Additional chromosomal abnormalities in core-binding factor acute myeloid leukemia

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ABSTRACT. Despite sharing a similar genetic abnormality, patients with core binding factor acute myeloid leukemia (CBF-AML), which is characterized by the presence of t(8;21) or inv(16)/t(16;16), show heterogeneous survival. Other molecular or cytogenetic factors are supposed to have an impact on the prognosis. We enrolled 24 CBF-AML patients to determine the impact of cytogenetic abnormality, and c-KIT, FLT3, NPM1, and CEBPA mutations on the prognosis. Only three patients had the c-KIT mutation (3/24, 12.5%) and one had the FLT3 mutation. However, over half of the patients (14/24) harbored additional cytogenetic changes, including ten with loss of sexual chromosomes (LOS) [all in the t(8;21) group], and six had additional abnormalities (two cases had both
LOS and additional abnormalities). From this small-number study, no association was found between c-KIT mutation and survival and relapse rate. However, additional chromosome abnormalities had a significant association with relapse of the disease ($P = 0.027$). Stem cell transplant had a trend of benefitting patients after relapse ($P = 0.065$). This implies that chromosome abnormalities occur in CBF-AML and might take part in the heterogeneous nature of CBF-AML.

Key words: Core binding factor; Acute myeloid leukemia; c-KIT; Loss of sexual chromosomes

INTRODUCTION

The chromosomal rearrangements t(8;21)(q22;q22) and inv(16)(p13.1q22)/t(16;16) (p13.1;q22) are among the most common recurrent genetic abnormalities found in core binding factor acute myeloid leukemia (CBF-AML) (Marcucci et al., 2005; Kuwatsuka et al., 2009). Although CBF-AML sufferers have a better prognosis than is normal in cytogenetic patients, the disease is still quite heterogeneous with a subset of patients responding poorly to therapy (Hart and Foroni et al., 2002; Swerdlow et al., 2008). Additional mutation and cytogenetic factors might take part in the pathomechanisms (Boissel et al., 2006).

c-KIT is a proto-oncogene located on chromosome 4q11-12, which encodes a 145-kDa transmembrane glycoprotein, a member of the type III tyrosine kinase receptor family (d’Auriol et al., 1988). After activation, the gene is integral to the proliferation, differentiation, and survival of hematopoietic stem cells because it modifies downstream signaling pathways. Mutated c-KIT genes have been reported in 12.8-46.1% of adult CBF-AML cases and also in around 12% of pediatric patients with a poor prognosis, but the impact remains equivocal (Hart and Foroni et al., 2002; Boissel et al., 2006; Paschka et al., 2006; Renneville et al., 2008; Pollard et al., 2010; Park et al., 2011; Cairoli et al., 2006, 2013).

In this study, we enrolled CBF-AML patients and reviewed their cytogenetic and molecular data to determine the effect of these abnormalities.

MATERIAL AND METHODS

Patients and treatments

We analyzed newly diagnosed AML patients in our hospital between January 2004 and December 2011, and enrolled 24 patients with CBF-AML in this study. The diagnosis of CBF-AML was arrived at following the discovery of either t(8;21) or inv(16)/t(16;16) in bone marrow or aspiration samples, or by detecting RUNX1-RUNX1T1 and/or CBFB-MYH11 fusion transcripts using reverse transcriptase polymerase chain reaction. Of the 24 patients, 17 harbored t(8;21)/RUNX1-RUNX1T1 and seven had inv(16)/CBFB-MYH11. All patients except one received standard induction chemotherapy with continuous intravenous infusion of cytarabine 100 mg/m² from Day 1 to Day 7, and idarubicin 45 mg/m² from Day 1 to Day 3. Complete remission was defined as recovery of blood cell count and less than 5% blast cells.
in the marrow. Patients achieving complete remission were prescribed high-dose cytarabine for consolidation treatment. Relapsed patients received induction therapy with a salvage regimen. Stem cell transplants were carried out on selected patients depending on their symptoms.

Mutations analysis

Bone marrow samples were used for the mutations survey. Polymerase chain reaction and direct sequencing were used to detect mutations in c-KIT (exons 8 and 17), as in the study by Park et al. (2011). The mutations in FLT3, nucleophosmin (NPM1), and CCAAT/enhancer binding protein (C/EBP) were performed as in previous studies (Fröhling et al., 2002; Preudhomme et al., 2002; Döhner et al., 2005). The results were identified for further statistical analysis.

Statistical analysis

All calculations were performed using SPSS 17.0 for Windows (SPSS Inc., IL, USA). Estimates of survival, overall survival, and event-free survival were constructed using Kaplan-Meier methods and were compared by a log-rank test. Pearson chi-square was used to compare categorical variables. For all analyses, tests were two-tailed and P values < 0.05 were considered to be statistically significant.

RESULTS

We studied a total of 24 patients, 17 male and 7 female, with a mean age of 43.7 years. Cytogenetic studies showed eight patients harboring standard t(8;21)/inv(16), six patients with additional abnormalities, and ten patients with loss of sexual chromosome (LOS), all of which were in the t(8;21) group (Table 1). The additional abnormalities included trisomy (8), trisomy (21), del (7), and del (9). There were two patients with additional abnormalities and LOS. Three patients harbored c-KIT mutation (3/24, 12.5%) and the other one had an Flt-3 mutation. There were no MPN1 or CEBPA mutations in the patients. All patients, except one owing to personal reasons, received standard induction chemotherapy with cytarabine 100 mg/m² from Day 1 to Day 7 and idarubicin 45 mg/m² from Day 1 to Day 3. Among the 23 intent-to-treat patients, 21 (91.3%) achieved complete remission after chemotherapy. However, 12 of the 21 remission patients eventually relapsed with a median progression-free survival of 12 months. Of the relapsed patients, four of five underwent salvage chemotherapy/stem cell transplant and survived, while only two of seven survived from salvage chemotherapy alone. There were three patients with the c-KIT mutation (two exon 17, one exon 8), and only one with the Flt-3 mutation. The remission status was not associated with c-KIT, Flt-3, additional chromosome, or LOS. Additional chromosome abnormalities were associated with disease relapse (P = 0.027), while c-KIT mutation, Flt-3 mutation, or LOS were not. With a median follow-up of 36 months, nine patients died including six patients from disease relapse, two from refractory disease, and one from sepsis during consolidation. Neither chromosome abnormalities nor gene mutation was associated with the overall survival. Relapse of disease had a trend of an impact on survival (P = 0.077) (Figure 1) and stem cell transplant after relapse had a trend to improve survival (P = 0.065).
Figure 1. Survival curve of patients with relapse of disease and non-relapse patients. (P = 0.077). OS = overall survival.

Table 1. Characteristics of patients.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
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<tbody>
<tr>
<td>Age</td>
<td>21–70</td>
</tr>
<tr>
<td>Gender</td>
<td>14 male</td>
</tr>
<tr>
<td></td>
<td>10 female</td>
</tr>
<tr>
<td>Cytogenetics</td>
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</tr>
<tr>
<td>t(8;21)</td>
<td>N = 17</td>
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<tr>
<td></td>
<td>Standard</td>
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<tr>
<td></td>
<td>Additional</td>
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<tr>
<td></td>
<td>LOS</td>
</tr>
<tr>
<td>Inv(16)/t(16;16)</td>
<td>N = 7</td>
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<tr>
<td></td>
<td>Standard</td>
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<tr>
<td></td>
<td>Additional</td>
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<tr>
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<td>Flt-3</td>
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<td>NPM1</td>
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</table>

LOS = loss of sexual chromosome.

DISCUSSION

Despite sharing a similar pathomechanism, the survival rate of CBF-AML patients is variable, which suggests its heterogeneous nature and the possible influence of other fac-
tors (Hart and Foroni et al., 2002; Marcucci et al., 2005; Swerdlow et al., 2008; Kuwatsuka et al., 2009). A relatively high incidence of c-KIT mutation has been noted in CBF-AML cases, which has an impact on prognosis (Boissel et al., 2006; Cairoli et al., 2006). Among the mutations involving “two-hit” therapy of AML pathogenesis, FLT3 has also been noted in CBF-AML with a lower incidence of around 5%, while NPM1 and CEBPA mutations are rare in CBF-AML. Therefore, we enrolled our patients to determine the effects of other factors on CBF-AML. In this study, the incidence of c-KIT mutations was low (3/24, 12.5%) and there was only one FLT3 mutation. However, the incidence of chromosomal abnormalities was high (16/24, 66%), especially LOS, and all of the LOS were in t(8;21) patients.

In previous studies, c-KIT mutations had an impact on prognosis, with poor outcomes and high relapse rates (Hart and Foroni et al., 2002; Boissel et al., 2006; Cairoli et al., 2006; Park et al., 2011), and greater difficulty in obtaining molecular remission (Park et al., 2013). However, some reports have shown equivocal results (Pollard et al., 2010; Cairoli et al., 2013). Owing to the small case number with c-KIT mutations in our study, we could not identify the effect of c-KIT on the disease with regards to relapse rate, overall survival, and progression-free survival. The real effect of these mutations on the disease should be elucidated by further studies.

Other cytogenetic abnormalities were high in this study, with ten cases of LOS and six cases of additional chromosome abnormalities. The incidence of chromosome abnormalities was higher than previously reported (Hart and Foroni et al., 2002; Cairoli et al., 2006, 2013; Park et al, 2013). The mean age of our patients was 43, which concurred with the study that showed that advanced age is associated with more frequent additional chromosome abnormalities, and predicted a higher cumulative incidence of relapse (Hart and Foroni et al, 2002). Though LOS showed no impact on the prognosis, additional chromosome abnormalities had an impact on relapse rate in our patients. This implies that chromosome analysis should be taken into consideration during management of this kind of patient.

In summary, there was a low incidence of c-KIT mutation in this small-number study. Moreover, chromosome abnormalities, especially additional chromosome abnormalities, in CBF-AML might explain the heterogeneous nature of CBF-AML survival.

Conflicts of interest

The authors declare no conflict of interest.

REFERENCES

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