Biological and clinical influences of NPM1 in acute myeloid leukemia patients with DNMT3A mutations

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Purpose: DNMT3A and NPM1 mutations are known to impact the prognosis of acute myeloid leukemia (AML). DNMT3A mutations are negative prognostic factors, while NPM1 mutations are low-risk factors and inclined to concurrently appear with DNMT3A mutations. In this study, we aimed to find out how NPM1 mutations affect patients’ outcomes in the background of DNMT3A mutations.

Patients and methods: We screened The Cancer Genome Atlas (TCGA) database and found 51 AML patients with DNMT3A mutations. Of them, 28 patients had a combination of NPM1 mutations.

Results: In all, NPM1 had the highest mutation frequency (n=28, 54.9%). DNMT3Amut/NPM1mut patients had higher bone marrow (BM) blasts (P=0.015), higher FLT3-ITD/TKD rate (P=0.004), and lower IDH2 mutation rate (P=0.014) than the DNMT3Amt/NPM1wt patients, while their prognoses were the same as the DNMT3Amt/NPM1mut patients (P=0.1). All 51 patients benefited from hematopoietic stem cell transplantation (HSCT) treatment (P=0.005 and 0.001 for event-free survival [EFS] and overall survival [OS], respectively). In the 23 patients with DNMT3Amt/NPM1wt, those who received HSCT had prolonged EFS and OS (P=0.043 and 0.008, respectively), while HSCT treatment did not produce a positive impact on EFS and OS in the remaining 28 patients with DNMT3Amt/NPM1wt (P=0.056 and 0.053, respectively).

Conclusion: Our study found that NPM1 mutations influenced BM blasts’ percentage, FLT3-ITD/TKD rate, and IDH2 mutation rate in AML patients with DNMT3A mutations but made little difference to the overall prognosis. While HSCT treatments benefited all DNMT3Amt patients, it was better for DNMT3Amt/NPM1wt patients to extend their EFS and OS.

Keywords: AML, DNA methyltransferases 3A, nucleophosmin 1, next-generation sequencing, prognosis

Introduction

Acute myeloid leukemia (AML) is a kind of heterogeneous disease characterized by the clonal expansion of hematopoietic stem cells (HSCs) or hematopoietic progenitor cells (HPCs) in the bone marrow (BM), blood, and other tissues without differentiation.1

As sequencing methods have technologically advanced in the past four decades, next-generation sequencing (NGS) is now available for a detailed understanding of the molecular pathogenesis of AML.

DNMTs are genes that encode DNMTs for the methylation of CpG islands. As methylations reduce the expression of downstream genes, spontaneous defects in
DNMTs lead to the instability of genome structure and thus increase the possibility of cancer. Since Ley et al. confirmed that DNMT3A mutations were highly recurrent in patients with de novo AML and associated with a poor outcome, many prognostic studies about DNMT3A-mutated AML have been carried out. Occurring in ~20% of AML patients and having a high proportion of R882 mutation, DNMT3A mutations are often accompanied by FLT3-ITD, NPM1, IDH1, and IDH2 mutations. NPM1 mutations were more common in patients with DNMT3A mutations than in DNMT3A wild-type patients. High-risk NPM1/FLT3 mutation (NPM1mut/FLT3-ITDneg, NPM1mut/FLT3-ITDpos, or NPM1mut/FLT3-ITDneg) was thought to be related with poor prognosis. Hematopoietic stem cell transplantation (HSCT) has been shown to benefit the survival of all AML patients with DNMT3A mutations. Gale et al. argued that the presence of DNMT3A mutations should be considered as a poor-risk prognostic factor, irrespective of the NPM1 genotype as it may disturb the outcome. There have been few studies concerning DNMT3A mutations alone in AML patients together with studying the biological and clinical features with companion genes. Therefore, understanding the biological and clinical characteristics of these patients with DNMT3A mutations is important for estimating their event-free survival (EFS) and overall survival (OS) and likely advantageous in deciding the most suited clinical treatment.

**Patients and methods**

**Patients**

We selected and enrolled 51 patients with diagnosed DNMT3A-mutated AML from The Cancer Genome Atlas (TCGA) database (https://cancergenome.nih.gov/). We collected data including age, gender, AML French-American-British classification subtypes, karyotype, cytogenetics’ risk, white blood cell (WBC) count, BM blasts percentage, peripheral blood (PB) blasts percentage, relapse, mutated recurrent genes, and combined mutation genes, which were highly related to AML such as FLT3, IDH1/2, TET2, NRAS, CEBPA, PTPN11, KARS, U2AF1, SMC1A, SMC3, and RAD21, with their clinical figures to include EFS and OS. Gene mutations were detected by NGS. Written informed consent was obtained from all patients. This study was approved by the Human Research Ethics Committee of Washington University.

**Statistical analyses**

The end points were EFS and OS. EFS is the time from the date of diagnosis to removal from the study due to the absence of complete remission, relapse, or death. OS is the time from the date of diagnosis to death due to any cause.

We compared different biological and clinical characteristics by using different statistical methods. The Student’s t-test was applied to two group comparisons, and Chi-square test was used to compare the rate between them. Survival analysis was performed by Kaplan–Meier method. A two-sided P-value of <0.05 was considered statistically significant. All statistical analyses were performed by the SPSS Version 20.0 software.

**Results**

**Biological and clinical characteristics**

In all of the 51 patients, NPM1 was the most frequently combined mutation gene (n=28, 54.9%), followed by FLT3 (n=21, 41.2%), IDH1 (n=11, 21.6%), and TET2 (n=6, 11.8%). The mutational spectrum of all genes with >5% mutation frequency is shown in Figure 1. The biological and clinical characteristics are summarized in Table 1.

Patients were divided into two groups depending on their NPM1 mutation status. There were 23 patients with DNMT3Amut/NPM1wild and 28 patients with DNMT3Amut/NPM1mut. The median age of the cohort was 58 years (24 men and 27 women; age range: 21–81 years), and 47.1% of the patients were ≥60 years. WBC counts ranged from 1.2 to 298.4×10⁹/L with a median of 45.0×10⁹/L, and 19 cases were not <50×10⁹/L. The median BM blast percentage and PB blast percentage were 76 and 36%, respectively, and ranged from 32 to 100% and 0 to 97%; there were 30 (58.8%) cases with BM blasts ≥70% and six (30.0%) cases with PB blasts ≥70%. In 28 of the 51 patients (58.8%) who had relapses, days from collection to first relapse ranged from 53 to 1230 days with a median of 324.7 days. There were two (8.7%) cases with FLT3-ITD mutation and two (8.7%) cases with FLT3-TKD mutation. HSCT treatments were received by 23 (45.1%) cases.

Upon comparing the two groups with DNMT3A mutations, we found that patients with DNMT3Amut/NPM1mut had higher BM blasts (P=0.015), higher FLT3-ITD/TKD (P=0.004), and lower IDH2 mutation (P=0.014). Other biological or clinical characteristics showed no significant differences between the two groups.

**Comparison of EFS and OS between different biological and clinical characteristics**

To determine what kind of biological or clinical characteristics were easily influenced by NPM1 mutation, we chose...
the following parameters for EFS and OS analyses: age (<60 vs ≥60 years), cytogenetic risk (intermediate vs poor), WBC count (<50×10⁹ vs ≥50×10⁹/L), BM blasts percentage (<70 vs ≥70%), PB blasts percentage (<70 vs ≥70%), mutated recurrent genes (<5 vs ≥5), genes with no <10 (19.6%) cases harboring FLT3-ITD/TKD and IDH1 changes (mutation vs wild type), and HSCT (yes vs no). The results are shown in Table 2.

The NPM1 mutation had no influence on any of the abovementioned parameters in DNMT3A-mutated AML patients. EFS and OS between DNMT3A\textsuperscript{mut}/NPM1\textsuperscript{mut} and DNMT3A\textsuperscript{mut}/NPM1\textsuperscript{wild} groups showed no difference (P=0.504 and 0.586, respectively, Figure 2A and B). Furthermore, there was also no difference in EFS and OS between groups of patients with only chemical therapy (P=0.938 for EFS and P=0.942 for OS, Figure 2C and D) or HSCT treatment (P=0.940 for EFS and P=0.790 for OS, Figure 2E and F).

HSCT was an effective way for the treatment of DNMT3A-mutated AML patients (P=0.005 for EFS and P=0.001 for OS) in all of the 51 patients (Figure 3A and B). We also compared the influence of different treatments in each group. The group of people with DNMT3A\textsuperscript{mut}/NPM1\textsuperscript{wild} derived the best of HSCT treatment and had longer EFS and OS than patients who received only chemical therapy (P=0.043 for EFS and P=0.008 for OS, Figure 3C and D).

Patients in the other group with DNMT3A\textsuperscript{mut}/NPM1\textsuperscript{mut} did not show similar results (P=0.056 for EFS and P=0.053 for OS, Figure 3E and F).

For further detailed analysis, we considered another subgroup of patients with DNMT3A\textsuperscript{mut}/FLT3\textsuperscript{mut}/NPM1\textsuperscript{wild} from the existing cohort of 51 patients. We compared the EFS and OS between DNMT3A\textsuperscript{mut}/FLT3\textsuperscript{mut}/NPM1\textsuperscript{wild} patients (n=19) and DNMT3A\textsuperscript{mut}/FLT3\textsuperscript{mut}/NPM1\textsuperscript{mut} patients (n=4) and found that there were significant differences between these two groups (P2=0.004 for EFS in Figure 4A and P2=0.001 for OS in Figure 4B), but there was no difference between the outcomes for the DNMT3A\textsuperscript{mut}/FLT3\textsuperscript{mut}/NPM1\textsuperscript{mut} and DNMT3A\textsuperscript{mut}/FLT3\textsuperscript{mut}/NPM1\textsuperscript{mut} groups (n=17, P1>0.1, Figure 4A and B).

**Discussion**

Our study showed that DNMT3A mutations in AML had higher BM blasts, higher FLT3-ITD/TKD mutation rate, and higher IDH2 mutation rate when combined with NPM1 mutation, but little difference in prognoses compared with NPM1 wild type. HSCT treatment benefited all patients who carried the DNMT3A mutation and was a better choice for the DNMT3A\textsuperscript{mut}/NPM1\textsuperscript{mut} group.

In Gale et al's\textsuperscript{15} study, 80% of 272 patients with DNMT3A mutation concomitantly had the NPM1 mutation. We analyzed the biological and clinical features of 51 patients...
| Characteristic                  | DNMT3A<sup>mut</sup>/NPM1<sup>mut</sup>, median (range) or n/% | WBC count (<10/L) or n/% | *P*  |
|-------------------------------|-----------------------------|-------------------------|------|
| Age (years)                   |                             |                         |      |
| <60                           | 63 (42–81)                  | 57 (21–81)              | 1.700<sup>a</sup> 0.095 |
| ≥60                           | 10/43.5                     | 17/60.7                 |      |
| Gender                        |                             |                         |      |
| Male                          | 12/52.2                     | 12/42.9                 | 0.440<sup>0</sup> 0.580 |
| Female                        | 11/47.8                     | 16/57.1                 |      |
| AML FAB subtypes              |                             |                         |      |
| M0                            | 3/13.0                      | 0                       | 3.880<sup>0</sup> 0.085 |
| M1                            | 7/30.4                      | 6/21.4                  | 0.539<sup>0</sup> 0.529 |
| M2                            | 5/21.7                      | 6/21.4                  | 0.001<sup>1</sup> 1.000 |
| M4                            | 5/21.7                      | 7/25.0                  | 0.075<sup>1</sup> 1.000 |
| M5                            | 2/8.7                       | 9/32.1                  | 4.104<sup>0</sup> 0.084 |
| M7                            | 1/4.3                       | 0                       | 1.242<sup>1</sup> 0.451 |
| Karyotype                     |                             |                         |      |
| Normal                        | 10/43.5                     | 23/85.2                 | 9.628<sup>0</sup> 0.003 |
| Complex                       | 3/13.0                      | 1/3.7                   | 1.472<sup>0</sup> 0.322 |
| Trisomy 8                     | 4/17.4                      | 1/3.7                   | 2.585<sup>1</sup> 0.167 |
| -7/tq                         | 2/8.7                       | 0                       | 2.446<sup>0</sup> 0.207 |
| Others                        | 4/17.4                      | 2/7.4                   | 1.172<sup>1</sup> 0.395 |
| Risk (cytogenetics)           |                             |                         |      |
| Good                          | 0                           | 0                       | 3.224<sup>0</sup> 0.121 |
| Intermediate                  | 17/73.9                     | 25/92.6                 |      |
| Poor                          | 6/26.1                      | 2/7.4                   |      |
| WBC count (>10/L)             |                             |                         |      |
| <5                            | 19/82.6                     | 13/46.4                 |      |
| ≥5                            | 4/17.4                      | 15/53.6                 |      |
| BM blasts                     | 62 (32–99)                  | 81.5 (41–100)           | 1.948<sup>a</sup> 0.057 |
| <70                           | 14/60.9                     | 7/25.0                  |      |
| ≥70                           | 9/39.1                      | 21/75.0                 |      |
| PB blasts                     | 32 (0–97)                   | 49 (0–91)               | 0.692<sup>a</sup> 0.492 |
| <70                           | 17/73.9                     | 18/66.7                 |      |
| ≥70                           | 6/26.1                      | 9/33.3                  |      |
| Relapse                       | 11/47.8                     | 17/60.7                 | 0.847<sup>1</sup> 0.407 |
| Mutated recurrent genes       | 6 (2–10)                    | 5 (2–11)                | 1.569<sup>1</sup> 0.123 |
| <5                            | 5/21.7                      | 10/35.7                 |      |
| ≥5                            | 18/78.3                     | 18/64.3                 |      |
| FLT3                          |                             |                         |      |
| FLT3-ITD                      | 2/8.7                       | 10/35.7                 | 9.785<sup>1</sup> 0.004 |
| FLT3-TKD                      | 2/8.7                       | 7/25.0                  |      |
| Wild type                     | 19/82.6                     | 11/39.3                 |      |
| IDH2                          |                             |                         |      |
| Mutation                      | 5/21.7                      | 0/0                     | 6.749<sup>1</sup> 0.014 |
| Wild type                     | 18/78.3                     | 28/100                  |      |
| IDH1                          |                             |                         |      |
| Mutation                      | 6/26.1                      | 5/17.9                  | 0.506<sup>1</sup> 0.514 |
| Wild type                     | 17/73.9                     | 23/82.1                 |      |
| TET2                          |                             |                         |      |
| Mutation                      | 5/21.7                      | 1/3.6                   | 4.015<sup>1</sup> 0.079 |
| Wild type                     | 18/78.3                     | 27/96.4                 |      |
| NRAS                          |                             |                         |      |
| Mutation                      | 2/8.7                       | 3/10.7                  | 0.058<sup>1</sup> 1.000 |
| Wild type                     | 21/91.3                     | 25/89.3                 |      |

(Continued)
also found that patients with DNMT3A mutations were more likely to have mutations in NPM1 with a trend toward a higher FLT3 mutation rate. In our study, the IDH2 mutation was seen in 21.7% patients in the DNMT3A\textasciimacronmut/NPM1wild group, while those in the DNMT3A\textasciimacronmut/NPM1mut group did not harbor the IDH2 mutation. Another study showed that the percentage of IDH2 mutations were 11.5 and 33.3% for DNMT3A\textasciimacronmut/NPM1wild and DNMT3A\textasciimacronmut/NPM1mut, respectively.15 We were not very consistent with their IDH2 mutation rates, and this may be related to the small number of cases.

Comparison of EFS and OS between different biological and clinical characteristics groups showed no difference. Similar to Xu et al’s23 conclusion, we also confirmed that HSCT is a better option for patients with DNMT3A mutations. We found that chemotherapy and HSCT treatment had the same effect in the DNMT3A\textasciimacronmut/NPM1mut group. We supposed that this may be due to high mutation rate of FLT3 in the NPM1-mutated group. Previous studies suggested that FLT3-ITD always results in significantly worse clinical outcomes and weakens the curative effect of conventional chemotherapy,24 as well as shortening the EFS and OS in DNMT3A-mutated patients.25 Only the R140 mutation in IDH2 seems to have prognostic meaning and is associated with a better outcome.15 In our study, we found that IDH2 mutation only occurred in DNMT3A\textasciimacronmut/NPM1wild patients and three of the five IDH2 mutations happened at the R140 locus. This could be another reason why the DNMT3A\textasciimacronmut/NPM1mut group showed similar results to the DNMT3A\textasciimacronmut/NPM1wild group.

There are some limitations to our study. First, the small sample size may have reduced the accuracy of our results. Second, our study has a retrospective design, the effectiveness of which is limited when compared to a prospective study.

**Conclusion**

All patients with the DNMT3A mutation benefited from HSCT, but those who also have NPM1 mutations should be treated carefully given their high FLT3-ITD/TKD rate, and hence, HSCT may not be better than chemotherapy for this group of patients.
Figure 2. Comparison of EFS and OS between different biological and clinical character groups.

Notes: (A and B) EFS and OS of DNMT3A<sup>mut</sup>/NPM1<sup>wild</sup> vs DNMT3A<sup>mut</sup>/NPM1<sup>mut</sup> in all of the 51 patients. (C and D) EFS and OS of DNMT3A<sup>mut</sup>/NPM1<sup>wild</sup> vs DNMT3A<sup>mut</sup>/NPM1<sup>mut</sup> in 28 patients with chemotherapy. (E and F) EFS and OS of DNMT3A<sup>mut</sup>/NPM1<sup>wild</sup> vs DNMT3A<sup>mut</sup>/NPM1<sup>mut</sup> in 23 patients with HSCT.

Abbreviations: EFS, event-free survival; HSCT, hematopoietic stem cell transplantation; OS, overall survival.
NPM1 mutations in patients with DNMT3A mutations

Figure 3 Comparison of chemotherapy and HSCT in different mutation groups.

Notes: (A and B) EFS and OS of all the 51 patients. (C and D) EFS and OS of the 23 patients with DNMT3A\textsuperscript{mut}/NPM1\textsuperscript{wild}. (E and F) EFS of the 28 patients with DNMT3A\textsuperscript{mut}/NPM1\textsuperscript{mut}.

Abbreviations: EFS, event-free survival; HSCT, hematopoietic stem cell transplantation; OS, overall survival.
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Disclosure
The authors report no conflicts of interest in this work.

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