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ORIGINAL ARTICLE

The prognostic implication of *SRSF2* mutations in Chinese patients with acute myeloid leukemia

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Abstract Recently, somatic mutations in *SRSF2* gene have been discovered in a proportion of hematologic malignancies including acute myeloid leukemia (AML). This study was aimed to investigate *SRSF2* mutations in Chinese AML patients. High-resolution melting analysis (HRMA) was developed to screen *SRSF2* mutations in 249 cases with AML, and then direct DNA sequencing was used to verify the results of HRMA. In this study, 3.6 % (9/249) of Chinese AML patients were found with heterozygous *SRSF2* mutations. Patients with *SRSF2* mutations were older than those with wild-type *SRSF2* ($P = 0.014$). No differences in the sex, blood parameters, French–American–British classification (FAB) subtypes, and karyotypes were observed between AML patients with and without *SRSF2* mutations. Although the overall survival (OS) of *SRSF2*-mutated patients was inferior to those without mutations in both whole AML patients (median 4 vs. 11 months, respectively; $P = 0.006$) and cytogenetically normal patients (median 2 vs. 12 months, respectively; $P = 0.008$), multiple analysis disclosed that *SRSF2* mutation was not an independent prognostic factor in AML patients. These results suggest that *SRSF2* mutation occurs at a low frequency in aged AML patients and might not be associated with adverse prognosis in Chinese AML patients.

Keywords *SRSF2* mutation · High-resolution melting analysis · Acute myeloid leukemia · Prognosis

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Introduction

Acute myeloid leukemia (AML) is the most common type of hematologic neoplasms in adults, which is characterized by an abnormal expansion of hematopoietic stem/progenitor cells with limited differentiation and excessive proliferation. Conventional cytogenetics analysis finds that approximately 55 % of AML patients exhibit chromosome abnormalities, which can be utilized widely to evaluate the risk status of AML [1]. With the fast development of genetic technology, the discoveries of somatic gene mutations involved in tyrosine kinase signaling, transcriptional, and epigenetic regulation have contributed to the improvement in risk stratification and prognosis prediction of AML [2, 3]. For example, the presence of *c-KIT* mutations in patients with core-binding factor (*CBF*) in AML confers a higher risk of relapse [4, 5]. *FLT3-ITD* mutations are considered to confer a significantly poorer outcome in cytogenetically normal patients [6, 7]. Recently, a new category of genes involved in RNA splicing pathway has been found to be mutated in myeloid neoplasms, most frequently (44–85 %) in myelodysplastic syndrome (MDS), less frequent (26 %) in AML transformed from MDS, and rarely (6.6 %) in de novo AML [8].

SRSF2 (also known as SC35), a member of the splicing factors, belongs to the Ser/Arg-Rich (SR) protein

family that plays a crucial role in the recognition of the 3′ splice site during RNA splicing pathway [9]. Hotspot mutations in the position codon 95 of *SRSF2* gene have been found in patients with MDS, chronic monomyelocytic leukemia (CMML), primary myelofibrosis (PMF), and AML [8, 10–14]. *SRSF2* mutation appears to predict adverse prognosis in MDS and PMF [12–15], while it is not associated with the survival of CMML patients [10, 11]. To date, little has been known about the clinical relevance and prognostic implications of *SRSF2* mutations in de novo AML patients [8, 16]. In the current study, we analyzed the frequency of *SRSF2* mutations and evaluated its clinical relevance in 249 Chinese AML patients.

Table 1 Distribution of *SRSF2* mutations in AML patients

	SRSF2 mutation ($n = 9$)	Wild-type ($n = 240$)	*P* value*
Sex, male/female	7/2	138/102	0.227
Median age at diagnosis, years (range)	69 (21–86)	50 (18–93)	0.014
Median WBC at diagnosis, $\times 10^9$/l (range)	35.8 (2.2–92.8)	12.65 (0.3–528.0)	0.334
Median hemoglobin at diagnosis, g/l (range)	83 (48–133)	74 (32–147)	0.602
Median platelets at diagnosis, $\times 10^9$/l (range)	39 (11–447)	40 (3–399)	0.565
FAB, no.			0.588
M1	0	28	
M2	5	99	
M3	0	35	
M4	2	45	
M5	2	22	
M6	0	11	
WHO			0.205
AML with t(8;21)	0	29	
APL with t(15;17)	0	35	
AML with 11q23	1	3	
AML without maturation	0	22	
AML with maturation	5	74	
Acute myelomonocytic leukemia	2	45	
Acute monoblastic and monocytic leukemia	1	21	
Acute erythroid leukemia	0	11	
Karyotype classification			0.698
Favorable	0	58	
Intermediate	8	138	
Poor	1	28	
No data	0	16	
Gene mutations			
C-KIT (+/−)	0/9	14/226	0.457
CEBPa(*dmCEBPA*/*smCEBPA*/*wtCEBPA*)	0/0/9	26/10/204	0.227
NPM1 (+/−)	1/8	22/218	0.844
FLT3-ITD (+/−)	0/9	8/232	0.578
RAS (+/−)	1/8	19/221	0.730
IDH1/IDH2 (+/−)	1/8	18/222	0.689
DNMT3A (+/-)	0/9	12/228	0.493
SF3B1 (+/−)	0/9	2/238	0.784
U2AF1 (+/−)	0/9	6/234	0.632

WBC white blood cell count, *FAB* French–American–British classification, *dmCEBPA* double mutated *CEBPA*, *smCEBPA* single mutated *CEBPA*, *wtCEBPA* wild-type *CEBPA*

*Results of statistical analysis between patients with mutated *SRSF2* and wild-type *SRSF2*

Materials and methods

Patients and DNA extraction

Two hundred forty-nine patients with de novo AML were diagnosed according to the French–American–British (FAB) Cooperative Group Criteria and the World Health Organization (WHO) classification [17, 18]. Bone marrow samples were collected at the time of initial diagnosis after the informed consent was written. The bone marrow mononuclear cells (BMNCs) were isolated by density-gradient centrifugation using Ficoll, and subsequent genomic DNA extraction from BMNCs was performed according to the manufacturer's instructions. This study was approved by the Ethics Committee Board of Affiliated People's Hospital of Jiangsu University.

Primer design and PCR conditions

Primers for PCR were designed with LightScanner primer design software v1.0 (Idaho Technology, Salt Lake City, Utah) and as follows: 5′-TGCAAATGGCGCGCTAC-3′ (Forward); 5′-GGCGGCTGTGGTGTGAG-3′ (Reverse). PCR was carried out in 25-μl volume in the presence of 1× PCR buffer (Invitrogen, Merelbeke, Belgium), 0.2 mmol/l of each dNTP, 0.5 μmol/l of both forward and reverse primers, 0.8 μmol/l of internal oligonucleotide calibrators [19], 1 × LCgreen Plus (Idaho, Salt Lake City, Utah, USA), 0.75 U hot start DNA polymerase (Takara, Tokyo, Japan), and 50 ng genomic DNA. PCR reactions were performed on a 7300 Thermo cycler (Applied Biosystems, Foster City, CA, USA). The PCR conditions were as follows: 98 °C for 10 min, followed by 40 cycles of 98 °C for 10 s, 63 °C for 30 s, and 72 °C for 30 s, followed by a final 7 min extension step at 72 °C.

High resolution melting analysis

PCR products were transferred to the LightScanner (Idaho Technology Inc. Salt Lake City, Utah, USA) for high resolution melting analysis (HRMA). Plates were heated from 55 to 95 °C with a ramp rate of 0.10 °C/s. The melting curve analysis was carried out by the LightScanner software package with CALL-IT® software (Idaho Technology Inc., Salt Lake City, Utah, USA).

Other nine genes recurrent in AML, including *C-KIT*, *CEBPa*, *NPM1*, *FLT3-ITD*, *RAS*, *IDH1/IDH2*, *DNMT3A*, *SF3B1*, and *U2AF1* mutations, were detected as reported previously [20–24].

DNA sequencing

To verify the results of HRMA, a separate PCR was carried out to generate a larger product (212 bp). Primers used as follows: 5′-TTCGCCTTCGTTCGCTTTCA-3′ (forward) and 5′-CCCCTCAGCCCCGTTTACC-3′ (reverse). PCR conditions were similar with that for HRMA except for the annealing temperature at 60 °C. PCR products were directly sequenced on both strands using an ABI 3730 automatic sequencer.

Statistics

Statistical analysis was performed using the SPSS 17.0 software package (SPSS, Chicago, IL, USA). The difference of discrete variables was compared by Pearson chi-square analysis or Fisher exact test. The difference of continuous variables was compared by Mann-Whitney's *U* test. Survival was analyzed according to the Kaplan–Meier method. The individual impacts of prognostic factors on OS were estimated using Cox

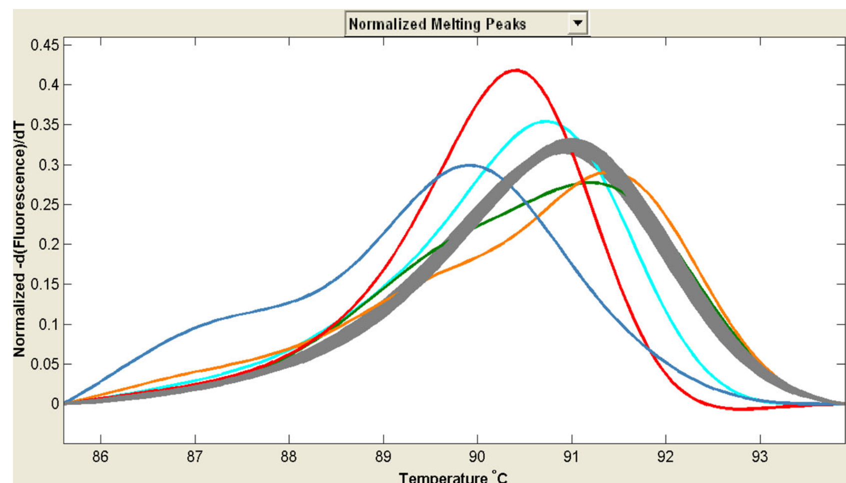


Fig. 1 Representative results of the HRMA for detecting *SRSF2*-P95 mutations in AML. *Gray lines* represented wild-type *SRSF2*; *pale blue* and *red lines* were validated as P95H mutations by Sanger sequencing; *green* and *blue lines* were validated as P95L mutations; *orange line* was validated as P95R mutation

proportional hazards regression. For all analyses, the *P* values were two-tailed, and less than 0.05 was considered statistically significant.

Results

SRSF2 mutations in AML patients

HRMA was performed to screen mutations in *SRSF2*. About 3.6 % (9/249) of cases were identified with heterozygous *SRSF2* mutations in 249 AML patients (Table 1), which showed complex changes of melting curve shapes, compared to smooth and symmetric melting curves in other 240 wild-type *SRSF2* patients (Fig. 1). The identical judgment was demonstrated by two blinded investigators. All HRMA results were further verified by direct sequencing which disclosed one heterozygous P95R (c.284C>G), two heterozygous P95L (c.284C>T), and six heterozygous P95H (c.284C>A) (Fig. 2). The clinical characteristics of *SRSF2*-mutated patients were shown in Table 2.

There was no difference in sex, blood parameters, WHO subtypes, and karyotype classification between cases with and

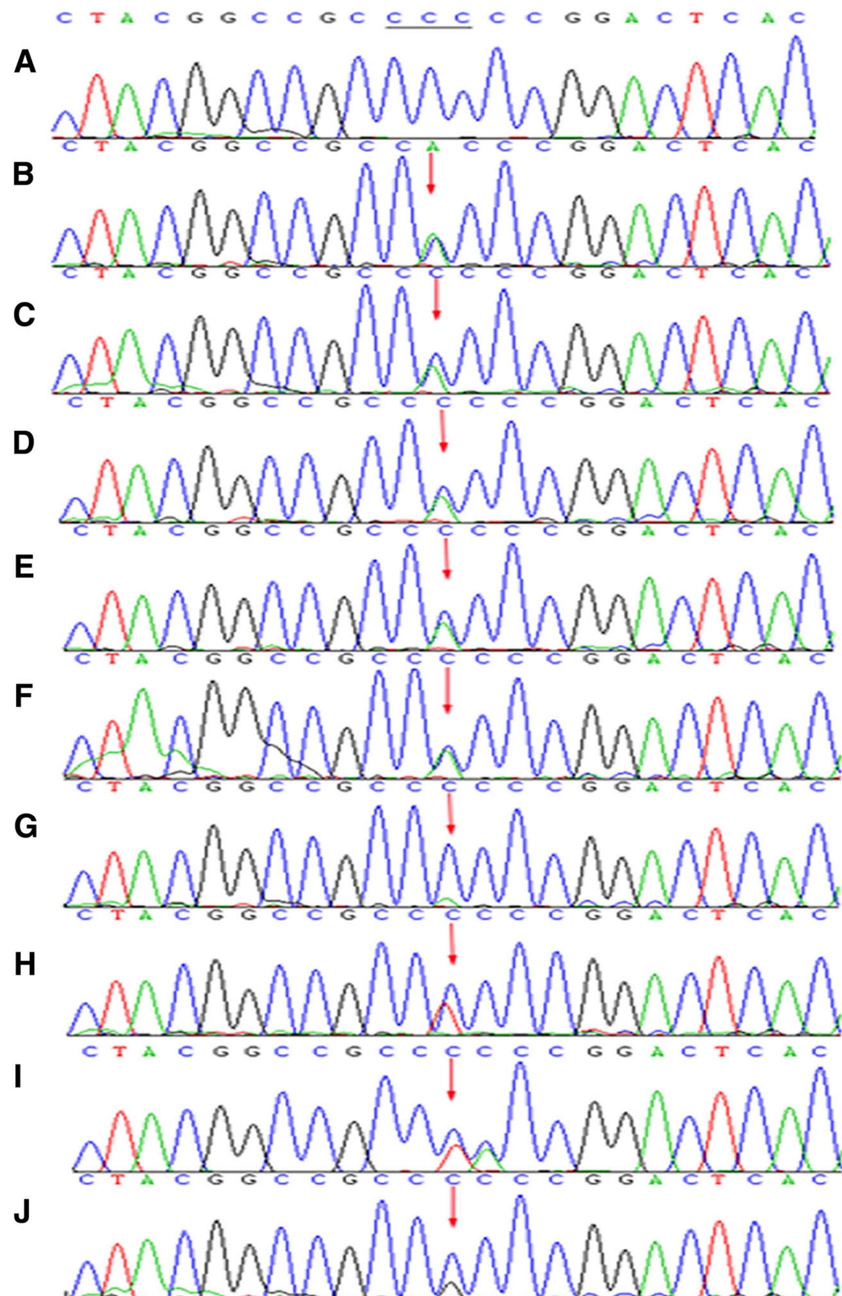


Fig. 2 Sequence chromatogram of *SRSF2*-P95 mutation. **a** wild-type *SRSF2*; **b**–**g** P95H mutation (c.284C>A); **h**–**i** P95L mutation (c.284C>T); **j** P95R mutation (c.284C>G). The *straight line* denoted the ninety-fifth codon of *SRSF2* gene. The mutated nucleotide was indicated by a *red arrow*

without mutations ($P>0.05$, Table 1). However, the patients with *SRSF2* mutations were older than those with wild-type *SRSF2* ($P=0.014$). There was also no significance in *SRSF2* mutation between cytogenetically normal (CN) AML (6/114, 5.20 %) and cytogenetically abnormal AML (3/119, 2.52 %) ($P=0.279$).

To investigate the prognostic impact of *SRSF2* mutation on AML, 173 cases with follow-up data were considered for survival analysis. Although the patients with *SRSF2* mutation had the lower rate of complete remission (CR) after induction chemotherapy than those without *SRSF2* mutation (28.57 vs. 50.88 %), the difference was not statistically significant ($P=0.249$). The median overall survival (OS) of patients with *SRSF2* mutations was 4 months (95 % confidence interval = 1.6–6.3 months), obviously shorter than those without *SRSF2* mutations (median = 11 months, 95 % confidence interval = 7.4–14.6 months) ($P=0.006$, Fig. 3a). Within patients with CN-AML, *SRSF2*-mutated cases also had a shorter OS than those without *SRSF2* mutations (median = 2 vs. 12 months, $P=0.008$, Fig. 3b). However, further multivariate analysis including *SRSF2* mutation and other six prognostic factors selected by univariate analysis ($P<0.1$) showed that significant impact of *SRSF2* mutation on OS was lost ($P=0.119$, Table 3).

Association of *SRSF2* mutations with other mutations

The mutational data of other nine genes recurrent in AML were available (*C-KIT*, *CEBPa*, *IDH1/IDH2*, *DNMT3A*, *NPM1*, *FLT3-ITD*, *RAS*, *SF3B1*, and *U2AF1*) in this cohort of AML. Overall, 44.2 % (110/249) of cases carried at least one gene mutation. Among the nine patients with *SRSF2* mutations, three patients simultaneously had the *IDH2* mutation, *NRAS* mutation, and *NPM1* mutation, respectively. No association was observed between *SRSF2* mutations and other gene mutations (Table 1). It was noted that the most commonly mutated genes in RNA splicing (*SF3B1*, *U2AF1*, and *SRSF2*) were occurred in a mutually exclusive manner.

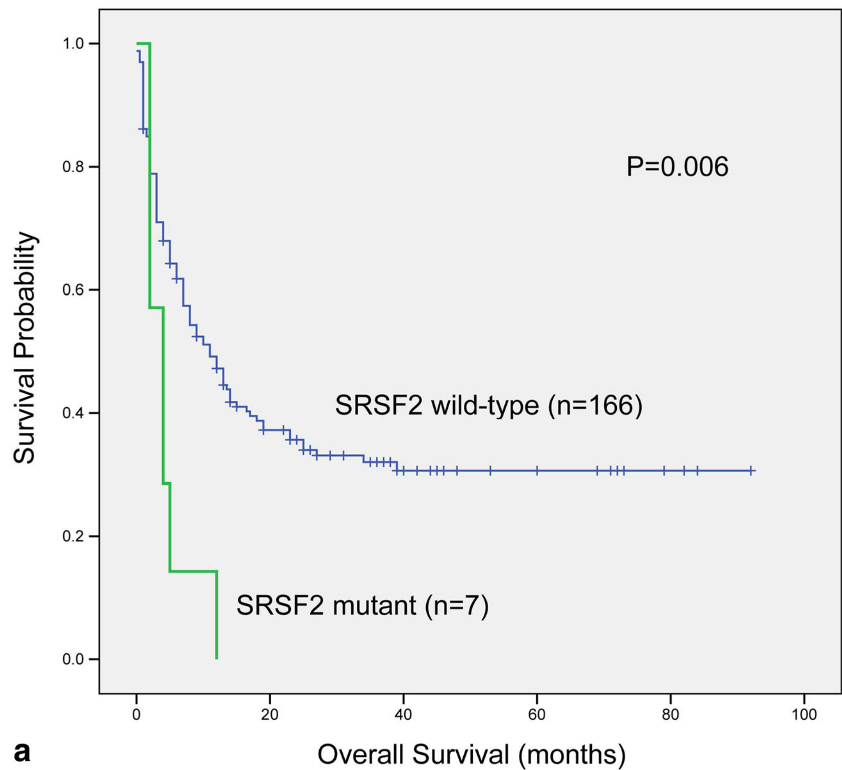
Discussion

Recurrent somatic mutations involved in RNA splicing machinery have been firstly reported in myelodysplasia by Yoshida et al. [8], suggesting that genetic alternations of the critical splicing components (mainly including *SF3B1*, *U2AF1*, and *SRSF2* genes) could contribute to the pathogenesis of disease. Later, increasing studies investigated these mutations in MDS and other hematologic diseases. In the present study, we identified a very low frequency of *SRSF2* mutations (3.6 %) in Chinese de novo AML patients. The low frequency was not caused by the methodology as the maximal sensitivity of HRMA could reach 10 % in a background of wild-type DNA, which was higher than those of direct DNA sequencing (25 %) and all HRMA results were further confirmed by direct DNA sequencing [25]. Besides, our result was in accordance with another two papers reported (0.7 and 5.6 %, respectively) [8, 16], implicating that *SRSF2* mutation is a rare event in AML patients.

All *SRSF2* mutations were heterozygous. In the previous studies, we have investigated the mutations in another two critical splicing factors *SF3B1* and *U2AF1* genes (1.1 and 2.5 %, respectively) [20, 21]. In total, 6.8 % (17/249) of AML patients harbored the three splicing genes mutations and we observed the same results in other studies that the three splicing factors mutated in mutually exclusive manner [8, 11, 14], suggesting that they might not affect RNA splicing and progression of disease cooperatively.

Table 2 The clinical and hematopoietic parameters of nine patients with *SRSF2* mutations

ID	Sex/Age (years)	Diagnosis	WBC (*109/l)	Hemoglobin (g/l)	Platelet (*109/l)	Karyotype	Survival time (months)	Mutation			
								SRSF2	*NRAS*	*IDH2*	*NPM1*
1	M/58	M4	37.9	93	16	N	No data	P95H			
2	M/64	M5	35.8	55	38	N	No data	P95H			+
3	M/21	M5	3.2	133	447	47,XY,del(9)(q11q22),del(11)(q23),+Mar[10]	12	P95H			
4	F/71	M4	47.0	95	125	N	4	P95H			
5	M/72	M2	62.2	67	264	+11[4]/46,XY[21]	5	P95L			
6	M/86	M2	27.7	107	39	N	2	P95R		+	
7	F/65	M2	16.8	48	23	+8	2	P95H	+		
8	M/76	M2	2.2	63	11	i(17q)	4	P95H			
9	M/69	M2	92.8	63	50	N	2	P95L			



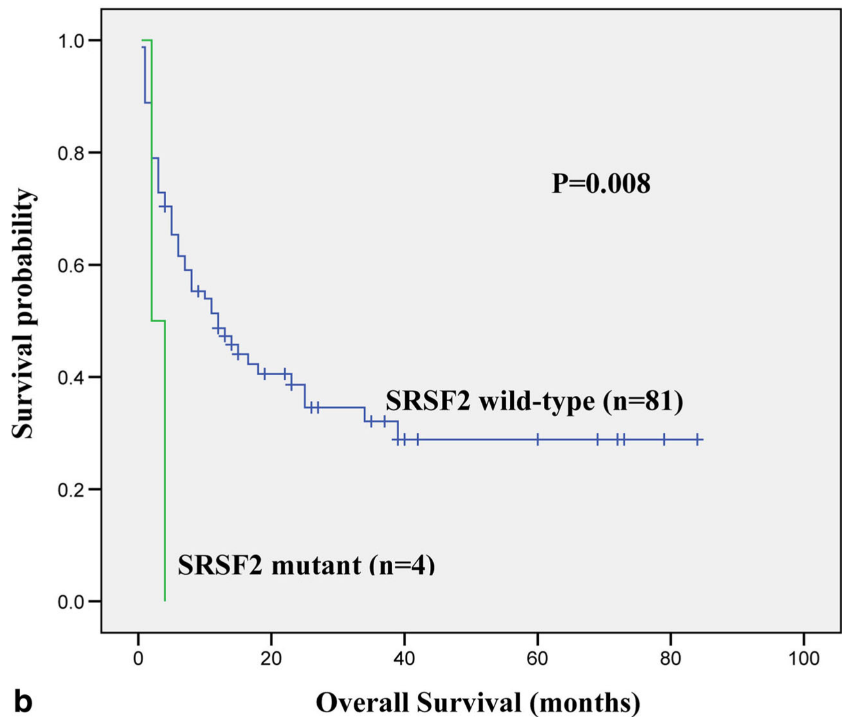


Fig. 3 Overall survival of AML patients according to Kaplan–Meier analysis. **a** Whole AML patients; **b** CN-AML

The presence of *SRSF2* mutations was significantly correlated with older age in our AML cohorts, which was in accordance with the observations in CMML, PMF, and MDS [10, 13, 15]. However, no correlations in sex, blood parameters, FAB subtypes, and karyotype classification were noted between cases with and without mutations. The prognostic impact of *SRSF2* mutations in MDS remains obscure: Makishima et al. reported that *SRSF2* mutations had worse

Table 3 Univariate and multivariate analyses for OS in MDS patients

	Univariate analysis			Multivariate analysis		
	HR*	95 % CI	*P* value	HR*	95 % CI	*P* value
Sex (male/female)	1.392	0.949–2.042	0.091	1.164	0.770–1.760	0.472
Age (≥60 vs. <60 years)	2.955	2.024–4.313	<0.001	1.082	1.299–2.140	<0.001
WBC ($\times 10^9$/l) (<30 vs. >30)	1.981	1.355–2.894	<0.001	1.687	1.152–2.470	0.007
Platelet ($\times 10^9$/l) (<100 vs. >100)	1.619	1.021–2.566	0.041	1.234	0.755–2.017	0.401
Karyotype (favorable/intermediate/poor)	1.831	1.478–2.268	<0.001	1.667	1.299–2.140	<0.001
U2AF1 mutation	3.194	1.386–7.358	0.006	2.744	1.158–6.502	0.022
SRSF2 mutation	2.734	1.257–5.948	0.011	1.924	0.846–4.375	0.119

OS overall survival, *CI* confidential interval, *HR* hazard ratio

*HR > 1 indicates an increased risk of an event for the first category listed

survival in low-risk MDS but not in the entire cohort of MDS patients [14]. Thol et al. reported that *SRSF2* mutations were correlated with inferior survival and a higher rate of progression to AML [12]. Wu et al. proposed that *SRSF2* mutation was associated with shorter OS but not an independent prognostic factor in MDS or in low-risk MDS and not associated with disease progression [15]. Our previous study also confirmed the adverse impact of *SRSF2* mutation on survival in MDS [20]. In PMF, *SRSF2* mutations predicted inferior OS and leukemia-free survival (LFS) [13]. Although Herold et al. identified *SRSF2* mutations in 81 % of AML patients with isolated trisomy 13 (+13) [26], no case with +13 was found in our group. So far, no prognostic relevance has been found in AML. Our data revealed that the presence of *SRSF2* mutations was significantly associated with worse OS according to univariate analysis; however, multivariate analysis did not disclose the association of *SRSF2* mutation with prognosis. Clonal hematopoiesis with somatic mutations including *SRSF2* mutation has been identified as an increasingly common event in aged people and is associated with increased risks of hematologic cancer [27–29]. Furthermore, a recent study has revealed that *SRSF2* mutation contributes to myelodysplasia by altering SRSF2's RNA binding activity and thereby altering the exon recognition to drive abnormal splicing of key hematopoietic regulators [30]. These results suggest that *SRSF2* mutation is an early driver mutation involved in leukemogenesis but not a prognosis-associated event.

Previous studies have shown that in CMML *SRSF2* mutations are frequently co-occurred with other mutations, such as *TET2*, *IDH1/IDH2*, *RAS*, *RUNX1*, and *ASXL1* mutations whereas mutually exclusive with *EZH2* mutation. Besides, patients with *RUNX1* mutations overlapped with *SRSF2* mutations have a favorable survival [10, 13–15]. Among the nine patients with *SRSF2* mutations in our study, three patients simultaneously had *IDH2* mutation, *NRAS* mutation, and *NPM1* mutation, respectively. No significant association of *SRSF2* mutation with them due to the low frequency in AML.

In summary, *SRSF2* mutations are rarely mutated and associated with older age in Chinese AML patients. Furthermore, *SRSF2* mutation might not be an independent prognostic event in AML.

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Compliance with ethical standards

Conflicts of interest None

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