LETTER TO THE EDITOR

Kinase-associated gene mutation pattern and clinical relevance in 205 patients with core binding factor leukemias

Blood Cancer Journal (2016) 6, e494; doi:10.1038/bcj.2016.107; published online 11 November 2016

Core binding factor (CBF) leukemia represents an individual subgroup of the disease, which accounts for 20% of acute myeloid leukemia (AML), characterized by the special t(8;21)(q22;q22) translocation most in AML-M2 variant (CBFa leukemia) or inv(16) (p13q22)/(t;16;16) rearrangement in AML-M4 with eosinophilia (CBFB leukemia), respectively. Chimerical fusion genes AML1-ETO and CBFB-MYH11 are formed by these two cytotgenetic changes, respectively, which finally lead to the leukemogenesis. Generally, CBF leukemias are considered to have favorable treatment outcome and prognosis and most centers regard CBF markers as ‘good’ cytogenetic factor, with a 5-year overall survival (OS) rate over 50%. However, given using similar treatment strategy, such as ‘3+7’ regimen in induction and high-dose Ara-C in consolidation, the treatment outcome of CBF leukemia in Chinese patients were not as good as reported by western groups. Interestingly, the incidence of CBF leukemia is even significantly lower than the western countries, as shown in our previous report; in 1185 AML patients, only 18 M4 with eosinophilia patients were identified. The difference of genetic background between Chinese and western population may be the reason, however, until now, evidence remains unavailable.

In mouse model, stepwise leukemogenesis in AML with t(8;21)/AML1-ETO is proved by the phenomena that coexpression of C-KIT NK822K and AML1-ETO induces the full development of AML, whereas single or C-KIT is not sufficient to lead to the leukemia. Similarly, transgenic mice of CBF-MYH11 only induce a myeloid maturation block. Therefore, it could be concluded that additional mutations, especially kinase-associated mutations, providing a second ‘hit’ may play a crucial role in the evolving of the disease.

In this study, we included 205 newly diagnosed AML patients, including 180 patients with CBFa and 25 patients with CBFb leukemia, to investigate the potential role of additional mutations beyond AML1-ETO and CBFB-MYH11 in these diseases. All the patients received standard first-line treatment of DNR (daunorubicin), A (Ara-c(cytarabine))-like regimen. In the consolidation therapy, young patients were treated with high-dose cytarabine-based chemotherapy. Allogeneic stem cell transplantation was not used as first-line treatment in first time to complete remission. This study was approved by the ethnic board of the participating centers. All patients were given informed consent for both treatment and cryopreservation of bone marrow and peripheral blood according to the Declaration of Helsinki.

Genomic DNA and total RNA were extracted as previously reported. We had screened the mutational status of FLT3-ITD and -TKD, C-KIT, N-RAS, CEPBA, WT1, ASXL1, DNMT3A, NPM1, MLL, IDH2 and TET2 genes by distinct approaches. A chip-based matrix-assisted laser desorption/ionization time-of-flight mass spectrometry analysis system (iPLEXTM, Sequenom, San Diego, CA, USA) was used to assess the mutational status of FLT3-TKD, N-RAS, NPM1, IDH1 and IDH2. For mutations of FLT3-ITD, and those in C-KIT, CEPBA, WT1, ASXL1, DNMT3A and TET2 genes, samples were analyzed by whole-gene sequencing. Six MLL-related common fusion genes, including MLL-AF9, MLL-AF10, MLL-AF6, MLL-ELL, MLL-ENL and MLL-AF17, were detected via multiplex RT-PCR strategy. Briefly, all samples were screened with two parallel multiplex RT-PCR reactions. If there were positive PCR fragments in the samples, split-out PCR was performed to determine the fusion gene type. The fusion genes, such as AML1(CBFa)-ETO and CBFB-MYH11, were determined by RT-PCR technique.

Kaplan-Meier and hazard ratio analyses were used to calculate and compare the OS and disease-free survival (DFS), respectively. Cox model was used for the multivariate analysis of associations of survival and potential prognostic factors.

The characteristics of the 205 patients with CBF AML, including 180 CBFa and 25 CBFb, are summarized in Table 1. The incidence of CBFb leukemia was significantly lower than CBFa leukemia, which was not concurrent with the western population, which might be contributed by the difference of genetic backgrounds between Chinese and Caucasian populations. In the 205 patients, 82 (40%) patients carried at least one mutation. C-KIT (55/205, 26.8%), and N-RAS (18/205, 8.8%) and CBFBPA (10/205, 8.8%) mutations were identified as the most common additional gene mutations, whereas another kinase-associated gene, FLT3 mutations were very few (2/205, 1.0%). When we combined C-KIT, N-RAS and FLT3 mutation together as a group of class I mutation, 67 (32.7%) patients contained such events. Other mutations could also be identified in relative low incidence (Supplementary Table 1). Interestingly, in CBF leukemia, mutual coexistence could be observed among the class I mutation (Figure 1a). Seven patients carried C-KIT and N-RAS and one patient carried C-KIT and FLT3 mutations together. We identified a similar incidence of C-KIT (30.2% and 13.6% in CBFa and CBFb, respectively) and N-RAS (8.4% and 19.0% in CBFa and CBFb, respectively), in contrast, FLT3 was very few identified in our group, which is different with the western reports (around 10%) but similar with the Asian series (1%) (Supplementary Table 2). This distribution of gene mutations is totally converse to acute promyelocytic leukemia, which have higher incidence of FLT3, whereas C-KIT are few identified.

As far as the possible association with clinic features, there was no significant difference regarding the age, gender and median

Table 1. Clinical characteristics of 205 CBF AML patients

| Characteristics | CBFa leukemia | CBFb leukemia |
|-----------------|---------------|---------------|
| Gender no. (%)  |               |               |
| Male            | 80 (44.4)     | 9 (36)        |
| Female          | 100 (55.6)    | 94 (64)       |
| Median age, years | 34 ± 19.1    | 40 ± 22.2     |
| Median WBC count, 10⁹/l | 8.95 | 48.1 |
| Range           | (0.8–177.9)   | (3.8–140.0)   |
| Median BM blasts, % | 56 | 78 |
| FAB subtype no. (%) |       |               |
| M2v             | 143           |               |
| M4              | 27            |               |
| M4eo            | 25            |               |
| M5              | 5             |               |
| Not classified  | 5             |               |

Abbreviations: BM, bone marrow; M4eo, M4 with eosinophilia; M2v, M2 variant; WBC, white blood cell.
WBC count in different mutation groups, with the exception of bone marrow blasts, which seemed higher in class I mutation group ($P < 0.001$, Supplementary Table 3).

Among 205 CBF leukemia patients, 160 (78.0%) achieved CR. CBF$\beta$ leukemia seemed to have a higher CR rate as compared with CBF$\alpha$ leukemia, however, no significance was achieved ($22/25$ (88.0%) vs $138/180$ (76.7%), $P = 0.302$). No adverse effects of FLT3-ITD/TKD, N-RAS, C-KIT and class I mutations were observed on CR induction either in CBF$\alpha$ leukemia or in CBF$\beta$ leukemia (Supplementary Table 4). Hence, no further multivariate analysis was performed.

The OS of CBF$\beta$ leukemia was significantly higher than CBF$\alpha$ leukemia (median OS: $40.0 \pm 1.1$ months, $P = 0.030$, median DFS: $21.0 \pm 5.5$ vs $18.0 \pm 4.0$ months, $P = 0.438$, Supplementary Figures 1A and 1B).

In 180 CBF$\alpha$ leukemia patients, C-KIT and class I mutations were associated with poor OS and DFS, respectively. For C-KIT mutant (−) and (+) patients, the median OS and DFS was $20.2 \pm 2.2$ and $14.0 \pm 3.5$ months ($P = 0.050$), and $25.0 \pm 10.0$ and $14.0 \pm 1.4$ months ($P = 0.046$), respectively. And for class I mutant (−) and (+) patients, the median OS and DFS was $23.0 \pm 3.4$ and $17.0 \pm 3.0$ months ($P = 0.030$), and $34.0 \pm 11.5$ and $15.0 \pm 1.3$ months ($P = 0.048$), respectively. The Kaplan-Meier survival curves were shown in Figure 1b and c. No significance was observed in different N-RAS mutant subgroup ($P = 0.497$ and 0.641, respectively), which might be caused by the small samples.

In 25 CBF$\beta$ leukemia patients, there is no significant difference between different mutant groups (OS: $P = 0.935$, 0.379 and 0.848 for class I, C-KIT and N-RAS, respectively; DFS: $P = 0.562$, not fit due to small sample, and 0.426 for class I, C-KIT and N-RAS, respectively).
In multivariate analysis of OS and DFS involving clinical parameters and class I mutation in 180 CBFα leukemia patients, class I mutation remained an independent prognostic factor for OS (P = 0.041), whereas no factor was the independent one for DFS (Supplementary Table 5).

CBF AML is a unique subtype of leukemia, whose diagnosis is so strongly upon detection of clonal genetic abnormalities of t(8;21) (q22;q22) and inv(16)(p13q22)/t(16;16), or their related gene fusion of AML1-ETO and CBF-MYH11, whereas the proportion of bone marrow blasts.12 It was reported that the incidence of CBFα and CBFβ AML was around 7% and 5–8%, respectively. Although lack of strong epidemiology data, our previous experience13 and this work proved that, in Chinese population, a slight more CBFα leukemia patients are presented, whereas CBFβ AML are very few. In this study, a similar incidence of C-KIT (30.2% and 13.6% in CBFα and CBFβ, respectively) and N-RAS (8.4% and 19.0% in CBFα and CBFβ, respectively) was identified; whereas FLT3 was very few in our group, similar with the Asian reports.

CBF AML is considered to have favorable prognosis when received standard DNR (daunorubicin), A (ara-c(cytarabine)) induction and high-dose Ara-C-based consolidation. However, in Chinese population, as presented in this study and previous reports, the treatment outcome was not as good as the western reports, given the similar treatment, especially for CBFα AML. Huang et al.12 even use allogeneic stem cell transplantation as the front-line treatment for t(8;21) AML patient, regarding the mutational status of C-KIT and minimal residual disease during the treatment. Our data proved that CBFβ AML have a better survival as compared with CBFα AML. In CBFα AML, kinase-associated mutation, when combined together, exerted a strong negative effect on survival (hazard ratio = 1.617 (95% confidence interval: 1.048–2.495), P = 0.030 and hazard ratio = 1.759 (95% confidence interval: 1.005–3.079), P = 0.048 for OS and DFS, respectively), which was further proved by multivariate analysis.

In conclusion, CBF AML is a heterogeneous disease, whose clinical behavior and treatment outcome is strongly dependent on additional gene mutations, especially for class I mutations.13 Further improvement of the prognosis of the disease should corporate TKI in the standard treatment algorithm, and several groups reported a promising result.14 This strategy may especially benefit Chinese CBF leukemia patients, new clinical trial integrating homoharringtonin,15 which is proved to improve the survival of non-CBF AML.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported in part by the National Natural Science Foundation of China (81370653), National Clinical Research Base construction Projects of Traditional Medicine (2012H01), and we are grateful to Shu-Min Xiong for performing the hematological morphological analysis.

AUTHOR CONTRIBUTIONS

SY and YY were the principal investigators who conceived the study, Y-SC, P-PW and YH performed most of the experiments. SY and YY coordinated and oversaw the study. J-YH was responsible for bioinformatics investigation. BC, Y-MZ and X-QW participated in sample bank management. J-ML gathered detailed clinical information for the study and helped to perform clinical analysis. YS wrote the manuscript.

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