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REVIEW ARTICLE

Prognostic value of *U2AF1* mutant in patients with de novo myelodysplastic syndromes: a meta-analysis

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Abstract

U2 small nuclear RNA auxiliary factor 1 (*U2AF1*) mutant is the most common molecular biological abnormality in patients with myelodysplastic syndromes. Some studies have reported the prognostic impact of *U2AF1* mutant in patients with de novo MDS, with discrepant results, so we do a meta-analysis about the relevant literatures to further investigate their prognostic impact on patients with de novo MDS. We conducted a literature search on databases such as PubMed, Embase, and the Cochrane Library to obtain studies on the prognosis of *U2AF1* mutant in patients with de novo MDS published up to August 9, 2018. The primary endpoint was overall survival (OS), and the secondary endpoint was acute myeloid leukemia (AML) transformation. We extracted the hazard ratios (HRs) of OS and AML transformation and their 95% confidence intervals (CIs). Meta-analysis was performed by selecting a fixed-effect model or a random-effects model based on the heterogeneity between studies. A total of 14 cohort studies were included in the final meta-analysis, including 3322 patients with de no MDS, in which 390 patients were associated with *U2AF1* mutant. The results showed that *U2AF1* mutant had an adverse prognostic impact on OS (HR = 1.84, 95% CI: 1.45-2.33, $P < 0.00001$) and AML transformation (HR = 2.47, 95% CI: 1.50-4.06, $P = 0.0004$). U2AF1 mutant was associated with shorter OS in subgroup analyses of low- or intermediate-1-IPSS, $U2AF1^{S34}$ and $U2AF1^{Q157/R156}$. Out meta-analysis indicates that *U2AF1* mutants are independent, detrimental prognostic factors for OS and AML transformation in patients with de novo MDS, as well as associating with shorter OS in subgroups of low- or intermediate-1-IPSS, $U2AF1^{S34}$ and $U2AF1^{Q157/R156}$. Further prospective studies are needed in the future, and subgroup analysis of *U2AF1* subgroups is needed to obtain a more reliable basis for the impact of *U2AF1* mutant on the prognosis of de novo MDS.

Keywords Myelodysplastic syndromes · *U2AF1* mutant · Prognostic value · Meta-analysis

Introduction

Myelodysplastic syndrome (MDS) is a group of highly heterogeneous pre-malignant clonal disorders originating from hematopoietic stem/progenitor cells. It is characterized by ineffective hematopoiesis, peripheral blood cytopenia, hypercellular bone marrow with morphologically defined dysplasia of one or more lineages and increased risk of progression to acute myeloid leukemia (AML) [16]. Although the current treatment for MDS is progressing rapidly, its meticulous prognostic stratification and individualized treatment are particularly important. At present, the main prognostic scoring systems, such as International Prognostic Scoring System (IPSS), Revised IPSS (IPSS-R), and WHO Prognostic Scoring System (WPSS), are scored based on the clinical characteristics and karyotype of the patients. Although the above scoring systems are widely used in clinical work to evaluate prognosis and guide treatment, its prediction of prognosis still needs improvement. It is urgent to select more reliable new molecular markers to guide prognostic stratification.

With the ubiquitous application of whole genome and exon sequencing in hematological tumors, a variety of somatic gene mutant have been found in patients with MDS, including RNA splicing factor genes (*SF3B1*, *U2AF1*, *ZRSR2* and *SRSF2*), histone modification genes (*EZH2* And *ASXL1*), DNA methylation (*TET2*, *DNMT3A* and *IDH1/2*), transcriptional regulatory genes (*RUNX1*), DNA repair genes (*TP53*), signal transduction genes

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(*CBL*, *NRAS*, and *KRAS*), and cohesin complexes (*STAG2*) [8]. The RNA splicing factors are the most frequent somatic gene mutant, with 50% mutant rate [19]. RNA splicing factors are involved in the key process of eukaryotic protein synthesis, RNA splicing, which removes the non-coding sequences of introns from the primary transcript (pre-mRNA) and performs exon splicing to generate mature messenger RNA (mRNA)[3, 29]. The splicing factor mutants alter the splicing process of RNA, producing a variety of different mRNAs that ultimately result in protein changes [3]. The splicing factor mutants in MDS widely and specially affect the major components of the splicing complexes E/A, in a mutually exclusive manner; the common consequence is logically the impaired recognition of three splice sites that lead to the production of aberrantly spliced mRNA species, which alter the hematopoiesis ultimately [35].

U2AF1, U2 small nuclear RNA auxiliary factor 1, also known as U2AF35, is located at 21q22.3 and has a molecular weight of approximately 35 KD [23, 32]. It encodes an auxiliary factor for the U2 pre-mRNA splicing complex, which recognizes and binds to the AG dinucleotide at the 3′ end of the intron and activates the splice complex [18]. *U2AF1* mutant leads to a change in the RNA splicing process, and jump splicing occurs [4, 9, 12, 18, 21]. Eleven distinct mutants have been reported in *U2AF1*, including nine missense mutants (resulting in A26V, S34F/Y, R35L, R156H/Q, Q157P/R, or G213A substitutions) and two frame-shift mutants (affecting codons Q157 or E159). Among them, S34 and Q157 are the most common mutant residues [5, 9, 15, 21, 27, 30, 35].

The exact mechanism of *U2AF1* mutant in the development of MDS is still unclear. Recent studies have shown that *U2AF1* mutant may be associated with the prognosis of MDS and leukemia transformation, but it is controversial. Studies by Thol et al. [27] and Kang et al. [13] showed that *U2AF1* mutants were not associated with MDS overall survival (OS) and leukemia transformation. Wu et al. [33] reported that U2AF1 mutant can predict a shorter OS of MDS and a higher risk of leukemia transformation. Hence, we performed a meta-analysis on data from related published studies to further explore the combined prognostic impacts of *U2AF1* mutant on patients with de novo MDS.

Materials and methods

Literature search

We conducted a literature search of several databases, including PubMed, Excerpta Medica database (Embase), and the Cochrane Library, for potentially relevant studies published up to August 8, 2018. No language limitations were added to the search strategy. The search formula for each database is as follows:

PubMed #1. "Myelodysplastic Syndromes"[Mesh]
#2. (MDS) OR (Myelodysplastic Syndrome) OR (Syndrome, Myelodysplastic) OR (Syndromes, Myelodysplastic) OR (Dysmyelopoietic Syndromes) OR (Dysmyelopoietic Syndrome) OR (Syndrome, Dysmyelopoietic) OR (Syndromes, Dysmyelopoietic) OR (Hematopoietic Myelodysplasia) OR (Hematopoetic Myelodysplasias) OR (Myelodysplasia, Hematopoietic) OR (Myelodysplasias, Hematopoietic)
#3. #1 OR # 2
#4. (U2AF1) OR (U2AF35) OR (U2 small nuclear RNA auxiliary factor 1)
#5. #3 and #4

Embase #1. 'myelodysplastic syndrome'/exp
#2. 'myelodysplastic syndrome'
#3. 'syndrome, myelodysplastic'
#4. 'syndromes, myelodysplastic'
#5. 'dysmyelopoietic syndromes'
#6. 'dysmyelopoietic syndrome'
#7. 'mds'
#8. #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7
#9. 'u2af1 gene'/exp
#10. 'u2af1'
#11. 'u2 small nuclear rna auxiliary factor 1'
#12. 'u2af35'
#13. #9 OR #10 OR #11 OR #12
#14. #8 AND #13

The Cochrane Library #1. Mesh descriptor: [Myelodysplastic Syndromes]
#2. "myelodysplastic syndrome"
#3. "MDS"
#4. #1 OR #2 OR #3
#5. "u2af1"
#6. "u2 small nuclear rna auxiliary factor 1"
#7. "u2af35"
#8. #5 OR #6 OR #7
#9. #4 AND #8

Inclusion and exclusion

Only papers that met all the following criteria were included: (1) The study focused on the prognostic impact of *U2AF1* mutant in de novo MDS patients. (2) The study provided sufficient survival data for patients with *U2AF1* mutant, for example OS or AML transformation. (3) Patients with *U2AF1* mutant in the study must be more than 5. (4) The study was published as a full article in English. Review articles, case reports, and laboratory studies were excluded. If the study

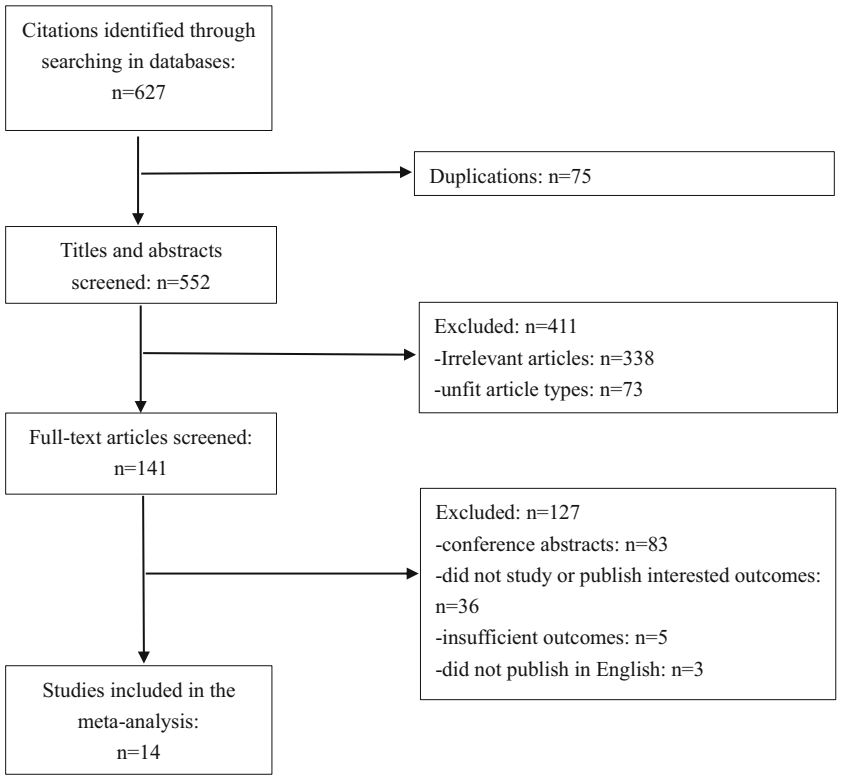

Fig. 1 Flow diagram of study selection

was reported in duplication, provided more detailed information or the highest quality study was included. Two reviewers screened the database and identified the eligible studies, independently. Disagreements were resolved by discussion.

Data extraction

Two reviewers independently extracted data from included studies: first author's name, year of publication, country of the population studied, number of patients, age of patients, MDS subtype, MDS classification criteria, karyotype, and IPSS classification. We selected OS as the primary endpoint and AML transformation as the secondary endpoint. OS was defined as the time from diagnosis of MDS to death due to any cause or censored or alive at last patient's follow-up. AML transformation is defined as beginning from the time the patient entered the trial to the time of AML diagnosis. We use hazard ratios (HRs) and their 95% confidence intervals (CIs) for OS and AML transformation from multivariate Cox proportional hazards models, univariate analysis hazards models, or Kaplan-Meier curves to evaluate the prognostic impact of U2AF1 mutant with MDS. For papers that only provided survival curves, it is preferred to send an e-mail to the authors to obtain data related to OS and AML transformation. If the original data is available, use IPSS 24.0 for statistical analysis. If the original data or related HR cannot be obtained, extract the data from the Kaplan-Meier curve using Engauge Digitizer 4.1 and use cal methods for incorporating 1745-625-8-S1 Worksheet to calculate HRs and their 95% CIs [20, 28]. Disagreements between reviewers regarding data abstraction were resolved through discussion.

Quality assessment

The Newcastle Ottawa quality assessment scale (NOS) was used to score the quality of each cohort study. This scale has nine items that are classified into the following three major categories: selection (whether the exposure group is representative, the selection method of the non-exposed group, the method of determining the exposure factor, whether there is an outcome indicator to be observed at the beginning of the study), comparability (the study controls the most important confounders, the study controls other confounding factors), and outcome (the adequacy of the evaluation of the results, the adequacy of follow-up, and the integrality of follow-up) [17]. The overall quality score was classified the following into three types: high quality (7–9 scores), intermediate quality (4–6 scores), and low quality (1–3 scores). Two reviewers independently assessed the quality of the included studies, and disagreements were resolved by discussion.

Statistical analysis

Review Manager version 5.3 software was used to calculate the combined survival impact of U2AF1 mutant. The prognostic effect of U2AF1 mutant on OS and AML

Table 1 Characteristics of studies in the meta-analysis

Study	Region	N/n	Age (year)	Sex (M/F)	MDS subtype					MDS criteria	IPSS	IPSS	U2AF1 (%)
					RA/RCUD/RARS	RAEB/RAEB-t	RCMD/RCMF-RS	CMML	Others		Low+Int-1/Int-2+high/Unknown	Low/intermediate/High/Unknown	
Tefferi 2018	America	357/52	74	250/107	NR	NR	NR	NR	NR	WHO	NR	59/30/11	15%
Gangat 2018	America	300/42	73 (28–96)	211/89	NR	NR	NR	NR	NR	WHO	NR	NR	14%
Li 2018	China	511/86	52 (14–84)	208/203	35	187	274	0	15	WHO	305/152/54	273/114/70/54	17%
Tefferi 2017	America	179/28	73 (28–96)	122/57	NR	NR	NR	NR	NR	WHO	NR	NR	16
Hwang 2016	Korea	58/9	69 (18–86)	37/21	16	20	13	0	9	WHO	36/22/0	NR	15.50%
Wu2016	China	304/26	57 (11–89)	162/142	29	123	145	0	7	WHO	212/90/2	NR	8.60%
Kang 2015	Korea	129/10	63.6 ± 12.5	71/58	19	46	56	0	3	WHO	NR	NR	7.70%
Hong 2015	Korea	58/10	67 (26–89)	46/12	20	29	0	9	0	FAB	34/24/0	28/16/14/0	17%
Wu 2013	China	478/36	66 (17/98)	318/160	208	216	0	0	0	FAB	236/176/0	248/81/83/0	7.50%
Thol 2012	Germany	193/14	65 (36–92)	119/74	58	53	30	0	52	WHO	96/51/43	109/20/23/41	7%
Qian 2012	China	96/6	58.5 (20–86)	58/38	11	43	39	0	3	WHO	69/23/4	68/17/8/3	6%
Bejar 2012	America	288/46	69 (15–90)	203/85	214	74	0	0	0	FAB	288/0/0	NR	16%
Damm 2012	France	221/12	71.5 (35–95)	129/92	92	100	39	0	21	WHO	165/51/5	155/38/19/9	5%
Graubert 2011	America	150/13	62 (20–87)	92/58	72	78	0	0	0	FAB	83/62/5	NR	8.70%

CMML chronic myelomonocytic leukemia, *F* Female, *FAB* French American British classification, *IPSS* International Prognostic Scoring System, *M* Male, *MDS* myelodysplastic syndromes, *n* number of patients with *U2AF1* mutant, *N* number of patients in total, *NR* not reported, *RA* refractory anemia, *RARS* RA with ringed sideroblasts, *RAEB* RA with excess blasts, *RAEB-t* RAEB in transformation, *RCMD* refractory cytopenia with multilineage dysplasia, *RCMD-RS* refractory cytopenia with multilineage dysplasia and ringed sideroblasts, *RCUD* refractory cytopenia with unilineage dysplasia, *WHO* World Health Organization

transformation was evaluated by calculation of the combined HRs and their 95% CIs with the generic inverse variance method in total population and subgroups [1]. The statistical heterogeneity of the studies was assessed by the chi-square based Q-test and quantified with the I^2 statistic (I^2 = 0–25%; low heterogeneity; I^2 = 25–50%; moderate heterogeneity; I^2 = 50–75%; large heterogeneity; and I^2 = 75–100%; extreme heterogeneity), with significance set at a *P* value of less than 0.10. The random-effect model was used if high heterogeneity ($I^2 > 50\%$ or $P < 0.10$) was observed; otherwise, a fixed-effect model (the Mantel-Haenszel method) was used for the meta-analysis [6].

Sensitivity analysis and meta-regression analysis of the total OS were carried out in Stata 15.1 software (Stata Corp, College Station, TX, USA) to further explore the impact of heterogeneity on the results. Begg's tests and Egger's tests were conducted to detect possible publication bias, and the funnel plot asymmetry or the two-tailed *P* value of less than 0.05 was considered to have publication bias [1, 6].

Results

Study identification and selection

As shown in Fig. 1, 627 records were obtained by a systematic literature search. After exclusion of 75 duplicates, 552 citations were further reviewed by reading the titles and abstracts, and 338 citations were then excluded for irrelevant subject and 73 were excluded for irrelevant article type. A total of 141 studies were left for full text review. Among them, 83 studies were excluded as conference abstracts, 36 were further excluded because they did not show interested outcome, five did not provide sufficient data, and three were not published in English. Ultimately, 14 studies were included in the meta-analysis [2, 5, 7, 9–11, 13, 14, 22, 25–27, 31] (Fig. 1).

Characteristics of included studies

The 14 included studies were all cohort studies, and their characteristics are listed in Table 1. These studies were published between 2011 and 2018, with seven studies from Asia, five from the Americas, and two from Europe. These studies included a total of 3322 patients with primary MDS, in which 390 patients harbored *U2AF1* mutant. For one study that included patients with both MDS and AML, only patients with MDS were studied in the meta-analysis. In the 14 included studies, the incidence of *U2AF1* mutant was 5–17%, and the overall incidence was approximately 11.7% (390/3322).

Table 2 Quality assessment of individual study

纳入研究	人群选择				可比性	结果评价			Nos. 总分
	①	②	③	④	⑤	⑥	⑦	⑧	
Tefferi 2018	1	1	1	1	2	1	1	1	9
Gangat 2018	1	1	1	1	2	1	1	1	9
Li 2018	1	1	1	1	1	1	1	1	8
Tefferi 2017	1	1	1	1	1	1	1	0	7
Hwang 2016	1	1	1	1	1	1	1	1	8
Wu 2016	1	1	1	1	2	1	1	1	9
Kang 2015	1	1	1	1	2	1	1	1	9
Hong 2015	1	1	1	1	1	1	1	1	8
Wu 2013	1	1	1	1	2	1	1	0	8
Thol 2012	1	1	1	1	1	1	1	0	7
Qian 2012	1	1	1	1	1	1	1	0	7
Bejar 2012	1	1	1	1	1	1	1	0	7
Graubert 2011	1	1	1	1	2	1	1	1	9
Damm 2012	1	1	1	1	1	1	1	1	8

① Representativeness of exposed cohort, ② selection of no exposed cohort, ③ascertainment of exposure, ④ outcome not present at start, ⑤comparability between exposed and no exposed cohort, ⑥ assessment of outcome, ⑦ follow-up length, ⑧ follow-up adequacy. In the NOS scale, the comparability score is 2 points, and the remaining small items are 1 point, with a total score of 9 points. In ⑤, if multivariate analysis is used to correct age-mixed factors, 2 points are obtained. If the age is not corrected and there is no significant difference in age, IPSS score, etc., 1 point is obtained; otherwise, 0 points are obtained. In ⑧, if the rate of loss to follow-up is < 20%, 1 point is scored. If the rate of loss to follow-up is not described or ≥ 20%, 0 point is obtained

Quality assessment of included studies

The median overall score of NOS results of the included studies was 8 (range 7-9), which indicated that the methodological quality was high (Table 2).

Clinical features of MDS patients with *U2AF1* mutant

In patients with de novo MDS, *U2AF1* mutants are more likely than genes such as *SF3B1*, *ASXL1*, *TET2*, and *TP53* to occur in younger patients [14, 33]. U2AF1 mutant is more likely to be isolated + 8, 20q- than unmutated *U2AF1* [14, 31]. In de novo MDS, the incidence of $U2AF1^{S34}$ mutant was significantly higher than that of $U2AF1^{Q157/R15\ 6}$[14, 22, 26, 33].

Analysis of outcome

Thirteen studies reported the prognostic effect of *U2AF1* mutant on OS in patients with de novo MDS, including 2926 de novo MDS, in which 327 had *U2AF1* mutant. Among the 13 studies, three studies only provided Kaplan-Meier curves (HRs extracted for OS as described above, which was regarded as universe HRs), and five studies reported

univariate HRs for OS, two studies reported multivariate HRs for OS, two studies reported both univariate and multivariate HRs for OS, and one study provided primary data which could be able to calculate univariate and multivariate HR for OS. First, the total OS is obtained by combining all univariate HRs and two only multivariate HRs for OS. Our results showed that *U2AF1* mutant was an independent hazardous factor for poor prognosis of OS in patients with de novo MDS (HR = 1.84, 95% CI: 1.45–2.33, $P < 0.00001$) with a high heterogeneity ($I^2 = 51\%$, $P = 0.02$) (Fig. 2a). Five studies have obtained multivariate HRs for OS, including 1119 primary MDS and 94 *U2AF1* mutants. The results were consistent with the total OS results, suggesting that the *U2AF1* mutants were associated with a shorter OS (HR = 1.59, 95% CI: 1.18-2.41, $P = 0.002$) with a low heterogeneity ($I^2 = 24\%$, $P = 0.26$) (Fig. 2b).

Four studies have obtained multivariate HRs for *U2AF1* mutant in AML transformation in patients with de novo MDS, including 981 patients with primary MDS, in which 67 patients had *U2AF1* mutant. The results showed that *U2AF1* mutants were a poor prognostic factor for AML transformation in patients with de novo MDS (HR = 2.47, 95% CI: 1.50–4.06, $P = 0.0004$) with no heterogeneity ($I^2 = 0\%$, $P = 0.47$) (Fig. 3).

A total of three studies that met the inclusion criteria provided the effects of *U2AF1* mutant on OS in patients with de novo MDS with low- or intermediate-1-IPSS risk. Meta-analysis results showed that the *U2AF1* mutants were associated with a shorter OS of patients with low- or intermediate-1-IPSS risk MDS (HR = 1.58, 95% CI: 1.14–2.17, $P = 0.006$) with a moderate heterogeneity (($I^2 = 47\%$, $P = 0.15$) (Fig. 4).

Several studies have reported the effects of different subtypes of *U2AF1*, such as $U2AF1^{S34}$ and $U2AF1^{Q157/R156}$, on OS in patients with de novo MDS. Among them, three studies reported the effect of $U2AF1^{S34}$ residues mutant on OS in patients with de novo MDS. The heterogeneity test results showed that there was between the studies, and a fixed-effect model was used for meta-analysis. The results showed that $U2AF1^{S34}$ subtype mutant had an adverse effect on the OS of patients with de novo MDS (HR = 1.58, 95% CI: 1.14–2.17, $P = 0.006$) with a moderate heterogeneity ($I^2 = 33\%$, $P = 0.22$) (Fig. 5a). Two studies involved the effect of $U2AF1^{Q157/R156}$ subtypes on OS in patients with de novo MDS. The results showed that $U2AF1^{Q157/R156}$ mutant had an adverse effect on OS of patients with de novo MDS (HR = 5.32, 95% CI: 1.06–26.78, $P = 0.04$) with an extreme heterogeneity ($I^2 = 91\%$, $P = 0.0008$) (Fig. 5b).

Subgroup analysis and meta-regression

Subgroup analysis and meta-regression were used to find the sources of heterogeneity in the analysis of the total OS and its impact on the results of the analysis. In the subgroup analysis of the region, publication year, sample size, and median age, the emerged HRs were similar between the subgroups, while the subgroup analysis of HRs sources was performed; the

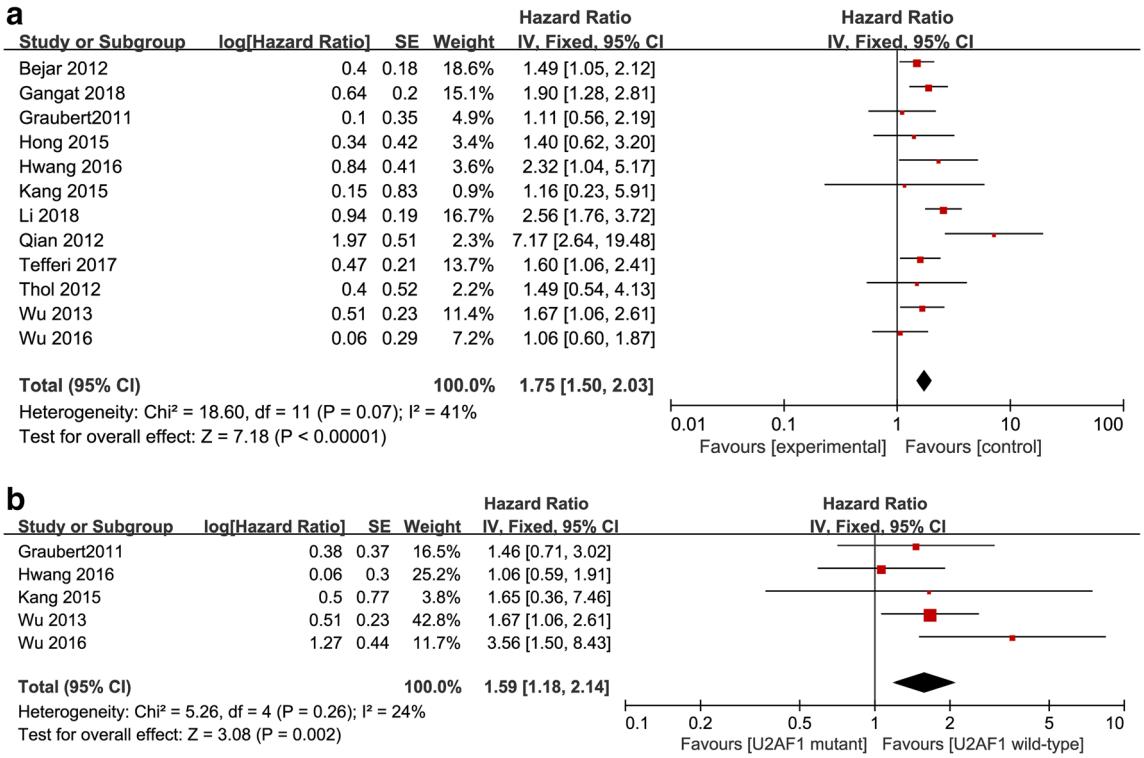

Fig. 2 Forest plot of pooled HRs and 95% CIs accessing the prognostic value of *U2AF1* mutant in patients with de novo MDS. **a** For total HRs of OS. **b** For multivariate HRs of OS

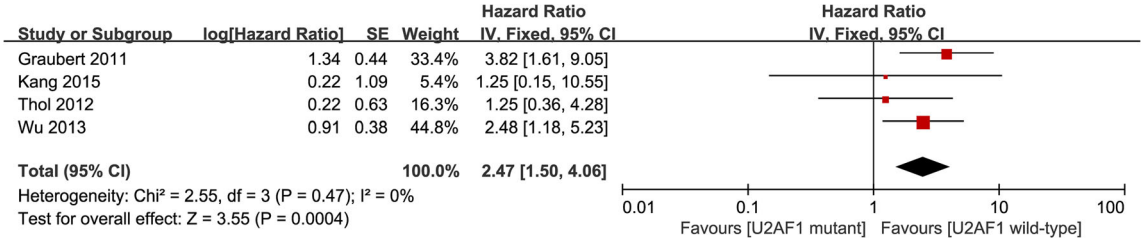

Fig. 3 Forest plot of pooled HRs and 95% CIs for AML transformation accessing the prognostic value of *U2AF1* mutant in patients with de novo MDS

pooled HR of the HR subgroup extracted from the survival curves was significantly higher than the subgroup of HR obtained from the original and original literature (Table 3). Similarly, meta-regression analysis was performed on each of the above subgroups individually. The results showed that the region, publication year, sample size, and median age had no effect on the combined HR, while HR sources had an effect on the combined HR results ($P = 0.001$). However, the results of the subgroup analysis of HR sources showed that the subgroups of extracting HR from the survival curves and obtaining HR from the original texts and the original literatures all had an adverse effect on the OS of the de novo MDS.

Sensitivity analysis and publication bias

We conducted a sensitivity analysis by excluding one study at a time to assess the effect of the study quality on the stability of this meta-analysis. Results did not show significant differences when any study was removed, which indicated that each single study did not influence the stability of the association between *U2AF1* mutant and OS in patients with de novo MDS (as shown in Fig. 6). The Begg's test (Fig. 7a) and the Egger's test (Fig. 7b) of *U2AF1* mutant in the total OS did not show evidence of published bias with *P* values of 0.502 and 0.585, respectively.

Discussion

In our meta-analysis, the results showed that patients with *U2AF1* mutant have shorter OS and more likely to convert to AML than unmutated patients with de novo MDS. While the HR of the total OS was combined in 13 studies, the heterogeneity between the studies was high (I^2 = 51%). The possible sources of heterogeneity are regions, ages of patients, the size of samples, year of publication, etc., as well as the differences in the way of HR extraction, including univariate analysis, multivariate analysis, and Kaplan-Meier curve. In order to find the source of heterogeneity and explore the impact of heterogeneity on the combined results, we first merged the five studies that can obtain multivariate HR for OS and found that the combined HRs for OS were consistent with the combined results of the 13 studies. Then, we divided the subgroups by region, publication year, sample size, median age, and HR source, and sub-groups were performed separately. The results of each subgroup were consistent with the total results. Therefore, we believe that the *U2AF1* mutants are independent prognostic factors in patients with de novo MDS. Similarly, in the subgroup analysis of low- or intermediate-1-IPSS risk, $U2AF1^{S34}$ and $U2AF1^{Q157/R156}$. Patients with *U2AF1* mutant are associated with shorter OS, indicating that *U2AF1* mutants are predictors of poor OS outcome in patients with low- or intermediate-1-IPSS risk and the major mutated residues of *U2AF1*.

Although the precise mechanisms of the contributions of *U2AF1* mutant to the pathogenesis of MDS remain largely unknown, a growing number of studies have explained the mechanisms of the adverse effects of *U2AF1* mutant on MDS patients. *U2AF1* mutant usually occur in funding clones, suggesting that they may play an important role in disease initiation [30]. Mutant *U2AF1* induces aberrant RNA splicing in HeLa and MDS-derived cell line (TF-1) cells, resulting in the appearance of un-spliced RNA and induction of nonsense-mediated mRNA decay (NMD) [35]. Compared to wild-type *U2AF1* cells,

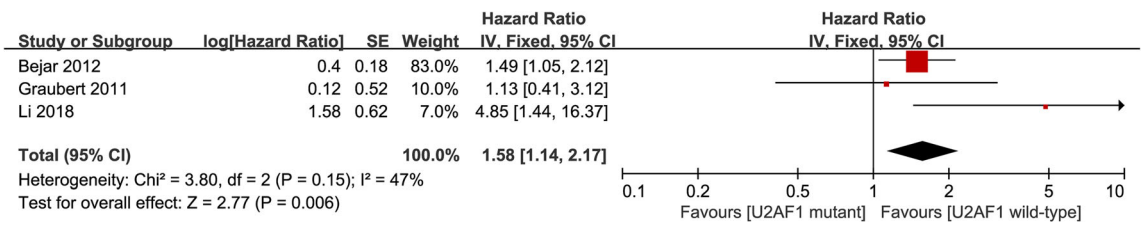

Fig. 4 Forest plot of pooled HRs and 95% CIs for AML transformation accessing the prognostic value of *U2AF1* mutant in patients with low- or intermediate-1-IPSS risk MDS

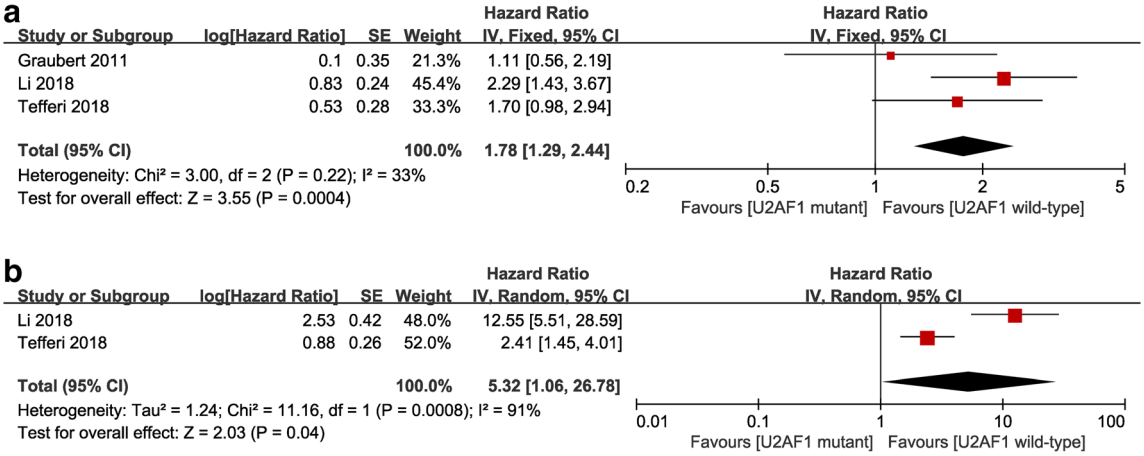

Fig. 5 Forest plots of pooled HRs and 95% CIs for OS accessing the prognostic value of **a** $U2AF1^{S34}$ and **b** $U2AF1^{Q157/R156}$ mutant in patients with de novo MDS

U2AF1 mutants inhibit the growth and proliferation of hematopoietic stem/progenitor cell growth and induce their apoptosis [35]. Overexpression of $U2AF1^{S34}$ mutant in mice induced by doxycycline leads to a decrease in peripheral blood leukocytes, increased apoptosis of mature cells in the bone marrow, and a common phenotype of MDS such as progenitor cell expansion, but no pathological hematopoiesis, and ultimately no development for AML/MDS [24]. Yip et al showed that the expression of $U2AF1^{S34F}$ in human hematopoietic progenitor cells erythroid differentiation and skewed granulomonocytic differentiation toward granulocytes [34]. This effect was achieved by $U2AF1^{S34F}$ impairing the expression of its downstream target gene H2AFY isoform 1.1 and STRAP. Furthermore, isoform modulation of H2AFY and STRAP rescues the erythroid differentiation defect in $U2AF1^{S34F}$ MDS cells, suggesting that splicing modulators could be used therapeutically [34]. In addition, studies have shown that *U2AF1* mutants are associated with aberrant splicing of genes involved in functionally important pathways, such as cell cycle progression and RNA processing. The alternative splicing patterns associated with *U2AF1* mutant were associated with specific sequence signals at the affected splice sites. Incorrect splicing patterns associated with the *U2AF1* mutant were observed in the exon and affected functionally related genomes, such as differences in mitosis genes for the stages (*CEP164*, *EHMT1*, *WAC*, and *ATR*) or RNA processing (*PTBP1*, *STRAP*, *PPWD1*, *PABPC4*, and *UPF3B*). Among them, CEP164 is one of the centrosome proteins involved in G2/M checkpoint

Table 3 Subgroup analyses for total OS according to the region, sample size, median age, survival analysis, and sequencing method

Variable	Number of studies	Heterogeneity		OS		Meta-regression Subgroup difference
		P	I^2(%)	HR (95%CI)	*P*	
Region						
Asian	7	0.02	59	1.96 (1.32–2.92)	0.0008	0.635
Non-Asian	6	0.11	44	1.72 (1.29–2.31)	0.0003	
Years of publish						
2014 and before	6	0.006	70	2.11 (1.3–3.41)	0.002	0.578
2015 and beyond	7	0.23	26	1.78 (1.4–2.27)	0.00001	
Sample sizes						
< 100	3	0.05	68	2.74 (1.13–6.64)	0.03	0.197
≥ 100	10	0.06	45	1.72 (1.37–2.17)	0.00001	
Median age (year)						
< 60	3	0.002	84	2.47 (1.05–5.84)	0.04	0.33
≥ 60	10	0.36	9	1.69 (1.4–2.04)	0.00001	
HR sources						
Original	10	0.84	0	1.55 (1.31–1.84)	0.00001	0.0001
Extracted from Kaplan-Meier curves	3	0.07	62	4.11 (2.06–8.2)	0.00001	

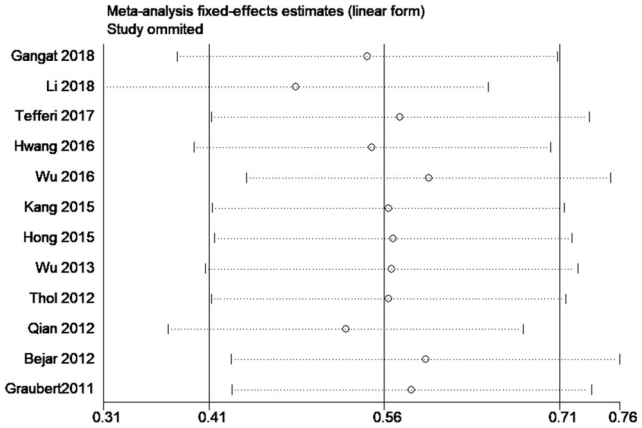

Fig. 6 Sensitivity analysis for individual studies on the summary HRs of total OS

control and nuclear division, while PTBP1 regulates alternative splicing events by interacting with pyrimidine-rich RNA sequences and may also inhibit the binding of U2 snRNP to certain pre-mRNAs [21]. Study on human primary CD34+ cells has shown that *U2AF1*^{S34F} affected the splicing of pre-mRNAs of multiple target genes, and uridines of e-3 nucleotides in the 3′ splice junctions were more frequently skipped, including both exon skipping events and alternative 3′ splice site usage. The effects may be due to the fact that U2AF1^{S34F} has a higher affinity for C than U, resulting in selective or altered binding to pre-mRNA at these junctions [18]. In the future, more reasonable experiments need to be designed to explore the effects of different mutant residues of *U2AF1* on its indirect function, hematopoiesis, and tumorigenesis mechanism. Clinical studies need to focus on residues to explore the relationship between different residues of *U2AF1* and clinical features and prognosis to more accurately guide risk stratification and prognosis assessment.

Our study is the first meta-analysis to assess the impact of *U2AF1* mutant on the prognosis of patients with de novo MDS. There are several limitations in the study, so the result should be cautiously viewed. The limitations of this study are listed as follows. First, although we use a comprehensive search strategy, and there is no obvious publication bias in Begg's test and Egger's test results, publication bias can still not be completely avoided. Second, we only include the full text published in English, so there may be a language bias. Third, in the total OS and U2AF1$^{Q157/R156}$ subgroup analysis, $I^2 > 50\%$, suggesting a high heterogeneity between studies, which may be due to different clinical characteristics of each study, or different data extraction methods. Fourth, in the case of total OS merger, because some studies cannot obtain univariate HRs or multivariate HRs, we combined HRs for OS with univariate HRs, multivariate HRs and HRs extracted from Kaplan-Meier curves (univariate analysis was superior to multivariate analysis, and multivariate analysis was superior to Kaplan-Meier analysis), during which bias may occur. Through subgroup analysis, the differences of HR sources have no disruptive effect on the result.

Conclusion

In conclusion, our results indicated that *U2AF1* mutant is an independent, adverse risk factor for OS and AML transformation in patients with de novo MDS. *U2AF1* mutants are also relevant with shorter OS in subgroups such as low- or intermediate-1-IPSS, *U2AF1*^{S34} and *U2AF1*$^{Q157/R156}$. The *U2AF1* mutants have not yet been included in the prognosis scoring system of patients with de novo MDS and are expected to be a new molecular marker for risk stratification and prognosis assessment in patients with de novo MDS based on the current studies. We recommended that patients with de novo MDS with *U2AF1* mutant should take more aggressive treatment strategy that may help improve the prognosis of these

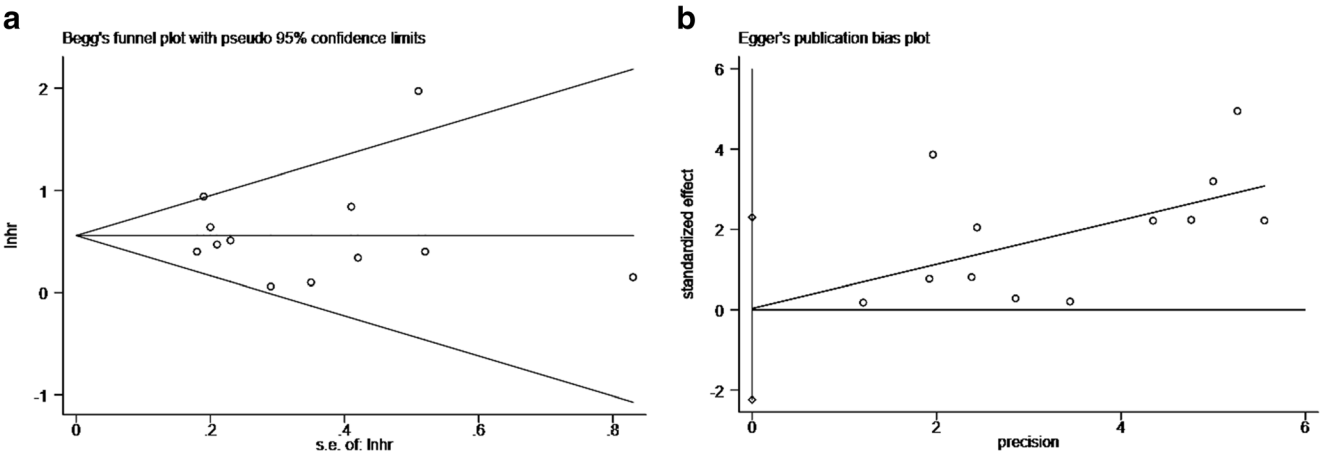

Fig. 7 **a** Begg's funnel plot. **b** Egger's funnel plot of total OS for publication bias analysis

patients. Further prospective studies are needed in the future, and subgroup analysis of *U2AF1* mutated residues is needed to obtain a more reliable basis for the impact of *U2AF1* mutant on the prognosis of de novo MDS.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of Interest.

Ethics statement This article does not contain any studies with human participants or animals performed by any of the author.

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