features, cytogenetics, and molecular abnormalities from 2 Chinese medical centers to identify the characteristics of Chinese CBF AML patients.

2. Materials and methods

2.1. Patients and treatment

From January 2008 to December 2010, 425 patients were diagnosed with de novo AML according to the French–American–British (FAB) criteria in 2 Chinese hematological centers: Tongji Hospital in Wuhan and Jiangsu Province Hospital in Nanjing. The diagnosis of CBF AML was based on cytogenetic findings of karyotype t(8;21) and inv(16) or detection of the fusion transcripts

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AML1-ETO and CBFβ-MYH11 by reverse transcription polymerase chain reaction. Related clinical parameters were collected for data analysis. All patients received induction therapy with cytarabine (100–200 mg/m^2/d for 7 days) in combination with daunorubicin (40–60 mg/m^2/d for 3 days). The post-remission therapy consisted of 3 or more courses of the administration of cytarabine 0.5–3 g/m^2 every 12 h on days 1–3. This study was approved by the ethics board of the two hospitals.

2.2. Detection of genetic mutations

Bone marrow aspirate was collected before treatment, and mononuclear cells were isolated by Ficoll density gradient centrifugation. Genomic DNA was prepared using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). Exon 8 of the c-kit gene was amplified using the primer pair F: 5′-GCT GAG GTT TTC CAG CAC TC- 3′ and R: 5′-AAT TGC AGT CCT TCC CCT CT-3′, and exon 17 was amplified using the primer pair F: 5′-TGA ACA TCA TTC AAG GCG TA-3′ and R: 5′-TCA CATG CCC CAA AAT TAC A-3′. The PCR products were subjected to direct DNA sequencing to detect mutations. Absolute quantitative PCR using a TaqMan® probe (ABI, Carlsbad, CA, USA) was employed to screen the common mutation types of NPM1, as previously reported [18]. The detection of CEBPα and WT1 mutations was performed by PCR amplification and subsequent sequencing as described previously [19,20]. FLT3-ITD was screened using 3% agarose gel electrophoresis of the PCR product spanning exons 14 and 15 [21]. Other mutations, including DNMT3A, N-RAS, K-RAS, IDH1, IDH2 and FLT3-TKD, were screened for point mutations by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). The c-kit mutations D816V and N822K were also validated by the more sensitive MALDI-TOF MS.

2.3. Statistical analysis

The analysis of categorical variables was performed using Fisher's exact test for 2×2 tables or Pearson's χ^2 test. Student's t test and the Mann–Whitney U test were applied to continuous variables. Complete remission (CR) was defined as bone marrow morphology with less than 5% blasts, a neutrophil count of 1×10^9 L^{-1} or more, a platelet count of 100×10^9 L^{-1} or more, and no evidence of extramedullary leukemia. Patients lost to follow-up were censored at the date of last contact. Multivariate models using Cox or logistic regression analysis were employed to assess the hazard ratios and odds ratios of patient characteristics on survival and CR. Differences in survival were compared using the log-rank test and estimated by the Kaplan–Meier method. All calculations were performed using SPSS Software version 16.0 (SPSS, Chicago, IL, USA). P values less than 0.05 (two-tailed) were considered to be statistically significant.

3. Results

3.1. General characteristics

Among the 425 AML patients, 76 patients (17.9%) were diagnosed with CBF AML, including 65 cases of t(8;21) and 11 cases of inv(16). As shown in Table 1, the FAB subtype was mainly M2 for t(8;21) patients and M4 for inv(16) patients ($P < 0.001$). Patients with inv(16) had considerably higher peripheral blood WBC counts ($P = 0.003$) and were more likely to have extramedullary involvement ($P < 0.001$), but no differences were found in bone marrow blast percentage, age or gender. Additional chromosomal abnormalities were observed in 41.5% of t(8;21) patients and 18.2% of inv(16) patients but showed no difference between the two groups ($P = 0.12$).

C-kit mutation was detected in 22 (28.9%) patients, including 21 cases of t(8;21) and 1 case of inv(16) ($P = 0.116$). The c-kit mutation subtypes were D816V (5/22, 22.7%), D816H (3/22, 13.6%), D816Y (3/22, 13.6%), N882K (5/22, 22.7%) and insertions/deletions at exon 8 (6/22, 27.3%). In addition, point mutations, including D816V and N822K, were further confirmed by MALDI-TOF MS. C-kit-mutated patients were younger than wild-type patients ($P = 0.042$) and had a marginally higher blast percentage in the bone marrow ($P = 0.057$). There was no significant difference in other clinical features between c-kit-mutated and wild-type patients.

3.2. Status of other mutations

The frequencies of additional gene mutations were compared between CBF AML patients and all non-CBF M2 and M4 patients in the study. Additional gene mutations were detected in 9 cases of CBF AML, including FLT3-ITD (1/76, 1.3%), CEBPα single mutation (3/76, 3.9%), DNMT3A (1/76, 1.3%), IDH1 (1/76, 1.3%), IDH2 (2/76, 2.6%) and K-RAS (1/76, 1.3%). As shown in Table 2, non-

Table 1
General characteristics of Chinese CBF AML patients.

Characteristic	AML-ETO $N = 65$	CBFβ-MYH11 $N = 11$	P	Wild-type c-kit $N = 54$	Mutated c-kit $N = 22$	P
Median age, years (range)	32 (13–70)	28 (16–64)	0.86	32 (13–70)	27 (13–50)	0.042
Gender, no. of patients (%)			0.099			0.133
Male	36 (55.4)	9 (81.8)		29 (53.7)	16 (72.7)	
Female	29 (44.6)	2 (18.2)		25 (46.3)	6 (27.3)	
Median WBC count, $\times 10^9$ L^{-1} (range)	9.5 (0.96–148.2)	60.5(1.08–191)	0.003	10.98 (0.96–191)	12.95 (3–100)	0.613
Median hemoglobin, g/L (range)	67.4 (28–147)	88.3 (31–111)	0.36	64.15 (28–147)	76 (41.6–123)	0.419
Median platelet count,$\times 10^9$ L^{-1} (range)	22 (4–292)	29 (10–53)	0.51	25.5 (4–292)	21.5 (7–201)	0.671
Median BM blasts, % (range)	54.9 (0.8–94.4)	59 (22–83.6)	0.87	51.6 (0.8–85.2)	66.15 (25–94.4)	0.057
FAB subtype, no. (%)			<0.001			0.214
M2	58 (89.2)	1 (9.1)		40 (74.1)	19 (86.4)	
M4	4 (6.2)	8 (72.7)		11 (20.4)	1 (4.5)	
M5	3 (4.6)	2 (18.2)		3 (5.6)	2 (9.1)	
Additional chromosome abnormalities, no. (%)			0.12			0.857
Yes	27 (41.5)	2 (18.2)		21 (38.9)	8 (36.4)	
No	31 (47.7)	8 (72.7)		29 (53.7)	10 (45.4)	
ND	7 (10.8)	1 (9.1)		4(7.4)	4 (18.2)	
Extramedullary involvement, no. (%)			<0.001			0.209
Yes	63 (93.8)	6 (54.5)		8 (14.8)	1 (4.5)	
No	2 (6.2)	5 (45.5)		46 (85.2)	21 (95.5)	

WBC, white blood cell; BM, bone marrow.