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Analysis of clinical and molecular features of MDS patients with complex karyotype in China

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ABSTRACT

We retrospectively analyzed 101 primary MDS patients with complex karyotype during January 2010 and April 2017.The median overall survival (OS) time was 13 (95% CI 9.98–16.02) months, and there was no significant difference in OS for different treatment. Chromosome 5/7 involvement was common (78.22%, 79/101) and associated with shorter OS (12 months vs. 28 months, $P < 0.01$) Monosomal karyotype (MK) is overlapped with CK in 79 patients, but was not statistically associated with shorter OS. While in 59 cases with genes sequenced, 57 (96.61%) patients were found to have at least one mutation of known significance, and *TP53* was the most frequent (74.58%, 44/59), the median OS of patients with *TP53* mutation was shorter than those without (10 vs. 27 months, $P < 0.01$). Multivariate analysis demonstrated that only *TP53* mutation was the strongest independent prognostic factor for OS. Moreover, high variant allele frequency (VAF) of *TP53* mutation (median VAF was 70.00%) was seen and associated with adverse survival (9 months vs. 13 months, $p = 0.04$). In conclusion, MDS patients with CK implied an unfavorable outcome regardless of any treatment, *TP53* mutation occurs at a high frequency and has a higher VAF, both were associated with worse survival.

1. Introduction

Myelodysplastic syndromes (MDS) consists of a group of clonal hematological stem cell disorders characterized by ineffective hematopoiesis, cytopenia, and a considerable risk of progression to acute myeloid leukemia (AML) [1,2]. The International Prognostic Scoring System (IPSS) and revised IPSS (IPSS-R) remain the most commonly used system for assessing the prognosis of primary untreated adult MDS, comprising 3 and 5 cytogenetic prognostic subgroups respectively [3,4]. Both scoring systems emphasize the important prognostic significance of cytogenetics. Among which, complex karyotype (CK) defined as more than or equal to 3 independent chromosomal abnormalities, was an independent poor prognostic factor, usually associated with a shorter median overall survival (OS), and propensity toward malignant transformation.

Owing to recent advances in whole genome sequencing, recurrent gene mutations which are associated with prognosis have been identified [5]. *TP53* mutations are found in 5–10% of MDS patients [6,7], especially enriched in patients with isolated del(5q), complex karyotype, or chromosome 17 abnormalities. Besides, *TP53* mutation has been identified to be associated with worse prognosis [8,9].

However, the clinical and molecular features of MDS patients with CK are not fully explored. Whether different WHO subtypes, treatment options, genetic mutations especially *TP 53* have any effect on survival is not fully understood. In this study, we performed a retrospective analysis of 101 MDS patients with CK in our center, and detected the gene mutation characteristics through next generation sequencing methods, and further compared the survival characteristics of this part of patients, thus trying to enrich the characteristics of MDS patients with CK.

2. Methods

2.1. Case selection and clinical information collection

This study was approved by the Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University. The study included all newly diagnosed MDS patients with complex karyotype according to the 2008 WHO classification during a period from January 2010 to April 2017. Cases with secondary MDS or combined with other

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malignant tumors were excluded from data analysis. Clinical parameters were obtained from the medical record. Bone marrow (BM) samples were obtained via aspiration from all of the patients when the diagnosis was made, prior to receiving any treatment with chemotherapy or hypomethylation agents, and mononuclear cells were subsequently collected via density gradient centrifugation. All patients gave their written consent to the use of their samples for research purposes.

2.2. Cytogenetic analysis

Bone marrow specimens were taken at the initial diagnosis and after short-term culture, cytogenetic slides were prepared with standard techniques and then were R-banded. Twenty metaphases were analyzed and the results were reported using the current International System for Human Cytogenetic Nomenclature [10,11]. Complex karyotype defined as more than or equal to 3 independent chromosomal abnormalities. Structural abnormalities or chromosome gains in < 2 cells and chromosome loss in < 3 cells were not counted as clonal unless the changes were detected in an earlier or subsequent bone marrow specimen. Monosomal karyotype (MK) was defined as at least two autosomal monosomies or a single autosomal monosomy associated with at least one additional structural abnormality.

2.3. Mutation analysis through next generation sequencing

Native genomic DNA (gDNA) was extracted from bone marrow mononuclear cells from the MDS patients at initial diagnosis. The purity (OD 260/280 > 1.8) and concentration (50 ng per μl) of the gDNA met the sequencing requirements. The 37 selected mutational gene targets related to MDS were examined for mutations in 59 cases have sequential samples available for analysis. A target-specific next generation sequencing (NGS) approach, which combines multiplex PCR-based target enrichment and library generation with ultra-deep high-throughput parallel sequencing using a Ion Proton platform was used [12,13], Target genes included *ASXL1*, *BCOR*, *BCORL1*, *CBL*, *CEBPA*, *CREBBP*, *CSF3R*, *CUX1*, *DNMT3A*, *DNMT3B*, *EP300*, *ETV6*, *EZH2*, *FLT3*, *GATA1*, *GATA2*, *IDH1*, *IDH2*, *IKZF1*, *JAK2*, *KIT*, *KMT2D*, *KRAS*, *NPM1*, *NRAS*, *PHF6*, *PTPN11*, *RUNX1*, *SETBP1*, *SF3B1*, *SRF2*, *TET2*, *TP53*, *U2AF1*, *U2AF2*, *WT1*, *ZRSR2*. The average sequencing depth of 1000×; and the sensitivity was 1–2%, mutations were annotated using the several databases including dbSNP, 1000 Genomes, Polyphen-2 and COSMIC.

2.4. Follow-up

The last follow-up was conducted on July 2017, and the overall survival was defined as the period from the day of diagnosis to the day of death regardless of the cause, or the last follow-up.

2.5. Statistical analysis

SPSS 22.0 software (SPSS Inc.; Chicago, IL, USA) was used for all analyses. Continuous variables were compared using Mann–Whitney *U* test. Categorical variables were analyzed with the chi-squared test and Fisher's exact test. Survival curves were constructed by the Kaplan–Meier method and compared by the log-rank test. Two sided *P*-values < 0.05 were considered statistically significant. Cox regression model was used to evaluate the effect of potential factors, including sex, age, WHO subtype, blood count, treatment, MK, IPSS and IPSS-R classification as well as gene mutations.

Table 1
Basic characteristics of a cohort of 101 patients.

	N(%)	%
Gender		
Male	57	56.44
Female	44	43.56
WHO type		
RCUD	5	4.95
RCMD	22	21.78
MDS-U	3	2.98
RAEB-1	37	36.63
RAEB-2	34	33.66
Karyotype		
CK with MK	79	78.22%
CK without MK	22	21.78%
IPSS classification		
Intermediate-2	75	74.26
High	26	25.74
IPSS-R classification		
High	11	10.89
Very high	90	89.11
Treatment		
Supportive care	50	49.50
Decitabine monotherapy	21	20.79
Combined chemotherapy	14	13.86
DAC + chemotherapy	11	10.89
HSCT	5	4.96
Outcome		
Leukemic transformation	21	28%(21/75)
Died	86	85.15

3. Results

3.1. Patient characteristics

One hundred and one eligible patients (57/44, male/female) with a median age of 62 years were enrolled in the study. Among which 5 patients were refractory cytopenia with unilineage dysplasia (RCUD), 22 patients were refractory cytopenia with multilineage dysplasia (RCMD), and 3 patients were myelodysplastic syndrome-unclassified (MDS-U), while the rest were refractory anemia with excess blasts (RAEB) (37 were RAEB-1 and 34 were RAEB-2). According to the International Prognostic Scoring System (IPSS), 75 (74.26%) patients were classified in the intermediate-2 risk IPSS category, and 26 (25.74%) were high risk. However, when classified through IPSS-R staging, 90 (89.11%) patients had very high risk, and only 11 patients had high risk. The median hemoglobin, neutrophil count, as well as platelet count were 74 g/L(IQR: 63.5–84.5), 1.2×10^9/L (IQR: 0.72–2.55) and 43×10^9/L(IQR: 22.5–76) respectively at the initial diagnosis (Table 1).

3.2. Treatment characteristics

From the detailed treatment history available, 50 (49.5%) patients received supportive care, including components of blood transfusion, hematopoietic growth factor and immunomodulatory, 21 (20.79%) patients received decitabine monotherapy, 14 (13.86%) patients received combined chemotherapy such as idarubicin/cytarabine (IA), aclacinomycin/cytarabine (AA) and homoharringtonine/cytarabine (HA) and another 11 (10.89%) patients received decitabine priming combined chemotherapy, only 5 (4.95%) patients underwent Hematopoietic stem cell transplant (HSCT) and all these patients received decitabine prior to the transplant.

3.3. Cytogenetic features

The karyotype of all patients were carefully classified according to each chromosome involved, the distributions were shown in Fig. 1. The most common chromosomal abnormalities was on chromosome 5 (65/

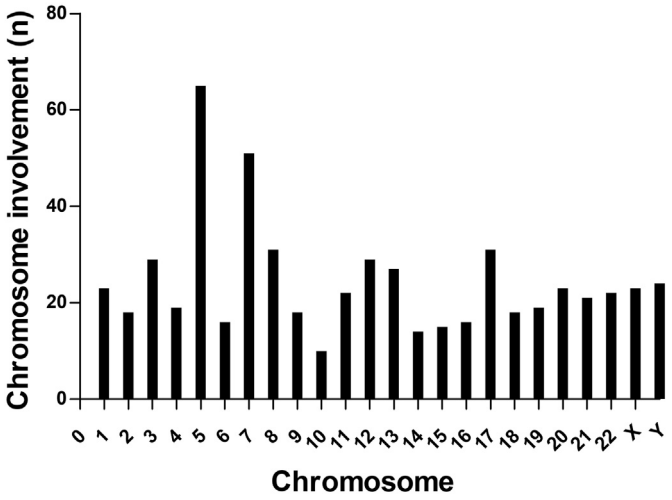

Fig. 1. Frequency of chromosome involvement of MDS patients with CK.

101), followed by chromosome 7 and chromosome 8 as well as chromosome 17, accounting for 50.50% (51/101) and 30.69% (31/101), 30.69% (31/101) respectively. Similar to previous studies, most abnormalities were the deletion of the chromosome. Del(5q)/-5 was identified in58(57.43%) patients, del (7q)/-7 in 41 (40.59%)and del (17p)/-17 in 25 (24.75%) patients. Besides, 29 (28.71%) patients with chromosome 3 and 12 involvement, followed by chromosome 13, 1 and 20, accounting for 26.73%, 22.77% and 22.77% respectively (Fig. 1). MK was considerably overlapped with CK and seen in 79 patients, accounted for 78.22%.

3.4. Survival analysis

One hundred and one patients were followed up for 60 months (95% CI 51.18–68.82 months). The median OS was 13 (95% CI 9.98–16.02) months, and 19 (95% CI 8.57–29.43) months for patients with blasts < 5%, 12 months (95% CI 8.28–15.72) for patients with RAEB-1 and only 11 months (95% CI 9.98–16.02) for those with RAEB-2 ($P = 0.04$) (Fig. 2A). Patients with intermediate-2 risk had longer OS (14 months with 95% CI 10.89–17.12) than those with high risk (12 months with 95% CI 8.51–18.72) by IPSS risk classification ($P = 0.007$) (Fig. 2B).

When OS analysis based on IPSS-R risk, patients had significantly shorter OS (13 months with 95% CI 9.66–16.34) with very high risk than those with high risk (29 months with 95% CI15.09–42.91) (Fig. 2C). However, there was no significant difference in OS for patients received different treatment ($P = 0.41$) (Fig. 2D).

Considering the higher rate of involvement of chromosomes 5, 7, and 17 in this cohort, we further compared the OS, patients with chromosome 5 or 7 involvement have a worse survival than those without (12 months vs. 28 months, $p < 0.01$) (Fig. 2E), but the involvement of chromosome 17 seems not to be associated with worse survival ($P = 0.746$, Fig. 2F). For patients with MK, the median OS was 11 with 95% CI 8.55–13.46 months, slightly shorter than that of patients without, 17 with 95% CI 12.52–21.48 months, but the difference was not significant ($P = 0.30$).

3.5. Gene mutation analysis

In total, 59 samples of bone marrow scrapes from patients were sequenced, the overall incidence of mutations was 96.61% (57of 59 patients). *TP53* mutation was most common, detected in 44 patients, followed by mutations in *TET2* in 10 (16.95%), *U2AF1* in 9 (15.25%), *KMT2D* in 9 (15.25%) patients respectively (Fig. 3).

Of the 44 (74.58%) patients harboring *TP53* mutations, 14 patients had *TP53* mutation only, and the remaining 30 patients had other combined mutations (Supplementary material 1). Additionally, *TP53* mutation was detected in 36, 25 and 10 patients with chromosome 5, 7 and 17 involvement, respectively. When the clinical features of patients with or without *TP53* mutation were compared, we found that among patients with *TP53* mutation, more patients had very high risk according to IPSS-R classification and 5/5q deletion as well as monosomal karyotypes, while other features were comparable between the two groups (Table 4).

Seventy-two *TP53* mutations were identified in the following sites: exon3 in 1 (1.39%), exon 4 in 4 (5.56%), exon 5 in 10 (13,89%), exon 6 in 8 (11.11%), exon 7 in 14 (19.44%), exon 8 in 15 (20.83%), exon 10 in 2 (2.67%), and splice sites in 4 (5.55%),while intron in 2 (2.67%). We next examined the numbers and sites of *TP53* mutation in MDS with chromosome 5, chromosome 7 and chromosome 17 involvement, all three types of MDS had predominantly one mutation and all had mutations clustered in DNA binding domain (exons 5–8). Exon7 and 8 mutations occurred more frequently but it was not no significantly different among the the patients with chromosome 5,7 and 17 involvement (24/54, 12/27 versus 7/15, $P = 1.00$) (Supplementary material 2).

3.6. Outcomes of patients with TP53 mutation

The median OS 59 patients with gene detected was 14 (95% CI 10.82–17.18) months, and the median OS of 44 patients with TP53 mutations was significantly shorter (10 with 95% CI 7.05–12.95 months) when compared to patients without (27 with 95% CI 11.28–58.72 months, $P < 0.01$) (Fig. 4A). A sub-group based on the number of mutations and combined other mutation was performed, and we found that there was no statistically difference in outcomes between patients with single or ≥2 TP53 mutations ($p = 0.879$) (Fig. 4C), The same results were seen in patients with or without other mutations ($P = 0.292$) (Fig. 4B).

We further compared VAF of *TP53* mutations in different types of MDS. The median VAF for non-RAEB, RAEB-1 and RAEB-2were 71.2% (range, 26.3–92.5%), 71.3% (49.5–76.7%) and 62.9% (range, 35.3–82.6%), respectively, and there was no significant difference ($P = 0.686$). We stratified VAF into different cohorts, 20% and 40% according to previous research, survival of patients with *TP53* VAF < 40% was not significantly different from those with VAF > 40%. Due to the median VAF of this cohort of patients was 70.00%, we analyzed the survival based on this cutoff, as shown in Fig. 4D, patients with > 70.00% VAF had a worse survival than patients with ≤70.00% VAF (9 months vs. 13 months, $P = 0.04$).

3.7. Impact on overall survival

We performed a univariate analysis to determine the independent impact of each variable examined on OS (Table 2). The variables included age, sex, WHO type, hemoglobin, neutrophils, platelet count, blast count, IPSS, IPSS-R risk groups, MK and *TP53* mutational status. It suggested that WHO subtype ($P = 0.024$), IPSS ($P = 0.007$), IPSS-R risk ($P = 0.037$) as well as *TP53* mutation status ($P < 0.001$) are associated with survival. But for the multivariate cox regression analysis, only *TP53* mutational status ($P = 0.001$) was significant unfavorable factors for OS (Table 3).

4. Discussion

In this study, we characterized the clinical and molecular features of MDS cases with CK from a cohort of 101patients. Our findings largely agree with previous work which showed that MDS with CK imply an unfavorable outcome. [8,14]. We also found that chromosome 5 or 7 were frequent involved, and most of which were the deletion of the chromosome. The median OS of patients with chromosome 5 or 7

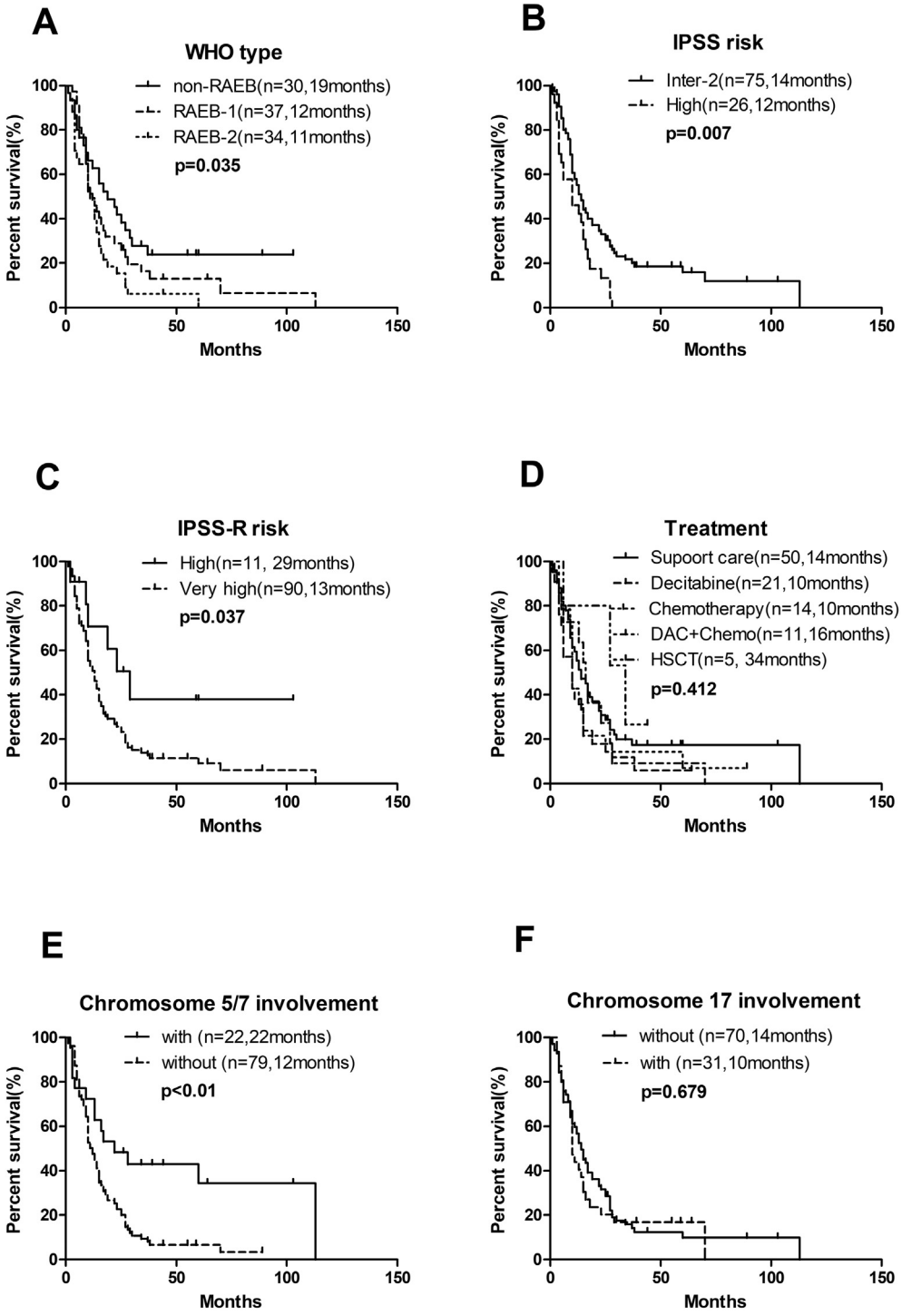

Fig. 2. The Kaplan–Meier curve for overall survival (OS) of MDS patients with CK. A, OS stratified by WHO subtype (non-RAEB including RCUD, RCMD and MDS-U). B, OS stratified by IPSS risk stratification. C, OS stratified by IPSS-R risk stratification. D, OS stratified by treatments. E, OS of patients with or without chromosome 5 or 7 involvement. F, OS of patients with or without chromosome 17 involvement.

involvement was shorter than that of patients without, while the chromosome 17 involvement seems not associated with survival. MK is considerably overlapped with CK and some studies indicated that MK was associated with a significantly inferior OS in MDS with CK [15,16,17], but others did not yield similar outcomes [18–20]. In our study, most patients had CK with MK, and the OS was a little shorter than those without, but the difference was not significant, large sample studies to explore the prognostic impact of MK in MDS are in need.

Previous research suggested that hypomethylating agents (HMA) alone or in combination with chemotherapy can improve outcome of AML/MDS with a complex karyotype [14,21,22]. Bejar's studies also suggested that HMA or bone marrow transplant may beneficial to CK patients without *TP53* mutation [9,23]. However, there was no significant difference in OS of patients received different therapies in our study, even decitabine. It may be associated with different patients included. Previous studies included some AML patients with a higher median white blood cell count, while in our study, all patients were MDS and the patient's treatment options were relatively randomized

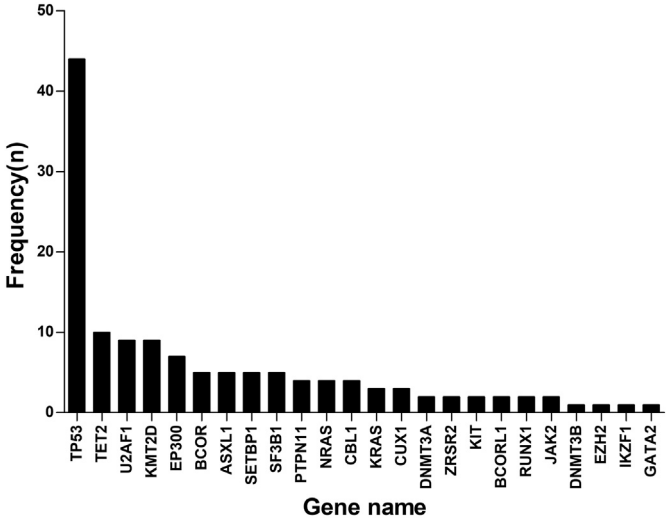

Fig. 3. Frequency of gene mutations identified by the sequencing screen in the cohort of 59 patients.

and the number of cases was not matched, both could affected the analysis of treatment outcome.

A variety of novel mutations have been identified in recent years and *TP53* mutations are enriched in patients with deletion of 5q or complex cytogenetics [6,7,9], other studies showed consistent association of *TP53* mutation with −7/7q- and 17p- [24,25]. In our cohort, gene mutations have been identified in 57 patients (96.61%), *TP53* mutation is most common and the frequency was comparable to previous study [26]. It was significant associated with chromosome 5

Table 2
Univariate analysis of prognostic factors associated with survival.

Category	Characteristics	*P* value
Gender	Male	0.482
	Female	
Age, y	≥60	0.834
	< 60	
Neutral count ($\times 10^9$/L)	≥0.8	0.918
	< 0.8	
Hemoglobin (g/L)	≥80	0.366
	< 80	
Platelet count ($\times 10^9$/L)	≥50	0.856
	< 50	
IPSS classification	Intermediate-2	0.007
	High	
IPSS-R classification	High	0.037
	Very high	
WHO type	Non-RAEB	0.024
	RAEB-1	
	RAEB-2	
Treatment	Support care	0.371
	Decitabine monotherapy	
	Combined chemotherapy	
	DAC priming chemotherapy	
	ASCT	
P 53 mutation	Mutation	< 0.001
	No mutation	
Monosomal karyotype	Yes	0.559
	No	

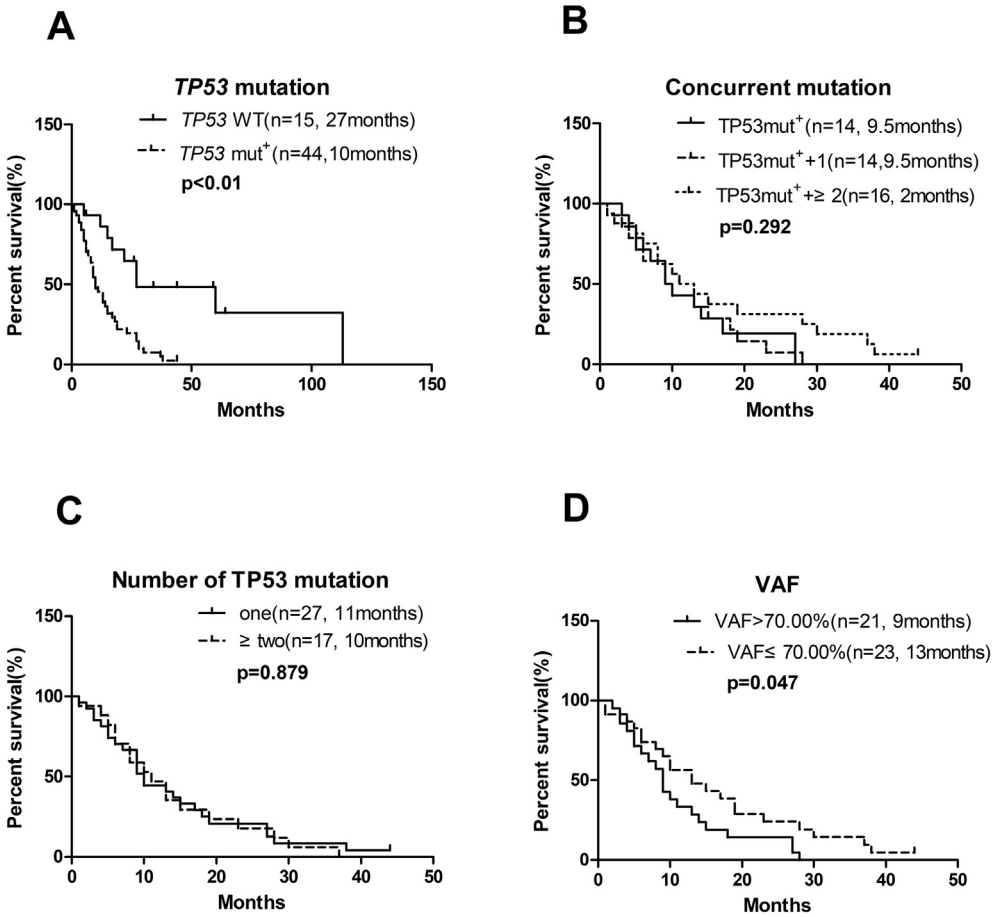

Fig. 4. The Kaplan–Meier curve for patients with gene sequenced. A, OS stratified by with or without *TP53* mutation. B, OS of patients with *TP53* mutation concurrent one or more other mutations. C, OS stratified by numbers of TP53 mutation. D, OS stratified by TP53 VAF.

Table 3
Multivariate Cox regression analysis for overall survival (OS).

Risk factors	*P*	HR (95% CI)
Age (≤60 versus > 60)	0.56	0.99 (0.96–1.02)
Sex	0.28	1.56 (0.70–3.46)
Neutrophils ($\geq 0.80 * 10^9$/L vs. $< 0.80 * 10^9$/L)	0.28	1.56 (0.70–3.48)
Platelet ($\geq 50 * 10^9$/L vs. $< 50 * 10^9$/L)	0.05	0.41 (0.16–1.00)
hemoglobin (≥80 g/L vs. < 80 g/L)	0.64	1.22 (0.59–2.86)
WHO subtype (Blast < 5% vs. 5–10% vs. 11–19%)	0.27	0.73 (0.41–1.28)
IPSS (intermediate-2 vs. high)	0.56	0.76 (0.30–1.93)
IPSS-R (high vs. very high)	0.79	0.85 (0.25–2.90)
Monosomal karyotype	0.43	0.69 (027–1.74)
TP53 mutation	0.001	0.20 (0.08–0.54)
Treatment (support vs. chemotherapy vs. DAC. vs. DAC + Chemo vs. HSCT)	0.78	1.04 (0.79–1.38)

Table 4
Comparison of clinical features of patients with and without TP53 mutation.

	TP53mutation	No mutation	p
No	44	19	
IPSS-R			< 0.001
High	4	2	
Very high	40	13	
WHO type			*P* = 0.181
RCUD	3	0	
RCMD	6	6	
MDS-U	2	0	
RAEB-1	18	6	
RAEB-2	15	3	
Del(5/5q)	32	3	P < 0.001
Del(17/17p)	9	2	*P* = 0.541
Del(7/7q)	18	6	*P* = 0.951
IPSS			*P* = 0.053
Intermediate-2	30	14	
High	14	1	
Neutrophils (median)	1.28(0.35–26.4)	1.2(0.5–5.3)	0.993
Hemoglobin	75(42–106)	64(44–123)	0.117
Platelet count	40(5–343)	25(3–158)	0.296
MK	39/44	5/15	0.023

involvement, but not notably associated with chromosome 7 and 17. We speculated that in MDS patients with complex karyotype, the abrogation of *TP53* activity may be more relevant to the mutations, rather than chromosome loss. A recent study suggested that *TP53* mutation clusters with MK in MDS patients and its adverse impact might be accounted for by this phenomenon [27]. While in our study, patients with complex monosomal karyotype had more frequent *TP53* mutation than those with complex non-monosomal karyotype, which further suggested that *TP53* mutation induced genetic/chromosome instability as the cause of the particular cytogenetic abnormality.

For survival analysis, *TP53* mutation predicated for an inferior OS as expected, confirming previously reported data.

TP53 mutation combined with additional molecular abnormalities was not associated with OS, and the number of mutations of *TP53* gene also had no significant effect on survival $P = 0.879$). Moreover, the frequency of these concurrent mutations is lower than that in general, such as the occurrence rate of 20–30% in *TET2* and 15–20% in *ASXL1* in MDS patients overall [28] which indicates the paramount role of *TP53* in determining prognosis.

Finally, there is some controversy as to whether the VAF of *TP53* mutations has an impact on survival. Some researchers found that patients with VAF over 40% had a worse survival than patients with VAF < 20% [29,30], whereas another study showed *TP53* mutations affected patient survival in an allele burden-independent manner [31]. In this study, the median VAF of our patients was 70.00% (46.1%–77.7%) and was higher than previous study [21], survival of patients with *TP53* VAF ≤ 40% was not significantly different from those with VAF > 40%, but when 70.00%% was determined as the optimal cut-off of VAF, patients with TP53 VAF > 70.00% showed a significantly diminished OS compared to those with TP53 VAF ≤70.00%. These findings suggest that high *TP53*-mutational burden > 70.00% might have an unfavorable prognostic on survival in MDS patients with CK.

Univariate analysis of factors influencing OS showed that higher risk stratification and more blast cells as well as *TP53* mutation were adverse prognostic factors, but only *TP53* mutational status was poor prognostic factor for survival in multivariate analysis. It further illustrates the important role of *TP53* mutation in complex karyotype MDS patients.

This single center study confirms that MDS patients with CK have a poor prognosis irrespective treatment. MK was considerably observed but not independent risk factor for OS. Frequent *TP53* mutation and high *TP53* VAF were observed in this study, and both were associated with shorter survival. Due to limited number of specimens that detected gene mutation in our study, further large cohort clinical studies are needed to clarify the significance of *TP53* mutation in MDS.

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HT and YR designed the study. YR and HT wrote the manuscript. YR, YL, and CM analyzed and arranged the data. HY, SZ and YL performed the gene mutation analysis. CH, YR, XZ, LM, WX, LY and provided patient samples and data. JJ guided the project design and article modification.

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Conflict of interest

The authors have no conflicts of interest. The funding sources had no involvement in study design; collection, analysis and interpretation of data; as well as in the decision to submit the article for publication.

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