

Study the ASXL2 mutations of acute myeloid leukemia patients with RUNX1-RUNX1T1

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Abstract: To assess the incidence of ASXL2 mutations in acute myeloid leukemia (AML) patients with RUNX1-RUNX1T1 fusion gene and the association with clinical features and prognosis, amplicon-targeted next generation sequencing of ASXL2, ASXL1, and KIT genes were carried out in 83 AML patients with RUNX1-RUNX1T1. ASXL2 and ASXL1 mutations of cases were 13 (15.66%) and 11 (13.25%), respectively. One patient has two genes mutation. The median white blood cell count of the 13 patients with ASXL2 mutations was $20.1 (3.4-139.03) \times 10^9/L$, which was higher than that of patients without ASXL2 mutations (20.1 vs. 13.14, $P = 0.025$). There was no significant difference in peripheral blood hemoglobin level, platelet count and bone marrow blast count between the two groups ($P > 0.05$). Overall survival and event-free survival between the two groups were also no significant difference ($P > 0.05$). These data suggest that ASXL2 mutations are associated with high level of white blood cell counts in AML patients with RUNX1-RUNX1T1.

Keywords: Acute myeloid leukemia; ASXL2; RUNX1-RUNX1T1; Gene mutation

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1. Introduction

Core-binding factor acute myeloid leukemias (CBF AML), which account for approximately 30% of pediatric and 15% of adult AML, contain chromosomal translocations or inversions that target the transcription factors RUNX1 and CBFβ[1]. CBF AML is generally a good prognosis by the relapse rate of patients receiving chemotherapy. It is still as high as 40%-50%, indicating a high clinical heterogeneous[2,3].

In recent years, more and more gene mutations have been found in CBF AML, such as KIT, FLT3, N/KRAS and ASXL1/2[3]. Different gene mutation profiles were shown in the two CBF AML subtypes. Mutations in genes of ASXL2, EZH2, KDM6A, RAD21, and SMC1A were observed in RUNX1-RUNX1T1 AML, and not in patients with CBFβ-MYH11 [3,4]. ASXL1/2 is frequently mutated in myeloid malignancies, and ASXL2 mutations are restricted to RUNX1-RUNX1T1 AML with a frequency of approximately 20% [5-7].

ASXL1 has attracted much attention due to its high mutation frequency in leukemia, while mutations in ASXL2 are not well understood. ASXL2 mutations were first discovered in pediatric AML and the mutation sites were mostly located in exon 11 and 12 of the gene [1,7,8]. Recent studies also reported that ASXL2 mutations or deletions can lead to the progression of leukemia in RUNX1-RUNX1T1 mice [9,10]. In this study, we assessed the incidence of

ASXL2 mutations in AML patients with RUNX1-RUNX1T1 fusion gene and the association with clinical features and prognosis.

2. Methods

2.1. Patients

83 AML patients with RUNX1-RUNX1T1 in Hebei Yanda Lu Daopei Hospital and Affiliated Hospital of Qingdao University from January 2014 to December 2017 were enrolled in this study. Among them, 51 were males and 30 were females, with a median age of 15 years (3-61). Patient diagnosis was confirmed by karyotyping analysis of t(8;21) rearrangements and/or detection of RUNX1-RUNX1T1 transcripts. The diagnosis of AML with RUNX1-RUNX1T1 was based on the WHO 2016 classification criteria[11]. This study was approved by the ethics committees at the above-mentioned hospitals, and all patients (or legal guardians) were enrolled in this study and signed informed consent. The end of the follow-up period was May 1, 2018. Median follow-up for survivors was 21 months (5-42).

2.2. Genomic DNA extraction

Genomic DNA was extracted from 3~5 ml bone marrow (38 cases) or bone marrow smears of patients retained at their first-diagnosis (45 cases). Fresh bone marrow samples were subjected to cell counting after lysis of red blood cells, and 1.0×10^7 nucleated cells

were used to extract genomic DNA. The bone marrow smear cells were repeatedly purged with a pipette and pure water, and the obtained cell suspension was used to extract genomic DNA. Genomic DNA was extracted using a silica column DNA extraction kit (Tiangen Biotech (Beijing) Co., Ltd., Cat. No. DP318-03).

2.3. DNA sequencing and mutation analysis

High-throughput targeted amplicon sequencing (Ion Torrent PGM platform) was used to detect ASXL1 exon13, ASXL2 exon11-12, and KIT exon8, Exon11, Exon17 as well as their splice sites[12]. The average sequencing depth was greater than 1000x. Single nucleotide variations (SNVs) and short fragment insertions or deletions were identified by TVC 5.0-13 software. A minimum coverage depth of 100x, and a variant allele frequency (VAF) >5% were used as thresholds for SNVs calling. The dbSNP, 1000 Genomes, ExAC, ClinVar, and COSMIC databases and bioinformatics software PolyPhen-2 and SIFT were used for mutation analysis.

2.4. Statistical analysis

Statistical analysis was performed using the SPSS 23.0 software. Quantitative data were analyzed by t test, while data that did not accord with normal distribution and uneven variance were analyzed using rank sum test. The categorical data was calculated using Chi-square or Fisher's exact tests.

OS and EFS[13] were evaluated by the Kaplan–Meier method, and the differences in survival were assessed using the Log-rank test. Two-sided P value < 0.05 was considered to be statistically significant.

3. Results

3.1. ASXL2 gene mutation analysis

ASXL2 mutations were detected in a total of 13 patients (15.67%), one of them carried two distinct mutations (Q592GfsX17; Q602X). There were 2 missense mutations (R591G/P), 4 nonsense mutations (S496X, Q602X, R614X, E742X) and 8 frame shift mutations in the 13 patients (Figure 1).

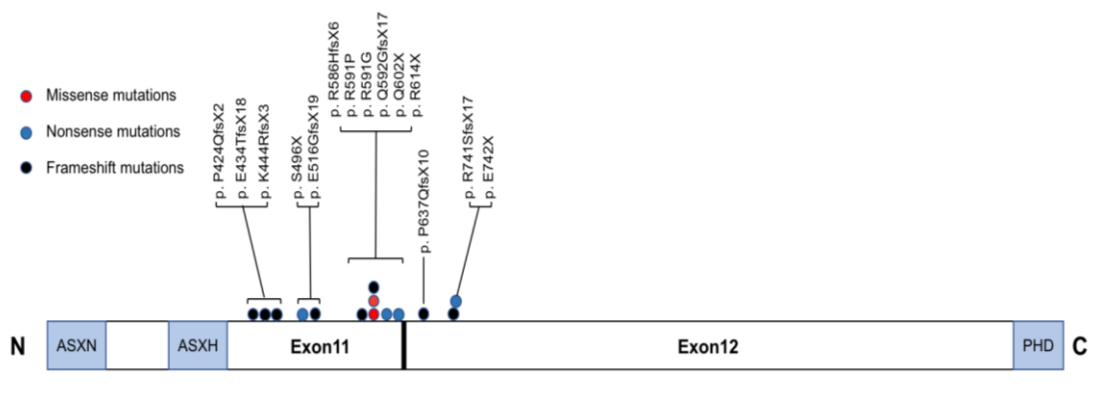


Figure 1. ASXL2 mutations detected in 83 AML patients with RUNX1-RUNX1T1

3.2. Correlation between clinical features and ASXL2 gene mutations in AML patients with RUNX1-RUNX1T1

Thirteen RUNX1-RUNX1T1 AML carried ASXL2 mutations, including 7 males and 6 females. The other 70 patients has negative relationship with ASXL2 mutations, including 44 males and 26 females. The median age of patients in the two groups was 15 years (4-42) and 15 years (3-61), respectively. The peripheral white blood cell counts of ASXL2-mutated patients were significantly higher than those of the ASXL2 wild-type group (20.1 vs. 13.14, P = 0.025). There was no statistical difference in age, hemoglobin, platelet and bone marrow blast cell ratio (P > 0.05). Only 1 patient carried mutations in both ASXL1 and ASXL2 genes. There was also no statistical difference in the incidence of concomitant ASXL1 or KIT mutations between the two groups (Table 1).

3.3. Prognosis of ASXL2-mutated AML patients with RUNX1-RUNX1T1

75 of the 83 patients were followed up for 5-42 months (median follow-up 21 months). All of them were received standard induction chemotherapy based on DA (Daunorubicin plus cytarabine) or IA (Idarubicin plus cytarabine). Among them, 58 patients underwent allogeneic hematopoietic stem cell transplantation (allo-HSCT) because of high risk factors[14], recurrence of disease, or demands of patients or their families (Figure 2).

Two (15.38%, 2/13) of the 13 ASXL2-mutated patients and 9 (14.52%, 9/62) of the 62 ASXL2 wild-type patients relapsed, respectively. Most relapsed patients underwent allo-SCT. There were no significant differences in OS and EFS between ASXL2-mutated and ASXL2 wild-type patients (OS, P = 0.164; EFS, P = 0.386) (Fig 3). Two ASXL2-mutated patients died of graft-versus-host disease after HSCT.

Table 1. Clinical features of patients with AML with RUNX1-RUNX1T1

Characteristics	Total N = 83	ASXL2 mut N = 13	ASXL2 wt N = 70	P-Value
Gender				
Male	51	7	44	
Female	32	6	26	
Age (years)				
Median, (range)	15 (3-61)	15 (4-42)	15 (3-61)	0.506
Median, WBC ($\times 10^9/L$)	14.57 (0.82-139.03)	20.1 (3.4-139.03)	13.14 (0.82-54.9)	0.025
Median, HB(g/L)	71 (32-194)	60 (41-107)	72 (32-194)	0.503
Median, PLT ($\times 10^9/L$)	28 (3-382)	21 (8-121)	28.5 (3-382)	0.506
Median, BM blast, %	45 (10-86)	38.8 (11.5-69)	47.14 (10-86)	0.254
KIT mut	48 (57.83%)	9 (69.23%)	39 (55.71%)	0.542
ASXL1 mut	11 (13.25%)	1 (7.69%)	10 (14.29%)	1

Abbreviation: Mut, mutated; wt, wild-type; N, number of subjects; WBC, white blood cell; HB, hemoglobin; PLT, platelet; BM, bone marrow.

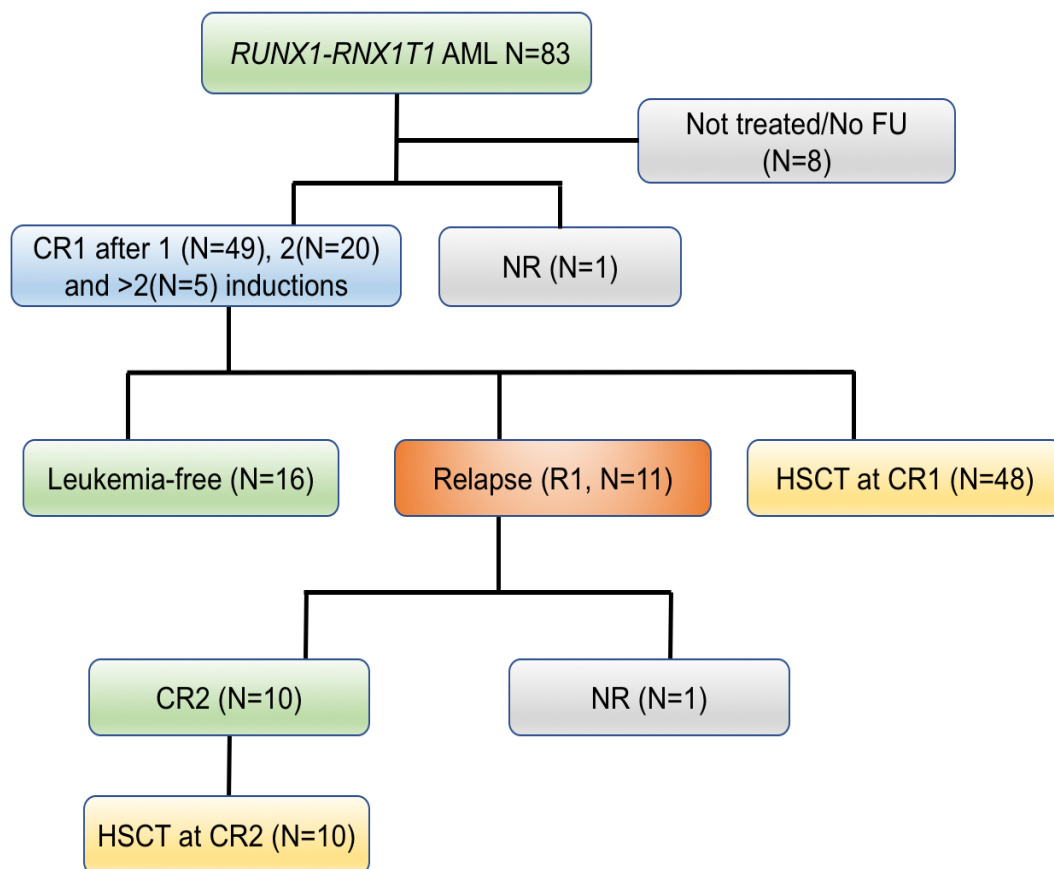


Figure 2. Treatment of the 83 patients with AML with RUNX1-RUNX1T1. Abbreviation: CR1, first complete remission; CR2, second complete remission; FU, follow up; HSCT, hematopoietic stem cell transplantation; NR, non-remission; R1, first relapse.

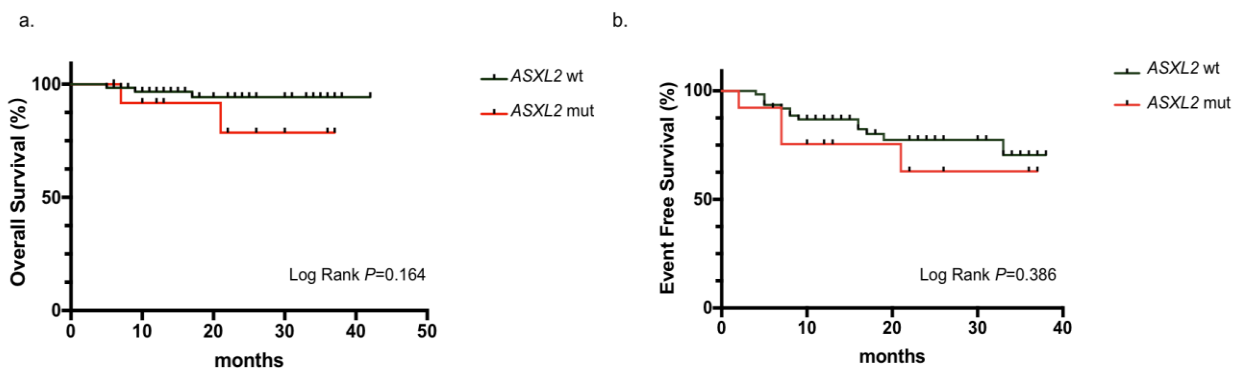


Figure 3. Effect of ASXL2 mutation on AML patients with RUNX1-RUNX1T1 Overall survival (OS) and Event-free survival (EFS). (a) OS of ASXL2-mutated and wild-type patients. (b) EFS of ASXL2-mutated and wild-type patients.

4. Discussion

In this study, the mutation rate of ASXL2 in patients with RUNX1-RUNX1T1 AML was 15.57%, which was consistent with the cohort of the Asian population[4]. It was slightly lower than the cohort study in Caucasians population[6,7]. Whether there is an interspecies difference in ASXL2 gene mutation rate needs further study.

In terms of clinical features, there was no statistically significant difference in peripheral hemoglobin, platelet count, and myeloid blast cell ratio between the ASXL2-mutated and the wild-type groups in our study. The peripheral white blood cell counts in the ASXL2-mutated group was significantly higher than those in the ASXL2 wild-type group and was consistent with the study of Micoll[7]. However, in most studies, no difference was found in ASXL2-mutated and wild-type patients[4,6]. Some studies reported that mutations in the ASXL2 and the ASXL1 genes are mutually exclusive[4, 5, 7]. In this study, only one of the 13 ASXL2-mutated patients was accompanied by the ASXL1 mutation, although the difference was not statistically significant. The mechanism by which mutations of the two genes are mutually exclusive is still unclear.

A follow-up of 75 patients with AML with RUNX1-RUNX1T1 found no statistically significant difference between the ASXL2-mutated group and the ASXL2 wild-type group in OS and EFS, which was consistent with other studies [4,6,7]. Jahn et al. studied a matched sample of 21 relapsed patients[6]. 2 of 4 ASXL2-mutated patients lost their ASXL2 mutations during relapse while 17 patients without ASXL2 mutations at diagnosis carried ASXL2 mutations during relapse, suggesting that the ASXL2 mutations are equivalent to cooperative events or passenger mutations, rather than driver mutations. This may be the reason why the prognostic significance of the ASXL2 gene is not very clear. In this study, we found there were no significant differences in OS and EFS

between ASXL2-mutated and wild-type groups.

There are a wide variety of mutated genes in AML, and there are also a large number of different combinations of gene mutations, which will affect the clinical characteristics and prognosis of patients. However, there may be multiple gene mutations in different patients, and the clinical significance of each gene mutation combination needs more research. With the accumulation of relevant medical data and the development and application of various targeted drugs, genetic mutation detection will provide more and more evidence for AML precision medicine.

5. Conclusions

The frequency of ASXL2 gene mutations in AML patients with RUNX1-RUNX1T1 was 15.66%. Peripheral white blood cell count in patients with ASXL2 mutations was significantly higher than that in patients without ASXL2 mutations. Overall survival and event-free survival were similar between RUNX1-RUNX1T1 positive AML patients with and without ASXL2 mutations.

Conflicts of interest

The authors have no conflicts of interest to disclose.

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