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Clinical features and prognosis of normal karyotype acute myeloid leukemia pediatric patients with WT1 mutations: an analysis based on TCGA database

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ABSTRACT

**Objectives:** To explore the clinical features and prognosis of normal karyotype acute myeloid leukemia (NK-AML) pediatric patients with WT1 mutations.

**Methods:** The clinical data and prognostic information of 220 NK-AML pediatric patients were selected from target-AML project of The Cancer Genome Atlas (TCGA) database. Survival analyses were performed for NK-AML pediatric patients with different combinations of mutations.

**Results:** We found that 28(12.7%) NK-AML patients harbored WT1 mutations. The positive rate of FLT3-ITD in the WT1-mutated group was higher than that in the WT1 wild-type group (P = 0.002). In contrast, WT1 mutation and NPM1 mutation were mutually exclusive (P = 0.013). Furthermore, the WT1-mutated group suffered lower rates of complete remission (CR) (P < 0.001 and P < 0.001, respectively) but higher rates of minimal residual disease (MRD) (P = 0.003 and P = 0.021, respectively) after both one and two courses of induction chemotherapy. Patients with WT1 mutations had significantly worse overall survival (OS) and event-free survival (EFS) in both univariate (P < 0.001 and P = 0.007, respectively) and multivariate survival analyses (P < 0.001 and P < 0.001, respectively). The stratification analysis showed that for FLT3-ITD positive patients, WT1 mutations predicted shorter OS (P = 0.003) and EFS (P < 0.001).

**Conclusion:** WT1 mutations conferred an independent poor prognosis for NK-AML pediatric patients.

1. Introduction

Acute myeloid leukemia (AML) is a group of heterogeneous diseases resulting from acquired somatic genetic lesions accumulated in hematopoietic progenitors [1]. The cytogenetic and molecular markers in the international risk stratification systems such as European Leukemia Network (ELN) [2] and National Comprehensive Cancer Network (NCCN) [3] can give guidance to the risk stratification of de novo normal karyotype AML (NK-AML) patients. Patients with NPM1 mutation and FLT3-ITD with a low allelic ratio (FLT3-ITD<sup>low</sup>) have a similar favorable outcome as patients with an NPM1 mutation but no FLT3-ITD [4–6]. In contrast, AML with wild-type NPM1 and FLT3-ITD with a high allelic ratio (FLT3-ITD<sup>high</sup>) has a poor prognosis and is considered as the adverse-risk patients [4]. RUNX1 and ASXL1 mutations [7,8] have also been added to the adverse-risk group when not accompanied by low-risk genetic alterations. But the prognostic significance of other gene mutations such as WT1 mutation is still unknown.

The WT1 gene, located on chromosome 11p13, encodes a zinc-finger protein [4]. It plays an important role in the development of genitourinary and hematopoietic systems and can act as a tumor suppress gene and an oncogene [9,10]. The truncated WT1 proteins, produced from frame-shift mutations, can induce the proliferation and block differentiation of stem cell, thereby contributing to leukemogenesis. WT1 mutations have been found in 10–20% of NK-AML patients [11–15], but its prognostic value remains controversial. Thus, it will be of major interest to explore the clinical features and prognosis of WT1-mutated NK-AML patients.

To further clarify the role of WT1 mutations in NK-AML pediatric patients, we compared the baseline data, treatment response and survival time between the WT1-mutated and WT1 wild-type NK-AML pediatric patients to evaluate its prognostic significance.

2. Material and methods

2.1. Patients

220 AML pediatric patients with normal karyotype selected from target-AML project (CCG-2961 [16], AAML0531 [17], AAML03P1 [18]) of The Cancer Genome Atlas (TCGA) database, were eligible for this study. Normal karyotype was defined by the
international system for human cytogenetic nomenclature (ISCN). Mutation analyses of FLT3-ITD, NPM1 and CEBPα were performed as previously described [19–21]. Information on WT1 mutations was available in all cases. Detail treatments and risk stratification of these studies have been previously described [22].

2.2. Statistical analysis

The statistical analyses were performed with the statistical software package SPSS (version 23.0; SPSS Inc.). The Mann–Whitney U test was applied for continuous variables. The χ² test or Fisher exact test was used to compare the frequencies of categorical data. CR1 and CR2 represent that complete remission (CR) was observed in patients after one course and two courses of induction chemotherapy, respectively. Overall survival (OS) was defined as the time from diagnosis until death or the last follow-up. Event-free survival (EFS) was defined as time between diagnosis and first event, including relapse, death, failure to achieve remission. The survival curves were estimated using the Kaplan–Meier method and compared using the log-rank test. Cox proportional hazard models were used to estimate hazard ratios (HR) for univariate and multivariate analyses for both OS and EFS.

3. Results

3.1. Patient characteristics

A total of 220 AML pediatric patients with normal karyotype, including 14 cases of CCG-2961, 183 cases of AAML0531 and 23 cases of AAML03P1, were analyzed. There were 121 males (55.0%) and 99 females (45.0%) with a median age of 12.4 (0.3–28.8) years. 179 (81.4%) were non-hispanic or non-latino, 32 (14.5%) were hispanic or latino, and 9 (4.1%) were unknown.

3.2. Comparison of baseline data and gene mutations

The clinical characteristics were compared between the WT1-mutated group and the WT1 wild-type group (Table 1). There are 28(12.7%) cases mutated in WT1 gene. There was no significant difference in age (P = 0.075), white blood cell (WBC) count at diagnosis (P = 0.169), percentage of bone marrow leukemic blast cells (P = 0.411) and percentage of peripheral blood blast cells (P = 0.565). What’s more, the WT1-mutated and WT1 wild-type AML patients were equally distributed over protocols (P = 0.240), gender (P = 0.061), race (P = 0.313), central nervous system (CNS) disease (P = 0.219) and chloroma (P = 0.743). However, the distribution of FAB type was different (P = 0.044) and WT1 mutations were more common in patients with French–American–British (FAB) class M4 (28.6% vs. 15.1%). And such mutations were also less frequent in a low-risk group (7.1% vs. 41.1%, P = 0.002). The positive rate of FLT3-ITD in the WT1-mutated group was higher than that in the WT1 wild-type group (67.9% vs. 36.5%, P = 0.002). In contrast, WT1 mutation and NPM1 mutation were negatively correlated (P = 0.013). There was no significant difference in the rate of FLT3 point mutation (7.1% vs. 8.9%, P = 1.000)
between the two groups. In addition, patients with WT1 mutations had a lower mutation rate of CEBPA, but this difference was not statistically significant (3.6% vs. 19.4%, \( P = 0.073 \)).

### 3.3. Comparison of patients’ response to treatment

Lower rates of CR were observed in patients with WT1 mutation than WT1 wild-type group after both one (CR1) and two (CR2) course of induction chemotherapy (Table 2). 148(79.1%) WT1 wild-type cases achieved CR1 while the rate of CR1 of patients with WT1 mutations was only 39.3% (\( P < 0.001 \)). Similarly, patients with WT1 mutations also had a lower CR2 (40.0% vs. 84.8%, \( P < 0.001 \)). There was no significant difference in the rate of stem cell transplantation (SCT) after CR1 (17.6% vs. 28.0%, \( P = 0.530 \)). Moreover, the WT1-mutated group had higher rates of minimal residual disease (MRD) (\( P = 0.003 \) and \( P = 0.021 \), respectively) after both one and two courses of induction chemotherapy. However, for the 119 and 117 patients obtained CR1 and CR2, the percentage of MRD between two groups had no statistical difference (\( P = 0.479 \) and \( P = 0.188 \), respectively). Details were shown in Table 3.

### 3.4. Survival analysis of WT1 mutated and WT1 wild-type patients

#### 3.4.1. Univariate analysis

The Log-rank method was used to compare the OS and EFS of pediatric patients in the WT1-mutated group and the WT1 wild-type group (Table 4 and Figure 1). The results showed that patients with WT1 mutations had significantly a shorter OS (\( HR = 2.861, 95\% \text{ CI: } 1.233–3.803, P < 0.007 \)) and EFS (\( HR = 2.547, 95\% \text{ CI: } 1.551–4.184, P < 0.001 \)) than patients without mutations. Meanwhile, the FLT3-ITD positive patients had an worse survival than the FLT3-ITD negative patients (shorter OS: \( P = 0.022 \), Figure 1(C) and shorter EFS: \( P = 0.04 \), Figure 1(D)) while patients with NPM1 mutations showed an improved survival than NPM1 wild-type patients (longer OS: \( P < 0.001 \), Figure 1(E) and longer EFS: \( P < 0.001 \), Figure 1(F)).

#### 3.4.2. Stratification analysis

Given the results of univariate analysis, the stratification analysis (Figure 2) was performed to further explore the prognostic significance of WT1 mutations. WT1-mutated and FLT3-ITD positive patients had the worst OS (\( P < 0.001 \), Figure 2(A)) and EFS (\( P < 0.001 \), Figure 2(B)), followed by patients with WT1 mutations but FLT3-ITD negative. WT1-mutated and FLT3-ITD positive patients suffered a worse survival than patients without WT1 mutations but FLT3-ITD positive (OS: \( P = 0.003 \), Figure 2(A); EFS: \( P < 0.001 \), Figure 2(B)).

#### 3.4.3. Multivariate analysis

COX proportional risk regression model was used to eliminate the effect of covariates FLT3-ITD and NPM1 mutations. Multivariate survival analysis suggested that WT1 mutation was an independent risk factor for OS (\( HR = 2.165, 95\% \text{ CI: } 1.233–3.803, P = 0.007 \)) and EFS (\( HR = 2.547, 95\% \text{ CI: } 1.551–4.184, P < 0.001 \)) in NK-AML patients (Table 4).

### 4. Discussion

In our study, we downloaded the high-quality clinical data and prognostic information of 220 NK-AML pediatric patients from TCGA database and compared the clinical features and prognosis between patients with WT1 mutations and patients without WT1 mutations.

The mutation rate of WT1 gene was 12.7% in our group, which was lower than that of Hollink et al. [15] (22%) but higher than that of Zidan et al. [23] (10.6%) and Virappane et al. [13] (10%). This difference may result from differences in race, sample size, and detection methods.

We found that WT1 gene mostly mutated in subtype M4 and standard risk group, which has not been previously reported. In addition, there was a substantial overlap between WT1 mutation and the class I mutation FLT3-ITD (\( P = 0.002 \)). Ho et al. [24] also supported this result. However, they failed to explain the role of WT1 mutations in leukemogenesis because a positive correlation between WT1 mutation and CBF translocation, a classic class II event, was also observed.

### Table 3. Comparison of MRD percentage after treatment between WT1-mutated group and WT1 wild-type group.

| Variables | Total number | WT1-mutated group | WT1 wild-type group | \( P \)  
<table>
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<tbody>
<tr>
<td>MRD% for CR1 patients</td>
<td>119</td>
<td>0(0–8.6)</td>
<td>0(0–44.0)</td>
<td>0.479</td>
</tr>
<tr>
<td>MRD% for CR2 patients</td>
<td>116</td>
<td>0(0–6.1)</td>
<td>0(0–10.0)</td>
<td>0.188</td>
</tr>
</tbody>
</table>

Note: MRD: minimal residual disease.
Generally speaking, our study was focused on NK-AML patients which was of less heterogeneity. WT1 mutation was inversely associated with NPM1 mutation in our study (\(P=0.013\)), which was consistent with the result of Zidan et al. [23]. It has been reported that NPM1 mutation was a common synergistic mutation with FLT3-ITD in the process of leukemogenesis [25]. Based on the above results, we can speculate that WT1 mutation plays the role of class II mutation in the pathogenesis of leukemia.

We suggested that patients with WT1 mutations had an inferior response to induction chemotherapy compared with patients without mutations. Strikingly, WT1 mutation was an independent adverse prognostic factor for NK-AML pediatric patients in both univariate and multivariate analyses. Our results also confirmed that FLT3-ITD was a poor molecular marker and NPM1 mutation was a good molecular marker for NK-AML, which were in line with the previous reports [26–30]. So far, there were very few studies on the prognosis of WT1 mutations in NK-AML. The United Kingdom Medical Research Council Adult Leukemia Working Party [13] screened exons 7 and 9 of the WT1 gene of 470 young adult NK-AML using a

<table>
<thead>
<tr>
<th>Mutated genes</th>
<th>Overall survival</th>
<th>Event-free survival</th>
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<tbody>
<tr>
<td></td>
<td>HR (95%CI)</td>
<td>HR (95%CI)</td>
</tr>
<tr>
<td>WT1</td>
<td>2.861(1.669–4.903)</td>
<td>3.430(2.155–5.459)</td>
</tr>
<tr>
<td>FLT3-ITD</td>
<td>1.670(1.078–2.587)</td>
<td>1.718(1.189–2.482)</td>
</tr>
<tr>
<td>NPM1</td>
<td>0.290(0.145–0.579)</td>
<td>0.248(0.139–0.442)</td>
</tr>
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Note: OS: overall survival; EFS: event-free survival; HR: hazard ratio.

Figure 1. Survival analysis of WT1, FLT3-ITD and NPM1 on NK-AML pediatric patients. A, C and E were the survival curves of OS in the WT1-mutated group, the FLT3-ITD positive group, the NPM1 mutated group and the corresponding wild-type group or negative group, respectively. B, D and F are the survival curves of corresponding EFS. OS: overall survival; EFS: event-free survival.
combination of direct sequencing and high-resolution capillary electrophoresis, its result was basically consistent with our study. Similarly, Egyptian research [23] showed that WT1 mutations were a negative prognostic indicator for disease-free survival (DFS) and OS in NK-AML patients. However, the report from the Japanese Childhood AML Cooperative Study Group [11] came to a different conclusion, which suggested that no significant differences were observed in the 3-year OS and DFS between patients with WT1 mutations and patients without. A comprehensive meta-analysis may be able to resolve this contradiction.

The prognosis of NK-AML pediatric patients with FLT3-ITD or NPM1 mutations still varies considerably. To provide further insight into the risk stratification, we further performed the stratification analysis according to their mutation status. Our result suggested that WT1 mutations predicted an even worse survival for FLT3-ITD positive NK-AML pediatric patients. In total, WT1 gene may function as an addition molecular marker for risk stratification.

In conclusions, WT1 mutation was an independent poor prognostic marker, which predicted a lower CR rate and worse OS and EFS for NK-AML pediatric patients. It may help to improve the risk stratification and prognostic evaluation of NK-AML pediatric patients in the future.

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Disclosure statement
No potential conflict of interest was reported by the author(s).

References


