Clinical and prognostic profile of SRSF2 and related spliceosome mutations in patients with acute myeloid leukemia

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Abstract

Background

Mutations in splicing factor (SF) genes are frequently detected in myelodysplastic syndrome, but rare data about the clinical and prognostic relevance of these mutations in acute myeloid leukemia (AML) have been reported.

Methods

A total of 368 newly diagnosed non-M3 AML patients were included in this study. Next generation sequencing including four SF genes was performed on the genomic DNA. The clinical features and survival were analyzed using statistical analysis. SRSF2<sup>P95H</sup> function was assessed by CCK8 assay.

Results

We found that 64 of 368 patients harbored SF mutations. The SF mutations were much more frequent in older or male patients compared with SF-wild patients. SRSF2 mutations were shown obviously co-existed with IDH2 mutation. The level of measurable residual disease after the first chemotherapy was higher in SF-mutated patients compared to that in SF-wild patients, while the complete remission rate was significantly decreased. And the overall survival (OS) of SF-mutated patients was shorter than that of SF-wild patients. Moreover, our multivariable analysis suggests that the index of male, Kit mutation or ZRSR2 mutation was the independent risk factor for OS. SRSF2<sup>mut</sup> was associated with older age, higher proportion of peripheral blasts or abnormal cell proportion by FCM (Flow CytoMetry). Functionally, the mutation of SRSF2<sup>P95H</sup> significantly promoted the proliferation of AML cells.

Conclusion

Spliceosome mutation is a distinct subgroup of AML frequently associated with clinic-biological features and poor outcome. SRSF2<sup>mut</sup> could be potential targets for novel treatment in AML.

Introduction

Acute myeloid leukemia (AML) is a group of myeloid malignancies characterized by clonal disorder of hematopoietic stem and progenitor cells [1, 2]. Although most patients with AML have great improvement upon induction chemotherapy, the risk of relapse is considerable [3]. AML had been preliminarily diagnosed according to the clinical manifestations and bone marrow (BM) cytological level, and molecular genetics plays an increasingly important role in the accurate diagnosis and prognosis of AML. There were plenty of prognostic genetic mutations included in the classical ELN and NCCN guidelines [4].
Among these genetic abnormalities, RNA splicing factors (SFs), spliceosome genes, were a kind of heterogeneous genomic categories, and patients with spliceosome mutations had poor outcomes [10]. However, the clinical and prognosis significance of the spliceosome mutations are not fully elucidated in AML.

Next-generation sequencing (NGS) has been developed for multiple genes determination and plays an important role in the diagnosis and prognosis of hematological diseases, especially for AML. Various gene mutations, including the SF mutations, can be simultaneously determined by NGS at the molecular level. The recurrent mutations in several important spliceosomes, including SF3B1, SRSF2, U2AF1 and ZRSR2, are frequently found in myelodysplastic syndromes (MDS) and AML [5–8]. These mutations commonly occur in genes controlling 3’ splicing and usually result in abnormal RNA splicing and compromised hematopoiesis [6, 9], so they are often called a class of cancer driver mutations [9]. The SRSF2 mutation was frequently found in patients with myeloid malignancies and was individually associated with poor outcomes [11]. SRSF2P95H animals could develop a disease characterized by the features of MDS/MPN in humans [35]. However, the clinical and prognostic significance of SRSF2P95H in AML has been rarely reported and its pathogenesis remains unclear.

In this study, we first investigated the clinical, cytogenetic and molecular features of AML patients with SF3B1, SRSF2, ZRSR2 or U2AF1 mutations. Then we clarified the survival outcomes of these patients and their correlations with mutation patterns. As the importance of SRSF2mut, we further examined its pathogenic mechanism by in vitro experiments. Our study will provide novel diagnostic and therapeutic approaches for AML patients with SF mutations.

Materials And Methods

Patients

A total of 368 newly diagnosed non-M3 AML patients were included in this study. AML patients were diagnosed according to the French-American-British (FAB) classification system. Patients were stratified into spliceosome mutated group (SF group, presence of SRSF2, SF3B1, U2AF1, or ZRSR2 mutations, n = 64) and non-spliceosome mutated group (SF-wild group, absence of anyone of these spliceosome mutations, n = 304). Complete response (CR) was defined based on the International Working Group Criteria [29]. This study was approved by the Medical Ethical Committee of Qilu Hospital of Shandong University, China. Informed consent was obtained from all patients before enrollment in the study in accordance with the Declaration of Helsinki.

Samples And Dna Isolation

Bone marrow or peripheral blood samples from patients with AML were collected and treated with erythocyte lysate to obtain leukocytes. Genomic DNA (gDNA) was extracted from leukocytes using the
TIANamp Genomic DNA Kit (TIANGEN, Beijing, China). Genomic DNA quality and quantification were determined using the DeNovix DS-11 ultrafine UV-Visible spectrophotometer (DeNovix Inc., Wilmington, Delaware, USA) and the Qubit 4.0 Fluorescence quantifier (Life Technologies, Carlsbad, USA).

Next-generation Sequencing

For NGS detection, we first constructed the DNA library using AML-MDS-MPN associated gene mutation detection kit (Shanghai Rightongene Biotechnology Co., Ltd, Shanghai, China). The quantity of library was assessed by capillary electrophoresis with Qsep-1 DNA Analyzer (BiOptic Inc., Taipei, Taiwan). Next generation sequencing was performed on the certificated gDNA. A sequencing panel consisting of 38 genes was used for the detection of somatic mutation in 368 patients by Novaseq (Illumina, San Diego, CA, USA). The panel consists of spliceosome mutations including SRSF2, SF3B1, U2AF1 and ZRSR2, and other relevant molecular marker genes, including Class I mutations, such as FLT3, NRAS, KRAS, JAK2, KIT mutations, and Class II mutations, such as CEBPA and RUNX1 mutations, as well as NPM1, WT1, TP53 and those genes related to epigenetic modification, such as ASXL1, IDH1, IDH2, TET2 and DNMT3A mutations. The mean depth for targeted sequencing was 2500 ×, and the average coverage was 0.9 at least for each amplicon. GATK (version 4.1.3.0) integrating the function of Picard was used to analyze variants. The annovar software and SnpEff were used to annotate the final obtained mutations in the coding region.

Chemotherapy

Induction regimes for young patients (< 60 years) included two convention schemes such as standard dose of cytarabine (Ara-C) (100–200 mg·m⁻²·d⁻¹×7d) combined with deoxydaunorubicin (IDA) (12 mg·m⁻²·d⁻¹×3d) or daunorubicin (DNR) (60 ~ 90 mg·m⁻²·d⁻¹×3d)[12, 13, 14] and other scheme included HHT (2 ~ 2.5 mg·m⁻²·d⁻¹×7d or 4 mg·m⁻²·d⁻¹×3d) combined with standard dose of Ara-C (100–200 mg·m⁻²·d⁻¹×7d)(HA). Three low-intensity chemotherapy regimens were used for elderly patients: decitabine (20 mg·m⁻²·d⁻¹×5d) [30, 31, 32], decitabine combined with low-dose chemotherapy or low-dose chemotherapy ± G-CSF [33, 34].

Measurable Residual Disease (Mrd) And Variant Allele Frequency (Vaf)

To assess the effect of chemotherapy on AML, MRD was applied using 8-color flow cytometry analysis (FCM). The result of MRD was present with the abnormal cell proportion by FCM. VAF was used to indicate the quantification of mutations. A threshold of ≥ 1% VAF for individual gene mutation was considered positive for all variants.

Follow-up
The follow-up data were obtained from outpatient cases, inpatient cases, and telephone follow-up records. Follow-up for these patients were ended on May 31, 2022. CR1 was defined as complete response after first induction chemotherapy. Overall survival (OS) was measured from the date of first diagnosis to the date of last follow-up or death from any cause.

Cell Cultures And Transfection

Human AML cell line Molm-13 was cultured in RPMI-1640 medium (Gibco, USA) with 10% fetal bovine serum (FBS, Gibco, USA) and 1% penicillin/streptomycin (Gibco, USA) in an incubator at 37°C and 5% CO₂. The homo-SRSF2<sup>wt</sup> (NM_003016.4) and homo-SRSF2<sup>P95H</sup> (c.284C A) were constructed into PCDNA3.1 vector respectively by BioSune Company (Shanghai, China) for cell transfection. Briefly, a total of 6 × 10<sup>5</sup> Molm-13 cells were seeded into each well of 6-well plates. Then, the mutant or wild-type plasmids was transfected into Molm-13 cells with Micropoly Transfecter Reagent (Invitrogen, USA). Four hours later, the culture medium was replaced with a new medium. After 48 h of transfection, the transfected cells were harvested for further assay.

Cell Viability Assay

The cell viability of Molm-13 was evaluated using the Cell Counting Kit-8 (CCK-8) (Bestbio Company, China) according to the manufacturer's guidelines. Briefly, the transfected cells were plated into 96-well plates at a density of 8×10<sup>3</sup> cells/well. After 0 h, 24 h, 48 h, 72 h and 96 h of incubation at 37°C in an atmosphere containing 5% CO₂, 10 µL of CCK-8 solution was pipetted into the wells and cultured for another 4 h. The optical density (OD) at 450 nm was finally measured.

Statistical analysis

Percentages and ranges were used to describe categorical values, and median and ranges were used to describe continuous variables. Patients' baseline characteristics were summarized using descriptive statistics. Mann-Whitney tests were used to calculate the significance if the continuous data were not normally distributed. The chi-square test was used to calculate the significance of categorical variables. Fisher exact test was used if any expected value of the contingency table was < 5. Survival analysis was applied using the Kaplan-Meier method followed by the log-rank test. Parameters that were significant in univariable analyses were included into multivariable analyses. <i>P-value</i> < 0.05 was considered statistically significant. All statistical analyses were performed with the SPSS 26.0.

Results

The general characteristics of AML patients
A total of 368 newly diagnosed patients with non-M3 AML were included in this study. There were 193 males and 175 females, with a median age of 53 years (range 14-87 years). The median white blood cell (WBC) count, platelet (PLT) count and hemoglobin (Hgb) were 8.35×10^9/L (range: 0.08-479.73×10^9/L), 40.5×10^9/L (range: 0-543×10^9/L) and 74.0 g/L (range: 6.7-162.0 g/L). Moreover, the median frequency of peripheral blasts, bone marrow blasts and abnormal cell proportion by FCM were 63% (range: 0-98%), 40% (range: 0-98%) and 38% (range: 0-99%). According to the FAB classification, patients were diagnosed as the following subtypes: M0 (n =1), M1 (n = 9), M2 (n = 23), M4 (n = 66), M5 (n = 223), M6 (n = 1) and unclassified (n = 45). For the 339 non-M3 AML cases available with risk data, they were divided into adverse risk (107, 31.5%), intermediate risk (126, 37.2%) and favorable risk (106, 31.3%).

**Molecular profile of SF mutations in AML patients**

After determining the mutations using NGS, we found that there were 17.4% (64/368) AML patients harboring the SF mutations (Figure 1a, b). We further analyzed the profile of SF mutations and found that the mutation frequency was 51.5% for SRSF2, 29.7% for U2AF1, 14.1% for SF3B1 and 4.7% for ZRSR2 (Figure 1c). The mutation in SRSF2 mainly occurred at the site of proline 95 and the most frequent mutation was a histidine (P95H) substitution (n=16, 48.5%). The mutations in U2AF1 were mainly missense mutations that affected S34 (S34F and S34Y, 55.6% and 5.6%, respectively) and Q157 (Q157R and Q157P, 11.1% and 11.1%, respectively). And all the mutations in SF3B1 were replacement mutations that caused original lysine to be substituted, in which c.A2098G (p.K700E) and c.G1998T (p.K666N) accounted for 55.6% and 45.4%, respectively. As for ZRSR2, three mutations occurred within the c.G397T (p.E133X), c.C376T (p.R126X) and c.837delA (p. E279fs) (Figure 1d).

**Co-mutation patterns of SF mutations in AML patients**

As co-mutations are important for AML progression, we explored the co-mutation patterns in SF-mutated patients. We found that SRSF2 mutations significantly co-existed with the mutations in IDH2 (r=0.47, P< 0.05) or NPM1 (r=0.33, P<0.05), but inversely associated with U2AF1 mutations (r=-0.33, P< 0.05). For U2AF1 mutation, it was found significantly associated with mutated JAK2 (r=0.30, P< 0.05) or KRAS (r=0.26, P< 0.05). And SF3B1 mutations were frequently co-occurred with SETBP1 (r=0.53, P< 0.05), EZH2 (r=0.27, P< 0.05), NF1 (r=0.27, P< 0.05) or IDH2 (r=0.27, P< 0.05) mutations (Figure 2).

**Clinical relevance of SF mutations in AML patients**

We evaluated the clinical relevance of SF mutations in AML patients. We first analyzed the association of SF mutations with FAB classification, and found that FAB subtypes of SF-mutated patients were significantly different from those of SF-wild patients (P=0.018) (Table 1). Moreover, our data showed that the SF mutations were much more frequent in older patients (median 64 years vs 52 years, P< 0.001), and there were more male patients harboring SF mutations compared with SF-wild patients (75.0% vs 47.7%, P< 0.001). There was no significant difference for WBC, HB, PLT, peripheral blasts, bone marrow blasts and abnormal cells by FMC between SF-mutated patients and SF-wild patients (Table 1). Given the important role of age in SF mutations, all patients were divided into older (≥60 years, n=132) and
younger (<60 years, n=236) groups. The older patients had higher WBC count than the younger patients (median 6.02×10^9/L vs 3.05×10^9/L, \( P=0.010 \)) (Figure S1a, see Supplemental Information), and no statistical significance of other index was found between older and younger patients (Figure S1b-f, see Supplemental Information). We further analyzed the VAF of SF mutations and found no significant difference of VAF among the mutations of SRSF2, SF3B1 and U2AF1. Although no statistical significance was found, the higher VAF of SRSF2 mutations had positive correlation with age and WBC at diagnosis.

**Table 1.** Baseline characteristics of the cohort of patients with SF and non-SF mutation AML
<table>
<thead>
<tr>
<th>Demographics</th>
<th>SF-mutated (n=64)</th>
<th>SF-wild (n=304)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, n (%)</td>
<td>48(75.0)</td>
<td>145(47.7)</td>
<td>0.001</td>
</tr>
<tr>
<td>Age, median (range), y</td>
<td>64(15-87)</td>
<td>52(14-83)</td>
<td>0.001</td>
</tr>
<tr>
<td>Median WBC (range), × 10^9/L</td>
<td>5.35(0.15-300.79)</td>
<td>3.35(0.08-479.73)</td>
<td>0.163</td>
</tr>
<tr>
<td>Median HB (range), × g/L</td>
<td>66.5(6.7-124.0)</td>
<td>70.0(21.0-162.0)</td>
<td>0.209</td>
</tr>
<tr>
<td>Median PLT (range), × 10^9/L</td>
<td>35(1-543)</td>
<td>33(0-483)</td>
<td>0.345</td>
</tr>
<tr>
<td>FAB classification</td>
<td>1/1/1/8/39/0/14</td>
<td>0/8/22/58/184/1/31</td>
<td>0.018</td>
</tr>
<tr>
<td>Median peripheral blasts % (range)</td>
<td>28.0(0.0-98.0)</td>
<td>44.0(0.0-98.0)</td>
<td>0.196</td>
</tr>
<tr>
<td>Median bone marrow blasts % (range)</td>
<td>46.5(0.0-98.0)</td>
<td>64.0(0.0-98.0)</td>
<td>0.113</td>
</tr>
<tr>
<td>Median abnormal cells by FMC % (range)</td>
<td>32.8(4.0~95.0)</td>
<td>38.7(0.0~99.0)</td>
<td>0.105</td>
</tr>
<tr>
<td>Cytogenetics—no. (%)</td>
<td></td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>ELN good risk</td>
<td>12(18.75)</td>
<td>95(31.25)</td>
<td></td>
</tr>
<tr>
<td>ELN Intermediate risk</td>
<td>14(21.87)</td>
<td>112(36.84)</td>
<td></td>
</tr>
<tr>
<td>ELN Adverse risk</td>
<td>32(50.00)</td>
<td>74(24.34)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>6(9.38)</td>
<td>23(7.57)</td>
<td></td>
</tr>
<tr>
<td>MRD%, median (range)</td>
<td>3.5(0.0-94.4)</td>
<td>0.8(0.0-88.8)</td>
<td>0.018</td>
</tr>
<tr>
<td>CR1 rate (%)</td>
<td>47.8(22/46)</td>
<td>75.2(182/242)</td>
<td>0.001</td>
</tr>
</tbody>
</table>
**SF mutations correlate to adverse risk of AML**

Furthermore, we analyzed the association of SF mutations with risk status. As shown in Table 1, for the 304 SF-wild patients, the patients with adverse risk cytogenetics were significantly fewer than the patients with favorable or intermediate risk (74 [24.34%] vs 112 [36.84%] or 95 [31.25%]). However, for the 64 SF mutated patients, their existed a higher proportion (n=32, 50%) of adverse risk patients compared with favorable or intermediate risk patients (12 [18.75%] or 14 [21.87%], \( P < 0.001 \)). Moreover, the most common SF mutations in adverse risk patients were U2AF1 (n=14, 43.8%) and SRSF2 (n=12, 37.5%) while less common for SF3B1 (n=5, 15.6%) and ZRSR2 (n=1, 3.1%). In addition, in 16 SF-mutated patients with abnormal karyotype, three mutations (SRSF2, U2AF1 and SF3B1) accounted for 43.75%, 25.00% and 31.25%, respectively. Therefore, the above findings indicated that the SF mutations may play important roles in adverse risk cytogenetics of AML patients (Figure 1a).

**SF mutations contribute to poor outcome of chemotherapy**

As high MRD level indicates the poor outcome of chemotherapy, we first analyzed the relationship of SF mutations with MRD. Our result showed that the level of MRD after the first course of induction chemotherapy was higher in SF-mutated patients compared to that in the SF-wild patients (median 3.5% vs 0.8%, \( P=0.018 \)). CR1 rate is another outcome index of chemotherapy. Next, we analyzed the effect of SF mutation on CR1, and found that CR1 rate of SF-mutated patients was lower as compared to that of SF-wild patients (median 47.8% vs 75.2%, \( P<0.001 \)) (Table 1). Considering the influence of age, we divided SF-mutated patients into older and younger groups, and found that the CR1 rate of SF-mutated patients was significantly decreased in both of these two groups compared with that in SF-wild patients (median 46.2% vs 71.7%, \( P=0.027 \); 50.0% vs 73.7%, \( P=0.027 \), respectively).

**SF mutated patients have poor overall survival**

The median follow-up for the entire cohort was 30 months (95% confidence interval [95% CI], 23.43-36.57). The OS of patients with SF mutations was significantly shorter than that of SF-wild patients (17 months [95% CI: 8.1-26.0] vs 24 months [95% CI: 18.5-29.5], \( P=0.041 \)) (Figure 3a). As compared to SF-wild patients (n=303), patients who had SRSF2 mutations (n=33) appeared to have a marginally shorter OS (13.0 months vs 24.0 months, \( P=0.064 \)) (Figure 3b). Among the SF-mutated patients, though the secondary AML (sAML) patients showed a decreased trend of survival compared with de novo patients (median OS: 13.0 months vs 22.0 months, \( P=0.227 \)), no statistical significance was found (Figure 3c). Moreover, as SRSF2 mutations have been reported to be frequently associated with IDH2 mutation in SF-mutated AML patients [22], we further explored the OS in patients with the mutations of SRSF2\textsuperscript{mut}IDH2\textsuperscript{wt} (n=14), SRSF2\textsuperscript{mut}IDH2\textsuperscript{R140Q} (n=20) and SRSF2\textsuperscript{wt}IDH2\textsuperscript{R140Q} (n=30). The results showed that there was significant difference of OS among the above three groups, with much shorter OS in SRSF2\textsuperscript{mut}IDH2\textsuperscript{wt} patients (10 months [95% CI: 0.0-20.8] vs 22 months [95% CI: 5.3-38.7] and 31 months [95% CI: 15.3-32.7] \( P=0.027 \)) (Figure 3d).
Furthermore, we applied the univariate Cox regression analysis to explore the clinical relevance for SF-mutated AML patients. The results showed that the index of male ($P=0.036$), ASXL1 ($P=0.035$), Kit mutation ($P=0.048$) or ZRMR2 mutation ($P=0.005$) was significantly correlated with the OS of AML patients. The other indexes, including age, WBC count, bone marrow blast, complex karyotype and other gene mutations, were not statistically associated with OS. Next, we included the above significant indexes for multivariate analysis. The results showed that the index of male (HR: 2.9, 95% CI:1.0-8.4, $P=0.047$), Kit mutation (HR: 20.9, 95% CI:2.1-207.0, $P=0.009$) or ZRMR2 mutation (HR: 4.8, 95% CI:1.0-22.0, $P=0.044$) was considered as the independent risk factor for OS (Table S1a, see Supplemental Information).

**Characteristics of patients with SRSF2\textsuperscript{mut}**

As SRSF2 mutations were the most common mutant type in our AML cohort, we further analyzed its clinical characteristics. We found that SRSF2\textsuperscript{mut} patients were much older (median 64 vs 61 years, $P=0.015$) and had higher proportion of peripheral blasts and abnormal cell proportion by FCM as compared to the other SF-mutated patients (47.5% vs 14.0%, $P=0.028$; 48.0% vs 23.3%, $P=0.014$, respectively) (Table S1b, see Supplemental Information). We further analyzed the subtypes of SRSF2 mutations, and found that the peripheral blasts and abnormal cell proportion by FCM in patients with SRSF2\textsuperscript{P95H} mutation were higher than those of the other mutation sites in SRSF2 (77% vs 32%, $P=0.031$; 78.6% vs 22.9%, $P=0.026$, respectively) (Table 2). These results indicate the important role of SRSF2\textsuperscript{P95H} mutation in AML.

**Table 2.** Characteristics of the cohort of patients with SRSF2P95H and other mutation AML
Demographics

<table>
<thead>
<tr>
<th>Demographics</th>
<th>SRSF2&lt;sup&gt;P95H&lt;/sup&gt; (n=16)</th>
<th>Other mutations (n=17)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, n (%</td>
<td>11 (68.8)</td>
<td>13 (76.5)</td>
<td>0.708</td>
</tr>
<tr>
<td>Age, median (range), y</td>
<td>65 (55-87)</td>
<td>64 (51-87)</td>
<td>0.127</td>
</tr>
<tr>
<td>Median WBC (range), × 10&lt;sup&gt;9&lt;/sup&gt;/L</td>
<td>4.12 (0.53-300.79)</td>
<td>17.67 (0.58-287.95)</td>
<td>0.051</td>
</tr>
<tr>
<td>Median HB (range), × g/L</td>
<td>66.5 (6.7-100.0)</td>
<td>67.0 (45.0-113.0)</td>
<td>0.843</td>
</tr>
<tr>
<td>Median PLT (range), × 10&lt;sup&gt;9&lt;/sup&gt;/L</td>
<td>29 (11-543)</td>
<td>40 (9-137)</td>
<td>0.460</td>
</tr>
<tr>
<td>FAB classification</td>
<td></td>
<td></td>
<td>0.723</td>
</tr>
<tr>
<td>n, M0/M1/M2/M4/M5/M6/unknown</td>
<td>0/1/3/10/0/2</td>
<td>1/1/0/1/12/0/2</td>
<td></td>
</tr>
<tr>
<td>Median peripheral blasts % (range)</td>
<td>77.0 (0.0-98.0)</td>
<td>32.0 (0.0-98.0)</td>
<td>0.031</td>
</tr>
<tr>
<td>Median bone marrow blasts % (range)</td>
<td>46.5 (15.0-98.0)</td>
<td>39.5 (0.0-95.0)</td>
<td>0.417</td>
</tr>
<tr>
<td>Median abnormal cells by FMC % (range)</td>
<td>78.63 (10.0-94.0)</td>
<td>22.89 (0.08-95.0)</td>
<td>0.026</td>
</tr>
<tr>
<td>MRD%, median (range)</td>
<td>0.3 (0.0-94.4)</td>
<td>2.8 (0.0-29.2)</td>
<td>0.347</td>
</tr>
<tr>
<td>CR1 rate (%)</td>
<td>41.7 (5/12)</td>
<td>47.8 (6/11)</td>
<td>0.537</td>
</tr>
</tbody>
</table>

**SRSF2<sup>P95H</sup> promotes the proliferation of AML cells**

To further investigate the biological function of SRSF2<sup>P95H</sup> on AML cells, we first constructed the vector of SRSF2<sup>P95H</sup> or SRSF2<sup>wt</sup> and transfected them into AML cells. Then we detected their effects on the proliferation of AML cells by using CCK8 assay after 24h, 48h, 72h and 96h. Our results showed that SRSF2<sup>P95H</sup> significantly promoted the proliferation of Molm-13 cells compared to SRSF2<sup>wt</sup> after 72h and 96h (P < 0.05)(Figure 4).

**Discussion**

Mutations in spliceosome components are the most common acquired lesions in MDS, and mutations in the spliceosome genes, U2AF1 and SRSF2, could predict for poor outcomes MDS [15–18]. However, less is known about SF mutations in AML. In this study, we recruited 368 newly diagnosed non-M3 AML patients and found that SF mutations were associated with distinct clinic-biological features and could be considered as a poor prognostic marker for AML patients.

Of the 368 AML patients included in this study, we found that 17.4% of them developed SF mutations, consistent with the result of one study in which spliceosome mutations were detected in 18% of AML patients [10]. As for SRSF2 mutations, our study showed that there were 51.6% SF mutated patients harboring SRSF2 mutation. Similarly, Pemmaraju et al reported that the SRSF2 mutation was in the
majority of 119 spliceosome mutated patients [19]. Among the SRSF2 mutations, nearly 50% of patients harbored P95H mutation, which was similar to Grimm's conclusion [20]. For other SF mutations, we found that the majority of the mutations occurred in hotspot areas, such as K666N and K700E in SF3B1, S34 and Q157 in U2AF1. Of interest, we found that the majority (83%) were S34 or Q157 mutations in U2AF1\textsubscript{mut} patients, which had been associated with inferior outcomes in myeloid malignancies [21]. Moreover, we demonstrated that there was a frequent overlap of mutations in IDH2 and SRSF2 by analyses of 64 patients with SF mutations, which was in accordance with previous report [22]. Therefore, we thought that the two mutations may have similar pathogenesis in AML.

For the clinical relevance, we found that SF mutations were associated with older age and the gender of male, which were in agreement with the previous observations [19, 23]. However, there was no significant difference between SF mutated and SF wild patients in WBC, HB, PLT, peripheral blasts, bone marrow blasts or abnormal cell proportion for FCM, which was similar with the results of Hsin-An Hou [18]. In addition, the VAF value of SRSF2 mutations had positive correlation with age and WBC count, which reminded us that the importance of quantitative mutation study. In a cohort of 39 AML patients with spliceosome mutations reported by Lachowiez [19], 54% patients had adverse risk according to ELN risk group, which was close to our study that 50% of adverse risk in SF patients and the proportion was significantly higher than that in SF wild patients. Further, we first reported that SRSF2 and U2AF1 mutations accounted for the majority of adverse risk or abnormal karyotype group in patients with SF mutations. In conclusion, the SF mutations may play a key role in adverse risk cytogenetics of AML patients.

The treatment outcome of SF mutations in AML was unsatisfactory. It was reported that SF mutated showed significantly lower CR rate compared to that in SF wild in AML patients [17, 19]. We also demonstrated that CR1 rate of SF mutated patients was lower as compared to that of SF wild patients. Considering the influence of age, we found that the CR1 rate of SF mutated patients in older and younger groups was significantly decreased compared with that in SF wild patients. Furthermore, we used abnormal cell proportion by FCM as MRD index, and found that the level of MRD after the first course of induction chemotherapy was higher in SF mutated patients compared to that in SF-wild patients.

As for the survival, it has been reported that SF mutations positively related to poor prognosis. In MDS patients, spliceosome mutations were shown to be associated with inferior survival [25, 27]. Furthermore, the event-free and overall survival of SF mutations were shorter than those of SF wild in AML [23]. Patients carrying mutations in U2AF1 had worse overall and relapse-free survival [24], and patients with SRSF2 mutations associated with poorer OS [28]. It was reported that mutation in ZRSR2 had negative impacts on OS in AML patients with wild-type TP53 [26]. In our study, the OS of patients with SF or SRSF2 mutations was also significantly shorter than that of SF wild patients. Interestingly, we also found the OS of patients with SRSF2\textsubscript{mut}IDH2\textsubscript{wt} was shorter than that of SRSF2\textsubscript{mut}IDH2\textsubscript{R140Q} or SRSF2\textsubscript{wt}IDH2\textsubscript{R140Q}. We speculated that IDH2\textsubscript{R140Q} may be associated with better prognosis, which could be demonstrated by previous results. IDH2\textsubscript{R172}, different from IDH2\textsubscript{R140Q}, was associated with DNA-methylation profiles and could lead to more severe aberrations in metabolic activity [10]. Moreover, it was
reported that IDH2\textsuperscript{R172} displayed better OS than the spliceosome mutation in AML [10]. Therefore, we suspect that patients with IDH2\textsuperscript{R140Q} mutation may show a better prognosis as compared to IDH2\textsuperscript{R172}.

Compare to patients with other mutations, we found that patients with SRSF2\textsuperscript{P95H} were more associated with poor prognosis of AML, such as the peripheral blasts and abnormal cell proportion by FCM. Moreover, the adverse effects of SRSF2\textsuperscript{P95H} mutation \textit{in vivo} experiments of MDS, MPN and AML have been reported in previous studies. A heterozygous SRSF2\textsuperscript{P95H} mutation in mice indicated significantly reduced numbers of hematopoietic stem and progenitor cells and differentiation defects [36]. Mice carrying SRSF2\textsuperscript{P95H} mutation rapidly succumbed to fatal bone marrow failure [37]. In the present study, we also verified that the SRSF2\textsuperscript{P95H} could promote the proliferation of leukemia cells. Therefore, SRSF2\textsuperscript{P95H} may impair normal hematopoiesis and contribute to the progression of AML.

In summary, our study indicated that SF mutated patients, particularly SRSF2 mutation, had distinct clinical and genetic features. Moreover, SF mutations were associated with poor outcome. It is remarkable that SRSF2\textsuperscript{P95H} should be considered a prognostic marker in newly diagnosed AML patients.

**Declarations**

**Ethics approval and consent**

All samples were collected after informed consent.

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**Authorship Contributions**

Professor Daoxin Ma designed and funded the research. Professor Chunyan Ji helped design the research. Wenbo Jia and Xiaodong Guo performed the research and wrote the manuscript. Yihong Wei, Jingting Liu and Can Can assisted with the research. Ruiqing Wang and Xinyu Yang analyzed the data.

**Conflicts of Interest Statement**

The authors declare no competing financial interests.

**Data availability**

The data that support the findings of this study are not openly available due to reasons of human data and are available from the corresponding author upon reasonable request.

**References**


Figures

Figure 1
Molecular landscape of the spliceosome mutated patients. **a** Oncoprint of molecular mutations in patients with spliceosome mutations associated with karyotype, prognosis, SF genes, response and sex. **b** Frequency of SF mutation in all patients. **c** Constituent ratio of four SF mutation. **d** Frequency and amino acid variation of the included spliceosome mutations.

![Oncoprint of molecular mutations in patients with spliceosome mutations](image)

**Figure 2**
The heatmap of co-occurring mutations and mutually exclusive mutations in SF-mutated patients. Each number represents the correlation coefficient between the intersecting genes.

Figure 3

Kaplan–Meier curves of overall survival of AML patients. a SF-mutated AML vs SF-wild AML patients. b SRSF2-mutated AML vs SF-wild AML patients. c SF-mutated AML patients stratified by s-AML vs de novo AML. d SRSF2\textsuperscript{mut}IDH2\textsuperscript{R140Q} (co-mutant) AML vs SRSF2\textsuperscript{mut}IDH2\textsuperscript{wt} AML (SRSF2 mutant only) vs SRSF2\textsuperscript{wt}IDH2\textsuperscript{R140Q} AML (IDH2 only)
SRSF2<sup>P95H</sup> promotes the proliferation of AML cells. CCK8 analysis of cell viability of Molm-13 with SRSF2<sup>P95H</sup> or SRSF2<sup>WT</sup> at 0, 24, 48, 72h, 96h is shown.

**Supplementary Files**

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