

RESEARCH ARTICLE

Cytogenetic and Genetic Mutation Features of *de novo* Acute Myeloid Leukemia in Elderly Chinese Patients

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Abstract

Objectives: The present study aimed to examine the cytogenetic and genetic mutation features of acute myeloid leukemia (AML) in elderly Chinese patients. **Methods:** A retrospective analysis of cytogenetics and genetic mutations was performed in 113 cases (age range 50-82 years) with *de novo* AML. **Results:** The most frequent cytogenetic abnormality was t (15;17) (q22;q21), detected in 10.0% (n = 9) of successfully analyzed cases, followed by t (8;21) (q22;q22) in 8.89% (n = 8), and complex karyotypes in 5.56% (n = 5). Those with complex karyotypes included 4 cases (4.44%) of monosomal karyotypes. The frequencies of NPM1, FLT3-ITD, c-kit, and CEBPA mutations were 27.4% (31/113), 14.5% (16/110), 5.88% (6/102), and 23.3% (7/30), respectively. The complete remission rates of patients in low, intermediate, and high risk groups were 37.5%, 48.6%, and 33.3%, respectively ($\chi^2 = 0.704$, $P = 0.703$) based on risk stratification. **Conclusion:** Cytogenetics and genetic mutations alone may not be sufficient to evaluate the prognoses of elderly AML patients. The search for a novel model that would enable a more comprehensive evaluation of this population is therefore imperative.

Keywords: Acute myeloid leukemia - cytogenetics - genetic mutations - Chinese elderly cases

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Introduction

Acute myeloid leukemia (AML) is a highly heterogeneous disease. Cytogenetics and genetic mutations play important roles in the diagnoses, risk stratification, and treatment of AML. In general, the clonal chromosomal abnormalities and genetic alterations observed in the leukemic cells from AML patients can be used to categorize cases into groups with similar clinical features and prognoses (Moorman et al., 2001). However, the prognosis of elderly patients with AML is very poor, and a higher incidence of adverse karyotypes is one of the factors associated with such unfavorable outcomes (Estey, 2007; Quintás-Cardama et al., 2012). In addition, we previously reported that the occurrence rates of favorable karyotypes decreased with age, whereas that of unfavorable karyotypes increased (Su et al., 2013). Furthermore, a number of genetic mutations have a particularly significant impact on AML prognosis, refining the disease's risk stratification, especially in patients with a normal karyotype. Mutations in the nucleophosmin (NPM1), CCAAT/enhancer binding protein alpha (CEBPA), and c-kit genes, as well as the internal tandem duplications of Fms-like tyrosine kinase 3 gene (FLT3-ITD) are listed as molecular predictors for AML in both, the European LeukemiaNet (ELN) classification and the National Comprehensive Cancer Network (NCCN) guidelines. Previous studies, including ours, reported

that the frequencies of NPM1 and FLT3-ITD mutations decreased in patients older than 60 years of age (Schneider et al., 2012; Su et al., 2013).

Although cytogenetics and molecular mutations of AML have been studied for many years, most investigations were conducted in young patients. Therefore, information available on elderly patients is scant. Furthermore, cytogenetic and molecular mutation data on elderly Chinese patients are even less readily available although China accounts for over 20% of the world's population. In the present study, we retrospectively analyzed the cytogenetics and genetic mutations in 113 consecutive Chinese patients with *de novo* AML.

Materials and Methods

Patients and induction therapy

From January 1, 2010 to August 31, 2013, 113 patients (62 men and 51 women), aged 50–82 years (median age, 59 years), with *de novo* AML were enrolled in this study. All patients were from the northeast region of China, including the Jilin, Heilongjiang, and Liaoning provinces. Those with a previous diagnosis of myelodysplastic syndrome (MDS) or chronic myeloproliferative disorder were excluded. Non-acute promyelocytic leukemia (APL) patients were treated with the standard “3 + 7” regimen for initial induction (daunorubicin/idarubicin + cytarabine). Some patients were also treated with

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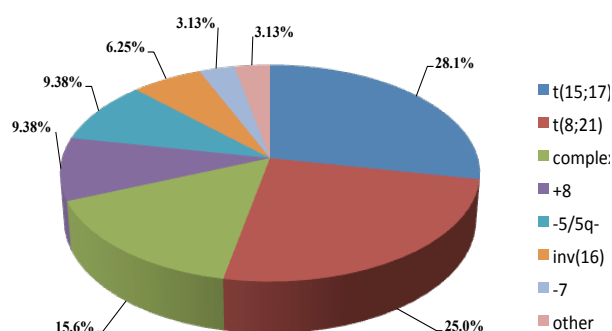


Figure 1. Percentages of Different Cytogenetic Abnormalities

CAG (cytarabine + aclarubicin + granulocyte colony-stimulating factor) regimen or decitabine. APL patients were treated with arsenic trioxide and all-trans retinoic acid (ATRA). Response was assessed by bone marrow aspiration performed on days 14 and 28 after treatment initiation. All participating patients provided informed consent prior to enrolment. This study was approved by the ethics committee of Jilin University and conducted in accordance with the Declaration of Helsinki.

Cytogenetic analyses

Cytogenetic analysis was conducted as previously described (Mir Mazloumi et al., 2013). Briefly, patients' bone marrow samples were cultured in RPMI 1640 medium containing 15% fetal bovine serum (Invitrogen Co., CA, USA) for 24 hours. Cells were then treated with colcemid, followed by trypsin, and stained with Giemsa. Clonal abnormalities were defined and described according to the International System for Human Cytogenetic Nomenclature (Shaffer et al., 2009).

NPM1, FLT3-ITD, c-kit, and CEBPA mutation analyses

Mutational status of molecular markers NPM1 (n = 113), FLT3-ITD (n = 110), c-kit (n = 102), and CEBPA (n = 30) were analyzed using polymerase chain reaction (PCR) as previously described (Han et al., 2006; Dai et al., 2009; Wang et al., 2009; Han et al., 2013). Briefly, genomic DNA was extracted from bone marrow leukemic cells using TRIzol reagent (GibcoBRL, Eggenstein, Germany). For NPM1 mutations, exon 12 of the NPM1 gene was amplified by PCR (forward primer [FP]: 5'-TTAACTCTC-TGGTGGTAGAATGAA-3', reverse primer [RP]: 5'-CAAGACTATTTGCCATTCTAAC-3'), and the products were directly sequenced using the following primer: 5'-AAAAGGACAGCCAGATATCAAC-3' (Hejun Co. Ltd., Shanghai, China). Similarly, FLT3-ITD analysis was performed on genomic DNA by PCR amplification (FP: 5'-GCAATTTAGGTATGAAAGCCAGC-3', RP: 5'-CTTTCAGCATTTTGACGGCAACC-3') (Haijie Co. Ltd., Shanghai, China), and PCR products were sequenced using high performance liquid chromatography. For c-kit mutation, exon 17 of the c-kit gene was amplified (FP: 5'-GTGGTTAAAGGAAACGCTCG-3', RP: 5'-CATAATTTCAGCAGGTGCG-3') (Cybersyn, PA, USA); PCR products were then digested with BsmA1/HinfI restriction enzymes to detect D816 mutation and directly sequenced to detect adjacent

mutations. For CEBPA mutations, genomic DNA was amplified by PCR using 4 pairs of primers (primer 1, FP: 5'-TCGCCATGCCGGGAGAACTCTAC-3', RP: 5'-ACGGCCGCCTTGGCCTTCTCCTGCT-3'; primer 2, FP: 5'-CTTCAACGACGAGTTCTGGCCGA-3', RP: 5'-AGCTGCTTGGCTTCATCCTCCT-3'; primer 3, FP: 5'-CCGCTGGTGATCAAGCAGGA-3', RP: 5'-CCGGTACTCGTTGCTGTTCT-3'; primer 4, FP: 5'-CCGCACCTGCAGTTCCAGAT-3', RP: 5'-CACGGTCTGGGCAAGCCTGGAGAT-3') (Shengong Co. Ltd., Shanghai, China). PCR products were then sequenced using an ABI 3130XL genetic analyzer (Applied Biosystems, CA, USA).

Statistical analyses

The Statistical Package for the Social Sciences (SPSS) version 16.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analysis. The chi-square test or Fisher exact test was employed to assess the statistical significance of the differences between groups, whereas independent sample t-test was used to compare between two groups. *P*-values < 0.05 were considered significant.

Results

French-American-British classification

The patients were categorized into FAB subtypes based on morphological diagnoses (Table 1). The most common subtype in the present cohort was M2 (42.5%, n = 48), followed by M4 (21.2%, n = 24) and M5 (16.8%, n = 19).

Therapeutic responses

APL patients treated with arsenic trioxide and ATRA were excluded from this analysis owing to their favorable outcomes. Of the 99 non-APL patients with available cytogenetic information, 35 did not elect chemotherapy, and the remaining 64 were induced with one course. Thirty cases achieved complete remission (CR), yielding a CR rate of 46.9%, whereas 16 patients achieved partial remission. The overall response rate was thus 71.9%.

Cytogenetics and their clinical significance

Successful cytogenetic analyses were achieved in 90 (79.6%) patients, among whom 32 (35.6%) had detectable clonal abnormalities, whereas 58 (64.4%) were considered cytogenetically normal. The most frequent cytogenetic abnormality was t (15;17) (q22;q21), detected in 10.0% (n = 9) of the successfully analyzed cases, followed by t (8;21) (q22;q22) in 8.89% (n = 8), and complex karyotypes in 5.56% (n = 5). Those with complex karyotypes included 4 cases (4.44%) of monosomal karyotypes. The constituent ratio of different karyotypes was listed in Figure 1.

All patients with t (15;17) (q22;q21) achieved CR (100%, 11/11) after one course of induction chemotherapy. Those with t (8;21) (q22;q22) and inv (16) (p13q22) had a 75.0% CR rate (6/8), which was higher than that of patients with a normal karyotype (43.2%, 16/37). Two of the 3 cases with complex karyotypes failed to achieve CR.

Genetic mutations and their clinical significance

The frequencies of NPM1, FLT3-ITD, c-kit, and

Table 1. Characteristics of the *de novo* AML Patients

	number or percent
Age (years), median (range)	59 (50-82)
Gender	
Male	62 (54.9%)
Female	51 (45.1%)
FAB subtypes	
M0-M1	5 (4.42%)
M2	48 (42.5%)
APL	14 (12.4%)
M4	24 (21.2%)
M5	19 (16.8%)
M6	3 (2.65%)
Cytogenetics	
Low risk	13 (14.4%)
Intermediate risk	72 (80.0%)
High risk	5 (5.56%)
Molecular markers	
NPM1 mutations	31 (27.4%)
FLT3-ITD	16 (14.5%)
CEBPA mutations	7 (23.3%)
c-kit mutation	6 (5.88%)

Table 2. Peripheral Blood Leukocytes and Marrow Blasts

	leukocytes ($\times 10^9/L$)	marrow blasts (%)	t	P
NPM1 mutations	64.3 \pm 70.3	72.8 \pm 19.2	2.908	0.004
NPM1 negative	30.0 \pm 49.8	62.2 \pm 23.1	2.27	0.025
FLT3-ITD	66.8 \pm 56.3	77.3 \pm 13.8	2.089	0.039
FLT3-ITD negative	34.6 \pm 57.0	63.4 \pm 22.7	3.337	0.002

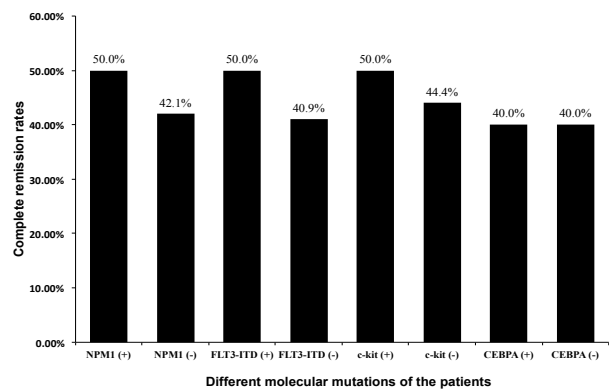
CEBPA mutations were 27.4% (31/113), 14.5% (16/110), 5.88% (6/102), and 23.3% (7/30), respectively (Table 1). Among patients with a normal karyotype, 36.2% (21/58), 17.5% (10/57), 5.56% (3/54), and 31.8% (7/22) carried NPM1, FLT3-ITD, c-kit, and CEBPA mutations, respectively. Both, NPM1 and FLT3-ITD mutations, were detected in 11 patients (10%). Two (33.3%) of the 6 c-kit mutated-patients also had t (8;21) (q22;q22).

NPM1 and FLT3-ITD mutations were associated with higher peripheral blood leukocytes and bone marrow blasts (Table 2). However, no significant difference in the CR rates after 1 course of induction therapy was observed between patients with and without different molecular mutations (all $P > 0.05$) (Figure 2).

Risk stratification based on cytogenetics and mutations

According to cytogenetic results, 13 patients (14.4%) were stratified as low risk, and the remaining 77 (85.6%) as intermediate (80.0%, $n = 72$) and high (5.56%, $n = 5$) risk (Table 1). Since genetic mutations could refine the risk stratification based on cytogenetics, we performed such risk analysis using both, cytogenetics and molecular mutations. Twenty-nine patients (32.2%) were classified as low risk, whereas 54 (60.0%) were of intermediate risk, and the remaining 7 (7.78%) were of high risk.

The CR rates of patients in the favorable, intermediate, and high risk groups were 37.5%, 48.6%, and 33.3%, respectively based on risk stratification. However, no statistically significant difference was observed ($\chi^2 = 0.704$, $P = 0.703$).

**Figure 2. CR Rates of Patients with and Without Different Molecular Mutations**

Discussion

The prognosis of elderly patients with AML is poor, with a median survival of 4–7 months despite intensive chemotherapy (Quintás-Cardama et al., 2012). A high frequency of adverse karyotypes was considered one of the factors associated with unfavorable outcomes. Considering the differences in genetic and ethnic backgrounds, the present study was conducted to investigate the prevalence of different karyotypes and genetic mutations in elderly Chinese patients with AML.

The frequency of aberrant karyotypes in our study was 35.6%, which was lower than the reported values for patients from the United Kingdom (UK) (52.0%, age range 44–91 years) (Grimwade et al., 2001), Hong Kong (58.0%, age range 60–89 years) (So et al., 2011), Austria (58.1%, age range 55–86 years) (Nakase et al., 2000), Japan (49.0%, age range 55–91 years) (Nakase et al., 2000), and south China (50.7%, age range 50–89 years) (Cheng et al., 2010).

The incidence rate of t (15;17) (q22;q21) was 10.0% in the present study, which was higher than the reported rates in the UK (4.04%) (Grimwade et al., 2001) and Hong Kong (2.96%) (So et al., 2011), but similar to those reported in Austria (11.6%) (Nakase et al., 2000), Japan (8.00%) (Nakase et al., 2000), and south China (9.18%) (Cheng et al., 2010). Our results indicated an occurrence rate of 8.89% for t (8;21) (q22;q22), which was higher than the reported rates in the UK (2.16%) (Grimwade et al., 2001), Austria (1.16%) (Nakase et al., 2000), and Hong Kong (3.70%) (So et al., 2011), but comparable to those reported in Japan (9.50%) (Nakase et al., 2000), and south China (5.96%) (Cheng et al., 2010). Furthermore, the frequencies of -5/5q- and -7/7q- were 3.33% and 1.11%, respectively in our study, which were lower than the reported rates in the UK (12.6% and 12.6%) (Grimwade et al., 2001), but similar to those in south China (1.15% and 1.38%) (Cheng et al., 2010). Complex karyotypes were detected in 5.56% of all patients enrolled in the present study, whereas a study on patients from south China (Cheng et al., 2010) reported an incidence rate of 4.82%, and one from the UK suggested a higher rate of 13.7% (Grimwade et al., 2001).

In the present study, the incidence rates of NPM1 and FLT3-ITD mutations in patients with a normal karyotype were 36.2% and 17.5%, respectively, which

were significantly lower than those reported in the United States (US) (56.1% and 31.1%) (Becker et al., 2010) and Germany (42.4% and 23.3%) (Schneider et al., 2012). However, CEBPA mutations were detected in 31.8% of those enrolled in this study, which was significantly higher than that reported in the US (11.5%) (Becker et al., 2010) and Germany (10.0%) (Schneider et al., 2012).

The above-mentioned observations may indicate that compared to those in western countries: 1) the proportion of Chinese patients with favorable cytogenetics was higher, whereas that with unfavorable cytogenetics was lower; 2) the frequencies of NPM1 and FLT3-ITD mutations were lower, and that of CEBPA mutations was higher in Chinese patients with AML. Such phenomena were also observed in a younger cohort (Su et al., 2013). The differences may arise from study cohort heterogeneity or diverse ethnic and environmental backgrounds.

Based on cytogenetics, 14.4% of our patients were of low risk, whereas the remainder were intermediate and high risk, consistent with a previous report from China (low: 16.0%; intermediate and high: 84.0%) (Liu et al., 2011). Furthermore, both Liu's and our studies demonstrated that, compared to younger patients (< 60 years), the proportion of elderly patients in the low risk group decreased (14.4–16.0% versus 30.2%). Such observation was consistent with previous reports (Estey, 2007; Quintás-Cardama et al., 2012) and indicated poor prognoses for elderly AML patients.

However, our data suggested that the CR rates were not significantly different among the 3 risk groups. Additionally, different molecular mutations had no influence on the CR rates after induction therapy. Since achieving CR is the prerequisite for long-term survival in AML patients, our results indicated that the poor prognoses of elderly AML patients may be attributed to many other factors, such as comorbid conditions, a higher incidence of secondary AML or evolution from MDS, and poor performance status. Unfortunately, the long term outcome was not assessed in the present study due to relatively short follow-up periods. Nevertheless, our findings suggested that cytogenetics and genetic mutations alone were not sufficient to evaluate the prognoses of elderly patients with AML.

In conclusion, although cytogenetic and genetic mutation features have been reported to be powerful prognostic markers for young patients with AML, they may not be sufficient to evaluate the prognoses in elderly patients. The search for a novel model that would enable a more comprehensive evaluation of elderly AML patients is therefore imperative.

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