U2AF1 Mutations in Chinese Patients with Acute Myeloid Leukemia and Myelodysplastic Syndrome

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Abstract

Somatic mutations of U2AF1 gene have recently been identified in myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). In this study, we analyzed the frequency and clinical impact of U2AF1 mutations in a cohort of 452 Chinese patients with myeloid neoplasms. Mutations in U2AF1 were found in 2.5% (7/275) of AML and 6.3% (6/96) of MDS patients, but in none of 81 CML. All mutations were heterozygous missense mutations affecting codon S34 or Q157. There was no significant association of U2AF1 mutation with blood parameters, FAB subtypes, karyotypes and other gene mutations in AML. The overall survival (OS) of AML patients with U2AF1 mutation (median 3 months) was shorter than those without mutation (median 7 months) (P = 0.035). No difference in the OS was observed between MDS patients with and without U2AF1 mutations. Our data show that U2AF1 mutation is a recurrent event at a low frequency in AML and MDS.

Introduction

Acute myeloid leukemia (AML) is characterized by autonomous proliferation and impaired differentiation of hematopoietic progenitors but is a genetically and phenotypically heterogeneous disease. The development of AML is associated with accumulation of acquired genetic alterations and epigenetic changes in hematopoietic progenitor cells that induce normal hematopoietic progenitor cell to lose the ability of self-renewal and differentiation to various mature cell lineages, to transform into a leukemic stem cell, and to accumulate in bone marrow [1,2]. In recent years, an increasing number of gene mutations have been identified involved in the pathogenesis of the disease and have been shown to be correlated with prognosis of AML patients [3]. Some gene mutations have been further introduced into the current World Health Organization (WHO) classification [4].

U2AF1 (U2AF35), an essential component of the U2 small nuclear ribonucleoprotein auxiliary factor (U2AF), plays an important role in the splicing process in which functional mRNA is generated from pre-mRNA [5,6]. The disruption of interactions of several factors involved in the splicing process can cause various types of mutations in an ever increasing number of genes [7]. Recently, somatic mutations in U2AF1 were discovered in myelodysplastic syndrome (MDS) and mainly occurred in two codons (Ser34 and Q157) [8,9]. Patients with U2AF1 mutations had an increased probability of progression from MDS to AML; however, they had similar overall survival as those with wild-type U2AF1 [8]. The prognostic impact and clinical characteristics of patients with U2AF1 mutations in AML are unknown. Thus, in this study we investigated the frequency and prognostic influence of U2AF1 mutations in a cohort of patients with AML, MDS and chronic myeloid leukemia (CML) patients.

Materials and Methods

Patients’ samples

This study was approved by the Ethics Committee Board of Affiliated People’s Hospital of Jiangsu University. Bone marrow aspirates or peripheral blood samples of patients with various hematologic malignancies were collected after informed consent written. The patients included 275 primary AML, 96 primary MDS, 81 CML (61 at chronic phase, 4 at accelerated phase, 16 at blast crisis). These hematological malignancies were diagnosed according to the French-American-British Cooperative Group Criteria and the 2008 World Health Organization proposal [4,10]. Karyotypes were classified according to reported previously [11,12]. Bone marrow specimens obtained at the time of complete hematologic remission from three patients (one AML, one RAEB-1 and one RAEB-2) with U2AF1 mutations at initial diagnosis and peripheral blood from 103 healthy individuals were used as control. The mononuclear cells were separated by density-gradient centrifugation using Ficoll. Subsequently, genomic DNAs were extracted using the Genomic DNA Purification Kit (Gentra, USA) according to the manufacturer’s instructions.

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**Table 1.** The clinical and hematopoietic parameters of 13 patients with *U2AF1* mutations.

| ID  | Sex/Age (years) | Diagnosis | WBC (<10^9/L) | Hemoglobin (g/L) | Platelet (<10^11/L) | Karyotype | Survival time (months) | U2AF1 mutation |
|-----|-----------------|-----------|---------------|------------------|---------------------|-----------|----------------------|----------------|
| 1   | M/20            | AML-M2    | 130.2         | 107              | 69                  | +8        | 3                    | S34Y           |
| 2   | M/76            | AML-M1    | 97.0          | 40               | 33                  | No data   | 1                    | Q157P          |
| 3   | F/29            | AML-M2    | 75.1          | 76               | 50                  | +8        | 1                    | S34Y           |
| 4   | M/44            | AML-M4    | 3.9           | 64               | 48                  | −7        | 9                    | Q157R          |
| 5   | M/60            | AML-M2    | 3.5           | 54               | 30                  | N         | 6                    | S34F           |
| 6   | F/80            | AML-M2    | 0.9           | 56               | 31                  | N         | 5                    | S34Y           |
| 7   | M/66            | AML-M5    | 37.2          | 65               | 42                  | 1p+, −5, 22q+, +mar1, +mar2 | No data | S34F |
| 8   | M/86            | RAEB-1    | 2.3           | 43               | 31                  | No data   | 4+                   | Q157P          |
| 9   | M/31            | RAEB-2    | 1.4           | 51               | 30                  | N         | 4+                   | S34Y           |
| 10  | F/31            | RCMD-RS   | 2.6           | 79               | 101                 | del(5)(q13q34) | 45 | S34F |
| 11  | M/28            | RAEB-2    | 2.4           | 56               | 44                  | N         | 40                   | S34F           |
| 12  | M/67            | RAEB-1    | 1.6           | 66               | 130                 | N         | 22                   | S34F           |
| 13  | F/40            | RCMD-RS   | 3.0           | 41               | 88                  | +8        | 1+                   | S34Y           |

M, male; F, female; N, normal.
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**Mutation scanning**

Primers to amplify the coding sequences of *U2AF1* (GenBank NC_000021.8) are listed in Table S1. Genomic DNA samples were amplified in a final volume of 25 μL containing 1× PCR buffer (Invitrogen, Merelbeke, Belgium), 0.2 mM/L of each dNTP, 2.5 mM/L of MgCl₂, 0.4 mM/L of both forward and reverse primers, 0.8 mM/L of oligonucleotide calibrators [13], 1× LCgreen Plus (Idaho Technology Inc. Salt Lake City, Utah), and 1 U Taq polymerase (MBI Fermentas, Canada). All PCR amplicons were generated on a 7300 Thermo cycler (Applied Biosystems, Foster City, CA, USA) using the PCR program as follows: an initial denaturation step was started at 95°C for 5 minutes, followed by 40 cycles of denaturation at 94°C for 30 seconds, annealing at melting temperature for 30 seconds, and an extension at 72°C for 30 seconds. Mutation scanning was performed for PCR products using high-resolution melting analysis (HRMA) with the LightScanner™ platform (Idaho Technology Inc. Salt Lake City, Utah) according to the protocol reported previously [14].

**DNA sequencing**

To confirm HRMA results, DNA sequencing was also performed in all samples identified by HRMA. PCR products were directly sequenced on both strands using an ABI 3730 automated sequencer.

**Results and Discussion**

HRMA could easily distinguish *U2AF1* mutations (S34Y and Q157R) with the sensitivity of 5% in a background of wild-type DNA, higher than that obtained by direct DNA sequencing (10%) (Figures S1, S2, S3 and S4).

In the cohort of 452 patients with myeloid malignancies, a heterozygous *U2AF1* mutation was found to be in 13 cases with AML or MDS, but in none with CML. The representative results of HRMA and direct sequencing of *U2AF1* mutations in AML and MDS were presented in Figures S5, S6, S7 and S8. The clinical characteristics of all patients with *U2AF1* mutations were shown in Table 1. *U2AF1* mutation, positive in the bone marrow samples from three individuals (case 4, 11 and 12, Table 1) at initial diagnosis, disappeared after the first complete remission. Furthermore, *U2AF1* mutation was not present in all healthy controls. These results support the somatic nature of *U2AF1* mutations.

Heterozygous *U2AF1* mutations were found in 7 (2.5%) of 275 AML patients (Table 1), including 3 S34Y, 2 S34F and 2 Q157 (1 Q157P and 1 Q157R) mutations. There were no difference in sex, age, blood parameters, FAB subtypes, and karyotype classification between cases with and without mutations (P=0.05, Table 2). Makishima et al reported higher occurrence (9.1%, 5/55) of *U2AF1* mutations in primary AML [19]. A larger cohort of Yoshida et al revealed 2% (3/151) of *U2AF1* mutations in Japanese AML population [9]. *U2AF1* mutations mainly occurred in the FAB subtypes of M1, M2, M4 and M5 [9,19]. Interestingly, all five *U2AF1* mutations found by Makishima et al occurred in cytogenetically abnormal AML, including two cases with monosomy 7 [19]. However, two cases with *U2AF1* mutations were identified with trisomy 8 besides one with monosomy 7 in our AML group. More patients should be investigated to determine the association of *U2AF1* mutations with karyotypes. C-KIT, FLT3-ITD, NPM1, IDH1/IDH2 and DNMT3A mutations were also detected. Among the patients with *U2AF1* mutations, only two cases had NPM1 mutation. There was no significant association of *U2AF1* mutation with other molecular alterations (Table 2).

Follow-up data were obtained for 150 AML patients. There was no significant difference in complete remission rate between patients with and without *U2AF1* mutation (57.1% vs 72.9%, P=0.398).
Table 2. Distribution of U2AF1 mutations in AML and MDS.

|                      | U2AF1 mutation | Wild-type | P    |
|----------------------|----------------|-----------|------|
| **AML**              |                |           |      |
| Sex, male/female     | 5/2            | 149/119   | 0.471|
| Median age at diagnosis, years (range) | 60 (20–80) | 47 (15–93) | 0.402|
| Median WBC at diagnosis, ×10^9/L (range) | 37.2 (0.9–130.2) | 14 (0.5–528) | 0.690|
| Median hemoglobin at diagnosis, g/L (range) | 64 (40–107) | 74 (32–147) | 0.196|
| Median platelets at diagnosis, ×10^9/L (range) | 42 (30–69) | 38 (3–447) | 0.634|
| **MDS**              |                |           |      |
| Sex, male/female     | 4/2            | 54/36     | 1.000|
| Median age at diagnosis, years (range) | 36 (28–86) | 60 (20–85) | 0.134|
| Median WBC at diagnosis, ×10^9/L (range) | 2.4 (1.4–3.0) | 2.8 (0.6–82.4) | 0.129|
| Median hemoglobin at diagnosis, g/L (range) | 54 (41–79) | 62 (26–128) | 0.237|
| Median platelets at diagnosis, ×10^9/L (range) | 66 (30–130) | 60 (1–1176) | 0.745|
| **Gene mutations**   |                |           |      |
| C-KIT (+/−)          | 0/7            | 13/255    | 1.000|
| NPM1 (+/−)           | 2/5            | 20/248    | 0.100|
| FLT3-ITD (+/−)       | 0/7            | 14/254    | 1.000|
| IDH1/IDH2 (+/−)      | 0/7            | 12/256    | 1.000|
| DNMT3A (+/−)         | 0/7            | 14/254    | 1.000|
| **WHO, no.**         |                |           |      |
| 5q−                  | 0              | 3         | 0.571|
| RA/RARS/RT           | 0              | 11        |      |
| RCMD/RCMD-RS         | 2              | 41        |      |
| RAEB-1               | 2              | 18        |      |
| RAEB-2               | 2              | 17        |      |
| **Karyotype classification** |            |           | 0.386|
| Favorable            | 4              | 64        |      |
| Intermediate         | 1              | 16        |      |
| Poor                 | 0              | 8         |      |
| No data              | 1              | 2         |      |
| **IPSS**             |                |           | 0.449|
| Low                  | 0              | 9         |      |
| Int-1                | 4              | 56        |      |
| Int-2                | 0              | 17        |      |
| High                 | 0              | 6         |      |
| No data              | 0              | 2         |      |

Gene mutations
The median follow-up duration of the patients was 7 months (range, 1–73 months). M3 subtype was excluded from survival analysis due to different therapy and prognosis. The estimated 50% survival time of the remaining 126 patients was 7 months. The overall survival (OS) of AML patients with \( U2AF1 \) mutation (median 3 months, 95% confidence interval 0–7.8 months) was shorter than those without mutation (median 7 months, 95% confidence interval 4.8–9.2 months) \( P = 0.035 \), Figure S9). However, there was no difference in disease-free survival between the patients with and without \( U2AF1 \) mutation. Two patients with \( U2AF1 \) mutation died early after initial diagnosis due to central nervous system involvement and sepsis respectively. Furthermore, a multivariate analysis for outcomes could not be performed because of the small sample size of patients with mutations. A larger cohort from a clinical trial will be needed to definitively address the effect of \( U2AF1 \) mutations on outcomes.

6 (6.3%) MDS cases were identified with heterozygous \( U2AF1 \) mutations (3 S34F, 2 S34Y, and 1Q157P) (Table 1). No significance in sex, age, blood parameters, WHO subtypes, and IPSS classification was observed between MDS patients with and without \( U2AF1 \) mutations \( P > 0.05 \), Table 2). 7.3%–8.8% of \( U2AF1 \) mutations have been reported in primary MDS recently [8,9,19–21]. All 72 \( U2AF1 \) mutations in MDS including ours, which could be almost found in each FAB or WHO subtype, exclusively occurred at the highly conserved sites of exon S34 and Q157 with a rare exception of A26V, E159, or R156H mutation [8,9,19–21]. Significant association of \( U2AF1 \) mutations has not been identified with specific karyotypes. Although Damm et al [21] found that the association of del20q with \( U2AF1 \) mutation, no \( U2AF1 \) mutation was observed in all four cases with del20q in our group. Survival analysis was performed in 76 MDS cases with follow-up information. No difference in OS was observed between patients with and without \( U2AF1 \) mutations \( P = 0.821 \), Figure S9). The impact of \( U2AF1 \) mutations on clinical outcome has been controversial in MDS [8,19,20]. Although Makishima et al considered \( U2AF1 \) mutation as a factor predictive for shorter survival [19], other three studies did not find the association of \( U2AF1 \) mutation with prognosis [8,20,21]. More cases with \( U2AF1 \) mutations should be further studied to determine its prognostic relevance.

The definite role of \( U2AF1 \) in the cancer pathogenesis has not been known. \( U2AF1 \) mutation induces abnormal global RNA splicing which has been described in a wide variety of cancers [22–24]. Reduced expression of \( U2AF1 \) was found in pancreatic cancer cells and correlated with mis-splicing of the cholecystokinin-B/gastrin receptor mRNA [25]. Additionally, knockdown of \( U2AF1 \) reduces cell proliferation, induces G2/M arrest, and enhances apoptosis [26]. Moreover, a recent study demonstrated that S34F mutant had the same effect as \( U2AF1 \) downregulation [9]. These results suggest that \( U2AF1 \) mutant leads to loss of function and contributes to ineffective hematopoiesis and the cytopenias seen in MDS. Furthermore, subjects with \( U2AF1 \) mutations were not restricted to a particular WHO subtypes, which indicates \( U2AF1 \) mutation should be an early, initial genetic event in MDS.

In summary, mutations in \( U2AF1 \) occur in patients with AML at a low frequency and are associated with a negative prognosis in AML which will require confirmation in a larger cohort.

### Supporting Information

**Figure S1** Results of a dilution series of S34Y \( U2AF1 \) mutation in a background of wild-type DNA detected by HRMA. 1: 0%, 1% and 2% mutant; 2: 5% mutant; 3: 10% mutant; 4: 25% mutant; 5: 50% mutant; 6: 100% mutant. A: normalized melting peaks; B: normalized difference curves. Mutated S34Y \( U2AF1 \) was identified by HRMA at the maximal sensitivity of 5%. Although the shapes were similar, homozygous mutants could be distinguished from wild-type amplicons by Tm shift.

**Figure S2** Results of a dilution series of S34Y \( U2AF1 \) mutant in a background of wild-type DNA detected by DNA sequencing. The maximal sensitivity of 10% was obtained. Arrow showed the mutation site.

**Figure S3** Results of a dilution series of Q157R \( U2AF1 \) mutant in a background of wild-type DNA detected by HRMA. 1: 0%, 1% and 2% mutant; 2: 5% mutant; 3: 10% mutant; 4: 25% mutant; 5: 50% mutant; 6: 100% mutant. A: normalized melting peaks; B: normalized difference curves.

**Figure S4** Results of a dilution series of Q157R \( U2AF1 \) mutant in a background of wild-type DNA detected by DNA sequencing. The maximal sensitivity of 10% was obtained. Arrow showed the mutation site.

**Figure S5** HRMA screening of S34 \( U2AF1 \) mutations in MDS patients. Grey lines represent wild-type S34 \( U2AF1 \); Blue line represents heterozygous S34F mutant in one MDS case; Orange line represents heterozygous S34Y mutant in one MDS case.

**Figure S6** Sequencing results of S34 \( U2AF1 \) mutations in AML and MDS patients. A: heterozygous S34Y mutation (TCT→TAT); B: heterozygous S34F mutation (TCT→TTT). Arrow denotes mutation site.

**Figure S7** HRMA screening of Q157 \( U2AF1 \) mutations in AML patients. Grey lines represent wild-type Q157 U2AF1; Red lines represent heterozygous Q157P mutant in one AML case.

| IDH1/IDH2 (+/-) | U2AF1 mutation | Wild-type | \( P \) |
|----------------|----------------|-----------|-----|
| 0/6            | 5/85           |           |     |

| DNMT3A (+/-)   | U2AF1 mutation | Wild-type | \( P \) |
|----------------|----------------|-----------|-----|
| 0/6            | 4/86           |           |     |

WBC indicates white blood cell count at diagnosis; IPSS, International Prognostic Scoring System; WHO, World Health Organization; FAB, French-American-British classification; RA, refractory anemia; RARS, refractory anemia with ringed sideroblasts; RT, refractory thrombocytopenia; RCMD, refractory cytopenia with multilineage dysplasia; RCMD-RS, refractory cytopenia with multilineage dysplasia with ringed sideroblasts; RAEB, refractory anemia with excess of blasts.
Figure S8 Sequencing results of Q157 U2AF1 mutations. A: heterozygous Q157P mutation (CAG→GGG) in one case with AML-M1; B: heterozygous Q157R mutation (CAG→GGG) in one case with AML-M4; Arrow denotes mutation site.

(DOC)

Figure S9 Overall survival of AML or MDS patients divided according to U2AF1 mutation status at diagnosis. A: AML; B: MDS.

(DOC)

References
1. Huntly BJ, Gilliland DG (2005) Cancer biology: summing up cancer stem cells. Nature 435:1169–1170.
2. Jordan CT, Guzman ML, Noble M (2006) Cancer stem cells. N Engl J Med 355:1253–1261.
3. Dobner H, Gaidzik VI (2011) Impact of genetic features on treatment decisions in AML. Hematology Am Soc Hematol Educ Program 2011:36–42.
4. Sverdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, et al. eds. (2008) WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon, France: IARC Press.
5. Zamore PD, Green MR (1991) Biochemical characterization of U2 snRNP auxiliary factor: an essential pre-mRNA splicing factor with a novel intranuclear distribution. Embo J 10:207–214.
6. Black DL (2003) Mechanisms of alternative pre-messenger RNA splicing. Annu Rev Biochem 72:291–336.
7. Baralle D, Baralle M (2005) Splicing in action: assessing disease causing sequence changes. J Med Genet 42:737–748.
8. Granbert TA, Siron D, Diog L, Okejo-Owor T, Lamm CL, et al. (2011) Recurrent mutations in the U2AF1 splicing factor in myelodysplastic syndromes. Nat Genet 44:53–57.
9. Yoshirin K, Sanada M, Shiraiishi Y, Nowak D, Nagata Y, et al. (2011) Frequent pathway mutations of splicing machinery in myelodysplasia. Nature 470:64–69.
10. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, et al. (1985) Proposed revised criteria for the classification of acute myeloid leukemia. A report of the French-American-British Cooperative Group. Ann Intern Med 103:620–625.
11. Stowka ML, Kopcicky KJ, Cassileth PA, Harrington DH, Theil KS, et al. (2000) Karyotypic analysis predicts outcome of preremission and postremission therapy in adult acute myeloid leukemia: a Southwest Oncology Group/Eastern Cooperative Oncology Group Study. Blood 96:4075–4083.
12. Greenberg P, Cox G, LeBeau MM, Fenaux P, Morel P, et al. (1997) International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood 90:2079–2088.
13. Qian J, Lin J, Yao DM, Chen Q, Xiao GF, et al. (2010) Rapid detection of JAK2 V617F mutation using high-resolution melting analysis with LightScanner platform. Clin Chim Acta 410:2679–2686.
14. Lin J, Yao DM, Qian J, Chen Q, Qian W, et al. (2011) Recurrent DNMT3A R882 mutations in Chinese patients with acute myeloid leukemia and myelodysplastic syndrome. PLoS One 6: e26906.
15. Kottaridis PD, Gale RE, Frew ME, Harrison G, Langabeer SE, et al. (2001) The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. Blood 98:1752–1759.
16. Szankasi P, Jama M, Bahler DW (2008) A new DNA-based test for detection of nucleophosmin exon 12 mutations by capillary electrophoresis. J Mol Diagn 10:236–241.
17. Lin J, Yao DM, Qian J, Chen Q, Qian W, et al. (2012) Recurrent DNMT3A R882 mutations in Chinese patients with acute myeloid leukemia and myelodysplastic syndrome. PLoS One 6: e26906.
18. Lin J, Yao DM, Qian J, Chen Q, Qian W, et al. (2012) IDH1 and IDH2 mutation analysis in Chinese patients with acute myeloid leukemia and myelodysplastic syndrome. Ann Hematol 91:519–525.
19. Makihiha H, Visconte V, Sakaguchi H, Jankowska AM, Abu Kar S, et al. (2012) Mutations in the spliceosome machinery, a novel and ubiquitous pathway in leukemogenesis. Blood 119:3205–3210.
20. Thel F, Kade S, Schlarmann C, Leffeld P, Morgan M, et al. (2012) Frequency and prognostic impact of mutations in SRSF2, U2AF1, and ZRSR2 in patients with myelodysplastic syndromes. Blood 119:3578–3584.
21. Damann F, Kosmider O, Gebi-Boyer V, Renneville A, Carubucci N, et al. (2012) Mutations affecting mRNA splicing define distinct clinical phenotypes and correlate with patient outcome in myelodysplastic syndromes. Blood 119:3211–3218.
22. Gross AR, Marins S, Carmo-Fonseca M (2008) The emerging role of splicing factors in cancer. EMBO Rep 9:1087–1093.
23. David CJ, Manley JL (2010) Alternative pre-mRNA splicing regulation in cancer: pathways and programs unlinked. Genes Dev 24:2343–2364.
24. Pajares MJ, Espanda T, Catena R, Calvo A, Pio R, et al. (2007) Alternative splicing: an emerging topic in molecular and clinical oncology. Lancet Oncol 8:349–357.
25. Ding WQ, Kunstz SM, Miller LJ (2002) A misspliced form of the cholecystokinin-B/gastrin receptor in pancreatic carcinoma: role of reduced cellular U2AF35 and a suboptimal 3′-splicing site leading to retention of the fourth intron. Cancer Res 62:947–952.
26. Pacheco TR, Mosta LF, Gomes AQ, Hacohen N, Carmo-Fonseca M (2006) RNA interference knockdown of U2AF35 impairs cell cycle progression and modulates alternative splicing of Cdc25 transcripts. Mol Biol Cell 17:4167–4199.

Table S1 The sequences of primers used in PCR for HRMA or direct sequencing.

(DOC)

Author Contributions
Conceived and designed the experiments: JL WQ JQ. Performed the experiments: DMY HYC YL JY. Analyzed the data: JL WQ JQ CZW XXC. Contributed reagents/materials/analysis tools: ZQD JCM. Wrote the paper: JL WQ JQ.