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SOX7 methylation is an independent prognostic factor in myelodysplastic syndromes

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ABSTRACT

Objective: *SOX7* downregulation caused by its promoter methylation was associated with poor survival in several types of human solid tumors. However, the pattern of *SOX7* methylation and its clinical significance are less studied in hematological malignancies. Herein, we evaluated the methylation pattern of *SOX7* in myelodysplastic syndrome (MDS) and determined its clinical implication in patients with MDS.

Methods: *SOX7* methylation was determined by real-time quantitative methylation-specific PCR (RQ-MSP) in 99 MDS patients. Bisulfite sequencing PCR was applied to confirm the results of RQ-MSP.

Results: *SOX7* methylation was detected in 55.6% of 99 patients but not in healthy donors. No correlation was found between *SOX7* methylation and clinical parameters including patient age, gender, white blood cell count, hemoglobin, and platelet count. However, patients with *SOX7* methylation harbored more *U2AF1* mutation than patients without *SOX7* methylation ($P = 0.015$). Kaplan-Meier curves indicated that the patients with *SOX7* methylation presented reduced overall survival (OS) ($P = 0.034$). Furthermore, subgroup analysis indicated that *SOX7* methylation was associated with poor OS in male patients ($P = 0.034$) and in patients older than 60 years ($P = 0.019$). According to the multivariate analysis, *SOX7* methylation remained as an independent prognosis factor in MDS patients both as dichotomous (HR = 2.14, $P = 0.041$) and as continuous (HR = 1.55, $P = 0.042$) variable. Importantly, *SOX7* methylation was significantly increased during progression from MDS to secondary acute myeloid leukemia (sAML).

Conclusions: Our findings demonstrated that *SOX7* methylation conferred adverse prognosis in MDS patients and was associated with leukemia progression.

1. Introduction

Myelodysplastic syndrome (MDS) is a group of phenotypically and prognostically heterogeneous diseases characterized by inefficient hematopoiesis and a risk of progression to acute myeloid leukemia (AML) [1]. Its incidence is prominently higher in men and individuals older than 60 years and has risen in recent years [2,3]. For risk stratification and optional treatment decisions of this disease, various prognostic scoring systems have been proposed, the most prevalent of them being the International Prognostic Scoring System (IPSS), although much efforts have been made to refine this system [4]. However, these models, which were mainly based on cytogenetic aberrations, do not fully reflect the molecular heterogeneity of the disease [5]. Gene mutation data and expression data, for instance, has already shown their potential superiority in prognostic stratification [6,7]. Indeed, accumulating evidence supports the view that epigenetic alternations, especially aberrant methylation of several regulatory genes, also play a key role in MDS. This could be demonstrated by the efficacy of hypomethylating agents in the clinical settings [8] and the prognostic significances of DNA methylation in MDS. Hypermethylation of the

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p15^{INK4B} gene, for example, is frequently observed in MDS and is correlated with high-risk subgroups [9], leukemic transformation [10,11], and worse prognosis [12]. A recent study has further demonstrated DNA methylation as a predictor for survival as well as treatment response in MDS [13]. Thus, it is of importance to identify methylated genes with clinical significance to refine estimations of prognosis in MDS.

SOX7 (Sex Determining Region Y-Box 7), a member of the F group of *SOX* (SRY-related HMG-box) family of transcription factors, has long been shown to participate in developmental processes, particularly in cardiogenesis, vasculogenesis, and lymphangiogenesis [14–16], and its role in hematopoiesis has recently been established [17,18]. Moreover, *SOX7* is frequently downregulated in various types of human solid tumors, which was associated with poor survival [19–28]. Functional studies have demonstrated *SOX7* as a potential tumor suppressor gene (TSG) which negatively regulates cell proliferation through the Wnt/β-catenin signal pathway [29]. However, few studies have addressed the pathogenesis role and clinical significance of *SOX7* in hematological malignancies. A recent study has shown that *SOX7* expression was silenced by DNA hepermethylation in AML, and confirmed its tumor suppressor role by demonstrating *SOX7* as a negative regulator of the Wnt/β-catenin pathway [30]. In MDS, it was found that *SOX7* gene was aberrantly methylated and was associated with advanced stages and poor survival; but *SOX7* gene methylation failed to be an independent prognostic factor [31]. Moreover, the methylation status of *SOX7* in this study was determined mainly by conventional MSP method which based solely on the presence of amplification products on an electrophoresis gel. Despite being highly sensitive and cost-effective, this technique has some limitations, such as not quantitative, low specificity, and false-positive results [32]. We therefore applied the method of real-time quantitative methylation-specific PCR (RQ-MSP) to examine the methylation pattern of *SOX7* in MDS patients, we also evaluated the association between *SOX7* methylation and the clinical characteristics of these patients.

2. Material and methods

2.1. Patients and samples

A total of 99 MDS patients diagnosed at the Affiliated People's Hospital of Jiangsu University were enrolled in this study. The patient cohort consisted of 57 men and 42 women and the median age was 60 years old (range, 20–86 years). All cases were classified according to the 2008 World Health Organization (WHO) classification criteria. The clinical and laboratory features of patients were shown in Table 1. Risk groups were classified according to International Prognosis Scoring System (IPSS) except for four cases without available karyotypic results. Patients with lower IPSS scores (Low/Int-1) received symptomatic and supportive treatment with/without thalidomide/lenalidomide or EPO or cyclosporine together with ATG. For those with higher IPSS scores (Int-2/High), treatment consisted of supportive care with/without chemotherapy comprising decitabine or the CHG regimen (low-dose cytarabine, homoharringtonine, and granulocyte colony stimulating factor) or the CAG regimen (low-dose cytarabine, aclacinomycin, and granulocyte colony stimulating factor). After informed consent was written, bone marrow (BM) samples from all patients were obtained at the time of diagnosis. BM samples collected from seven healthy donors of BM transplantation were used as controls. The study was conducted in accordance with the principles of Helsinki declaration and was approved by the Institutional Review Board. The follow-up data were obtained for 72 patients. The median follow-up duration of the patients was 13 months (range, 1–89 months).

2.2. Cytogenetic analysis

Cytogenetic analysis was carried out on BM samples obtained at diagnosis using a direct method or short-term culturing. Karyotypes were analyzed on R-banded metaphases, and chromosomal abnormalities were described following the International System for Human Cytogenetic Nomenclature.

Table 1
Correlation between *SOX7* methylation and patients parameters.

Patient's parameters	Status of *SOX7* methylation			
	Unmethylated (n = 44)	Methylated (n = 55)	Total (n = 99)	*P* value
Median age, years (range)*	62 (20–86)	53 (27–86)	60 (20–86)	0.422
Sex, male/female	29/15	28/27	57/42	0.134
Median WBC, × 109/L (range)	2.5 (0.9–82.4)	2.8 (1.2-19.5)	2.7 (0.9–82.4)	0.122
Median hemoglobin, g/L (range)	64 (26–117)	62 (38–128)	63 (26–128)	0.706
Median platelets, ×109/L (range)	68 (12–654)	54 (0–1176)	60 (0–1176)	0.586
WHO				0.922
RA (RS)	7 (16%)	5 (9%)	12 (12%)	
RCMD (RS)	17 (39%)	24 (44%)	41 (41%)	
RAEB-1	7 (16%)	9 (16%)	16 (16%)	
RAEB-2	11 (25%)	14 (25%)	25 (25%)	
5q-	2 (4%)	2 (4%)	4 (4%)	
MDS-U	0 (0%)	1 (2%)	1 (1%)	
Cytogenetics				0.340
Good	35 (80%)	38 (69%)	73 (74%)	
Intermediate	7 (16%)	8 (15%)	15 (15%)	
Poor	2 (4%)	6 (11%)	8 (8%)	
No data	0 (0%)	3 (5%)	3 (3%)	
IPSS				0.597
Low	5 (11%)	6 (11%)	11 (11%)	
Int-1	27 (61%)	28 (51%)	55 (56%)	
Int-2	8 (18%)	13 (24%)	21 (21%)	
High	4 (9%)	5 (9%)	9 (9%)	
No data	0 (0%)	3 (5%)	3 (3%)	
Gene mutations				
C/EBPA (±)	2/42	1/52	3/94	0.589
IDH1/2 (±)	2/42	1/52	3/94	0.589
DNMT3A (±)	0/44	3/50	3/94	0.249
U2AF1 (±)	0/44	7/46	7/90	0.015
SF3B1 (±)	1/43	5/48	6/91	0.216
SRSF2 (±)	4/40	1/52	5/92	0.173
SETBP1 (±)	1/43	1/52	2/95	1

WBC, white blood cells; IPSS, International Prognostic Scoring System; WHO, World Health Organization; RA, refractory anemia; RARS, RA with ringed sideroblasts; RCMD, refractory cytopenia with multilineage dysplasia; RCMD-RS, RCMD with ringed sideroblasts; RAEB, RA with excess of blasts. Int-1, intermediate-1; Int-2: intermediate-2.

2.3. Gene mutation detection

The detection of *IDH1/2, DNMT3A, U2AF1, SF3B1, SRSF2, SETBP1* mutation were performed using high-resolution melting analysis on the LightScanner platform (Idaho Technology Inc., Salt Lake City, Utah) and positive samples were validated by direct DNA sequencing [33–36]. *C/EBPA* mutation was measured by direct DNA sequencing [37].

2.4. DNA isolation and bisulfite modification

The BM mononuclear cells (BMNCs) were separated by density-gradient centrifugation using Lymphocyte Separation Medium (TBD sciences, Tianjin, China). Genomic DNA was isolated from BMNCs using genomic DNA purification kit (Gentra, Minneapolis, MN, USA) and modified by the CpGenome™ DNA Modification Kit (Chemicon, Ternecula, Canada) according to the manufacturer's instructions. The bisulfite-modified genomic DNA was eluted in ultra-pure water and used immediately or stored at −80 °C until used.

2.5. RQ-MSP

RQ-MSP reaction was performed on a 7300 Thermo Cycler (Applied Biosystems, CA, USA). The primers for unmethylated *SOX7* (U-*SOX7*) were 5′- GGGAAGTTTGTTTAGATGGAGGAT -3′(forward) and 5′- TAC ACAACTCAAACAAAAAACA -3′ (reverse), and the primers for the methylated *SOX7* (M-*SOX7*) were 5′-GATGGAGGACGTATTTTTTGTA AAC-3′ (forward) and 5′- AACCGCTACACTAATAAACCGAA -3′ (reverse). The RQ-MSP reaction was carried out with the following reaction mixture: 10 μM of AceQ™qPCR SYBR Green Master Mix (VazymeBiotechCo., Piscataway, NJ, USA), 0.4 μM of ROX Reference Dye 1 (Invitrogen, Carlsbad, CA, USA), 0.8 μM of primers, 20 ng of modified DNA and distilled water brought the final volume to 20 μL. The PCR condition was performed at 95 °C for 5 min, 40 cycles for 10 s at 95 °C, 30 s at 67 °C (M) or 61 °C (U),30 s at 72 °C, and 77 °C (M) or 75 °C (U) for 30 s, followed by a melting program of 95 °C for 15 s, 60 °C for 60 s, 95 °C for 15 s, and 60 °Cfor 15 s to validate the specificity of the expected PCR product. Negative control samples without DNA were included for each set of PCR. PCR products were analyzed on 2% agarose gels and visualized under UV illumination. The normalized ratio ($N_{M\text{-}SOX7}$) was applied to evaluate the level of *SOX7* methylation. $N_{M\text{-}SOX7}$ was calculated using the following formula: $N_{M\text{-}SOX7} = (E_{M\text{-}SOX7})^{\Delta CTM-SOX7(\text{control-sample})} \div (E_{ALU})^{\Delta CT\ ALU(\text{control-sample})}$.

2.6. Bisulfite sequencing PCR(BSP)

The primers for bisulfite modified*SOX7* were 5′-TTAAGGGTTTTGT TGGAGGTATA-3′ (forward) and 5′- TAAATTTACCCCTCTTTTCCAA -3′ (reverse). BSP was conducted as previously described [38], with the amplification of the bisulfite-treated *SOX7* promoter encompassing 18 CpG sites. Then 20 ng of sodium bisulfite-treated DNA was used as the template for PCR. The PCR cycles were as follows: 95 °C for 5 min, followed by 40 cycles of denaturation (30 s at 94 °C), annealing (30 s at 57 °C) and extension (30 s at 72 °C) in 50 μL reaction mixture containing 10 × PCR buffer (KCl 0.25 mM), dNTP Mixture 6.25 μM, primers 0.5 μM, hot start DNA polymerase 0.75 U (Takara, Tokyo, Japan). The PCR yields were analyzed on 2% agarose gels and purified with AxyPrep DNA Gel Recovery Kit (Axygen, CA, USA). The purified products were ligated intopMD19 T Vector (Vazyme Biotech Co, Piscataway, NJ, USA), and then transfected into DH5 A competent cells (Vazyme Biotech Co., Piscataway, NJ, USA). Six clones from each sample were sequenced (BGITech Solutions Co., Shanghai, China).

2.7. Reduced representation bisulfite sequencing (RRBS)

To assess methylation changes of *SOX7* during progression from MDS to sAML, we utilized a RRBS data recently generated in our laboratory. Briefly, genomic DNA (gDNA) was isolated from five paired MDS/sAML samples. The DNA concentration and purity were assessed using Nanodrop2000 (Thermo Scientific), then gDNA was digested with the restriction enzyme MspI (NEB), followed by end-repair, A-tailing of digested gDNA, and methylated adapter ligation. After gel extraction purification (TIANgel Midi Purification Kit), samples were subject to sodium bisulfite treatment using the EZ DNA Methylation™ Kit. To prepare for sequencing libraries, the sequences were subjected to PCR amplification followed by library quality assessment. Final libraries were sequenced on the Illumina HiSeq 2500 platform using paired-end 150-bp reads. After quality control, resultant reads were aligned to the reference genome (hg19) using Bismark [39]. Methylation status of each CpG site was determined based on the ratio of methylated reads to that of methylated and unmethylated reads combined. Mean Methylation level of chr8: 10587304-10591109, a CpG island locates on the first exon of *SOX7* gene, was used to represent the methylation status of *SOX7*.

2.8. Statistical analysis

Statistical analysis was performed using the SPSS 17.0 software package (SPSS, Chicago, IL) and the R statistical package 3.5.1 (http://www.r-project.org). Pearson Chi-square analysis or Fisher's exact-test was applied to compare categorical variables between groups. The correlation between the frequency of *SOX7* promoter methylation and the clinical and hematologic parameters was analyzed with Spearman's rank correlation. For time-to-event analyses, the Kaplan-Meier method was used to calculate survival estimates, and the log-rank test was used to compare groups. Multivariate Cox proportional hazards model was used to calculate the hazard ratios and 95% confidence intervals of the associations between risk factors and survival. To select the best multivariate model, we used a stepwise selection procedure which starts with an empty model and adding or removing one variable at each step. Models were compared using the Akaike Information Criteria (AIC) method [40]. The AIC allows the measure of goodness of fit of a model, and a lower AIC value generally indicates a better model. The model's discriminatory ability was evaluated using the Harrell's concordance index (C-index) [41], which was bias-corrected by bootstrapping with 1000 resamples (for internal validation). AIC value and C-index were calculated using the stepAIC function (from the "MASS" package) and the validate function (from the "rms" package), respectively. Age and *SOX7* methylation were analyzed not only as categorical but also as continuous variables, considering that dichotomizing continuous data may compromise the accuracy and stability of the model [42,43]. Templeton's two-step normalization method [44] was applied to reduce the skewness *SOX7* methylation data. Paired *t*-test was used to test methylation differences between paired MDS/sAML samples. For all analyses, the *P* values were two-tailed, and the threshold of statistically significant was a *P*-value of less than 0.05.

3. Results

3.1. Methylation pattern of SOX7 in MDS

In this study, we performed RQ-MSP to explore the methylation pattern in 99 primary MDS patients. We found that 55.6% (55/99) of the patients had methylated CpG islands of the *SOX7* gene, whereas BM samples from healthy donors (n = 7) were unmethylated for *SOX7* gene (data not shown) as previously reported [31]. Representative RQ-MSP results are shown in Fig. 1. Briefly, RQ-MSP sensitivity was tested in 10-fold serial dilutions of recombinant *SOX7* methylated plasmids, and the maximal reproducible sensitivity was 10^2 copies (Fig. 1A). The dissociation curve with single peak shows that the primers yielded specific PCR products and no primer dimers (Fig. 1B). To evaluate the RQ-MSP results, we randomly selected two hypomethylated and two hypermethylated MDS samples, using BSP to define the methylation density encompassing the whole MSP region in the *SOX7* promoter. Our results showed that *SOX7* hypomethylated patients had a quite lower density of the region (17% and 22%) than the *SOX7* hypermethylated patients (82% and 67%) (Fig. 2). Moreover, there was a significant correlation between the methylation density and methylation level ($R = 0.913$, $P = 0.002$), thus confirming our RQ-MSP results were reliable. To strengthen the above findings, we further explored *SOX7* methylation status using a DNA methylation array data from the Gene Expression Omnibus (GSE51758, containing 4 MDS patients and 4 controls). Expectedly, as shown in Fig. 3, the methylation level of *SOX7* in MDS was significantly higher than normal controls ($P = 0.006$).

3.2. Association between SOX7 methylation and clinical characteristics in MDS

To analyze whether *SOX7* methylation was associated with clinical characteristics, the cases were divided into two groups: *SOX7* unmethylation and *SOX7* methylation, depending on whether methylation

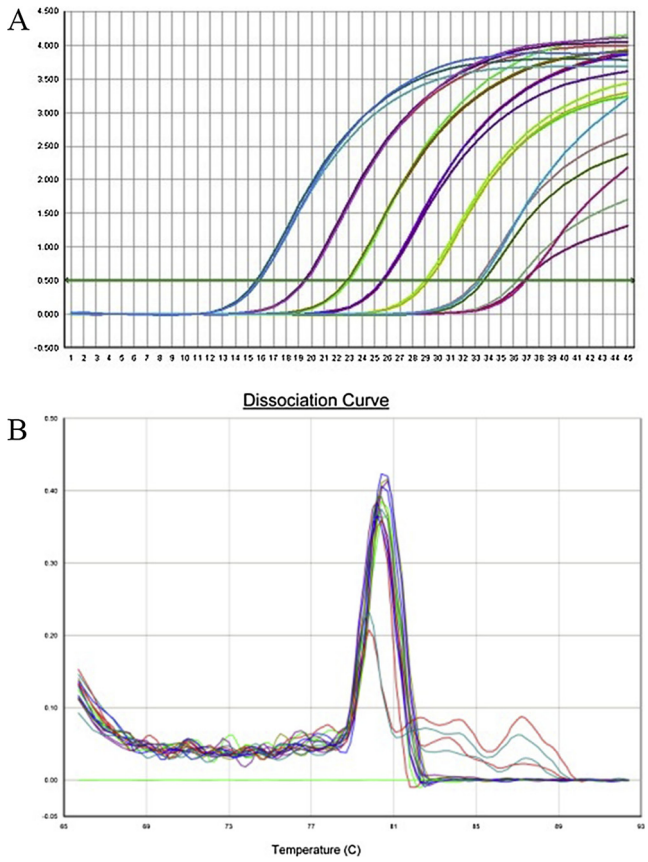

Fig. 1. Representative RQ-MSP results for recombinant *SOX7* methylated plasmids in different concentrations. A: represented amplification curves for M; B: represented dissociation curves of M. M: methylation.

was detected by RQ-MSP. We found there was no relationship between *SOX7* methylation and clinical parameters including patient age, gender, white blood cell count (WBC), hemoglobin, and platelet count (PLT). Likewise, *SOX7* methylation was unrelated to karyotypes, IPSS or WHO subtypes ($P > 0.05$) (Table 1). We further investigated the relationship between *SOX7* methylation and common mutations in MDS. Results showed that patients with *SOX7* methylation had a significantly higher incidence of *U2AF1* (7 of 46, 15.2%) than those without *SOX7* methylation (0 of 44, 0%, $P = 0.015$). No significant association was found between *SOX7* methylation and other gene mutations in our cohort.

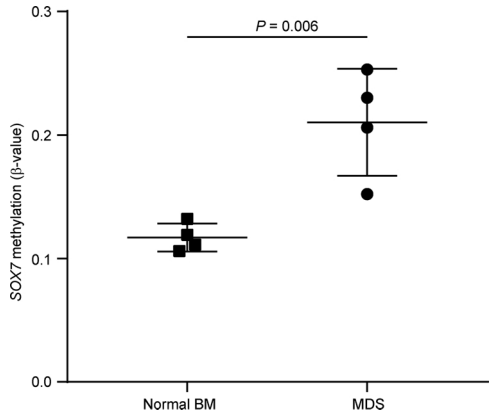

Fig. 3. The levels of *SOX7* methylation in MDS patients and controls in a published dataset (GSE51758). *SOX7* methylation levels was significantly higher in MDS patients (n = 4) as compared to normal controls (n = 4).

3.3. *SOX7* methylation predicts poor outcome in MDS patients

Next, we investigated the influence of *SOX7* methylation status on the prognosis for 72 patients with follow-up data. Distributions of overall survival (OS) were estimated between *SOX7* methylation group and unmethylation group on the basis of the log-rank test. Kaplan-Meier curves indicated that the patients with *SOX7* methylation presented reduced OS (Fig. 4A), and the median survival time of patients with *SOX7* methylation and unmethylation was 13 and 33 months, respectively ($P = 0.034$). We then evaluated whether methylation of *SOX7* was associated with poor prognosis among the patients with a normal karyotype. As shown in Fig. 4B, a trend for an adverse OS in the *SOX7* methylation group was observed ($P = 0.082$). Since MDS incidence is significantly higher in men and in people older than 60 years, we further assess the prognosis impact of *SOX7* methylation separately within these two groups. Our results indicated that methylation of *SOX7* was also associated with poor OS in male patients ($P = 0.034$, Fig. 4C) and in patients older than 60 years ($P = 0.019$, Fig. 4D). In the last step, Cox proportional hazards models (univariate and multivariate analyses) were conducted to evaluate the prognostic significance of the following variables: age, sex, *SOX7* methylation, IPSS status, and gene mutations (as described in Table 1). Variables with $P < 0.20$ in the univariate analysis (data not shown), including age, sex, *SOX7* methylation, IPSS status, and *DNMT3A* mutation, were initially included in a multivariate analysis. After a stepwise backward selection procedure, *DNMT3A* mutation was eliminated from the final model (AIC = 228.59). AIC values and c-indexes of each model were described in Table 2. In the final model, age and *SOX7* methylation were evaluated as both

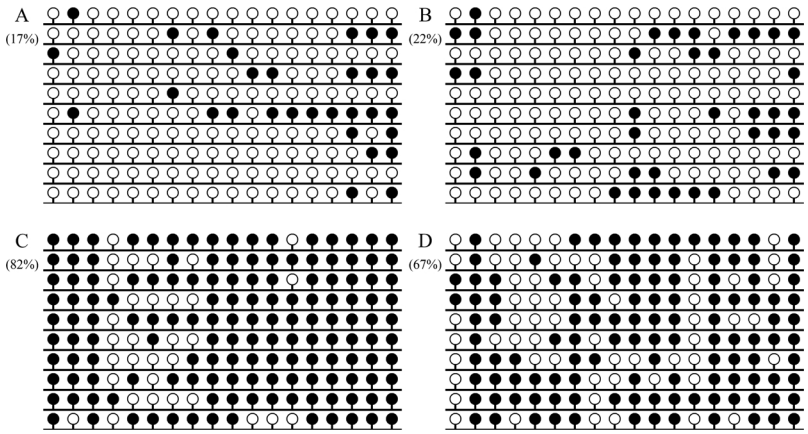

Fig. 2. Bisulfite sequencing analysis of the methylation status of *SOX7* CpG island in four MDS patients. Black circle: methylated CpG dinucleotide; White circle: unmethylated CpG dinucleotide. A, B: the samples with *SOX7* hypomethylation; C, D: the samples with *SOX7* hypermerthylation.

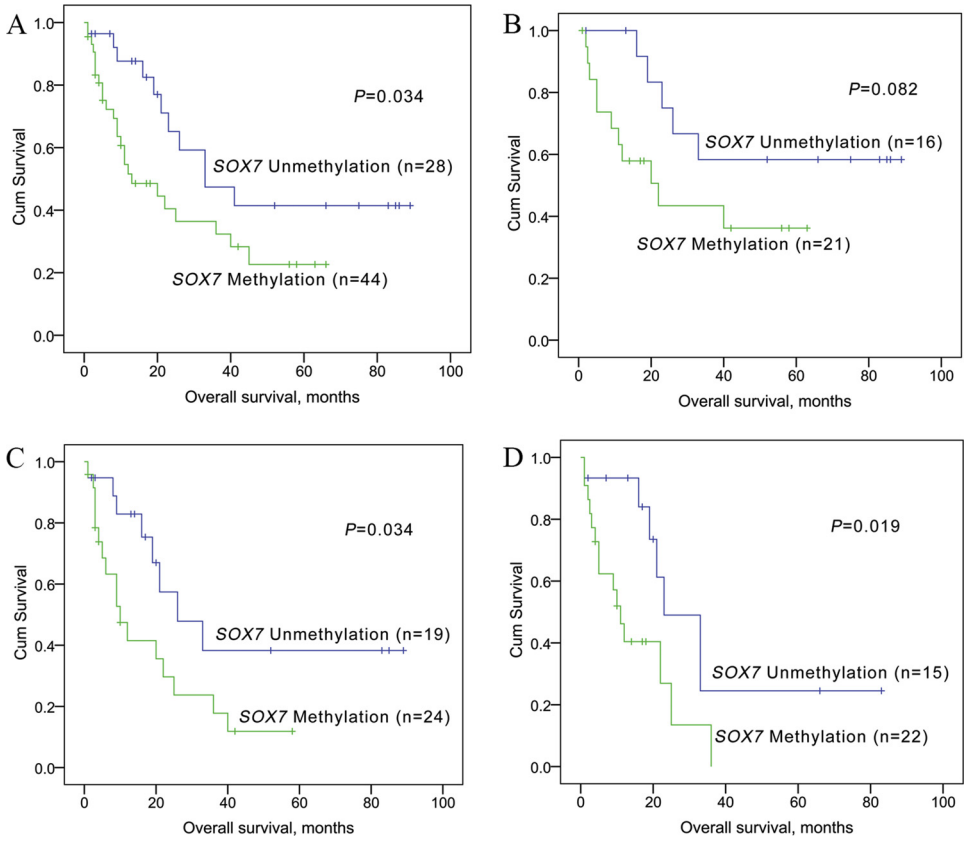

Fig. 4. The impact of *SOX7* methylation on overall survival in MDS patients. A: all MDS patients; B: cytogenetically normal MDS patients C: male MDS patients; D: MDS patients with age older than 60. Survival analysis was analyzed by Kaplan-Meier method.

Table 2
Akaike information criteria and c-index of different models regarding overall survival for MDS classifications.

No. of variables	Variables included in the model	AIC value	C-index
1	Age	233.68	0.68
2	Age, IPSS status	231.55	0.70
3	Age, IPSS status, *SOX7* methylation	229.81	0.73
4 (final model)	Age, IPSS status, *SOX7* methylation, Sex	228.59	0.75
5	Age, IPSS status, *SOX7* methylation, Sex, DNMT3A	229.19	0.75

Abbreviations: AIC, Akaike information criteria; C-index, Harrell's concordance index.

dichotomous and continuous variables (Table 3). When analyzed as categorical variables, the c-index of the model was 0.71. After bootstrapping with 1000 resamples, the corrected C-index was 0.68. By including age and *SOX7* methylation as continuous data, the predictive accuracy increased to 0.75 (0.71 for bootstrap corrected C-index). This indicates the reliability of both models, suggesting the better statistical power of continuous prognostic data. Importantly, *SOX7* methylation, both as dichotomous (HR = 2.14, $P = 0.041$) and as continuous (HR = 1.55, $P = 0.042$) variable, remained as an independent prognostic factor of MDS patients (Table 3). Age and IPSS status were also independent prognosticators in both models (Table 3).

3.4. *SOX7* methylation was increased during MDS transformed into AML

To investigate whether *SOX7* methylation was changed during disease progression of MDS patients, we first examined the RQ-MSP result in 99 MDS patients and 8 sAML patients, our data showed that *SOX7* methylation level was significantly higher in sAML patients compared

Table 3
Multivariate analyses of prognostic factors for overall survival in MDS patients.

	Categorical Form		
Variables	Hazard Ratio	95% CI	*P*
Age (> 60/≤60)	2.41	1.15-5.05	0.019
Sex (Men/Women)	2.00	0.94-4.24	0.071
IPSS status (High/Int-2/Int-1/Low)	1.79	1.13-2.84	0.014
SOX7 methylation (yes/no)	2.14	1.03-4.45	0.041
	Continuous Form		
Variables	Hazard Ratio	95% CI	*P*
Age	1.04	1.01–1.06	0.008
Sex (Men/Women)	1.94	0.92–4.11	0.084
IPSS status (High/Int-2/Int-1/Low)	1.69	1.06–2.68	0.027
SOX7 methylation	1.55	1.02–2.36	0.042

Note: Age and *SOX7* methylation were evaluated as both categorical and continuous variables. *SOX7* methylation levels were normalized using the Templeton's two-step procedure to approximate a normal distribution.

to MDS patients ($P < 0.0001$, Fig. 5A). Our laboratory has recently performed reduced representation bisulfite sequencing (RRBS) on BM samples from five paired MDS/sAML patients (unpublished data), using this data, we confirmed the dynamic change of *SOX7* methylation in MDS progression to AML individually ($P = 0.017$, Fig. 5B).

4. Discussion

A previous study found that *SOX7* was methylated in MDS patients [31]. However, *SOX7* methylation status was mainly detected by gel-based MSP method, which could have some limitations such as low

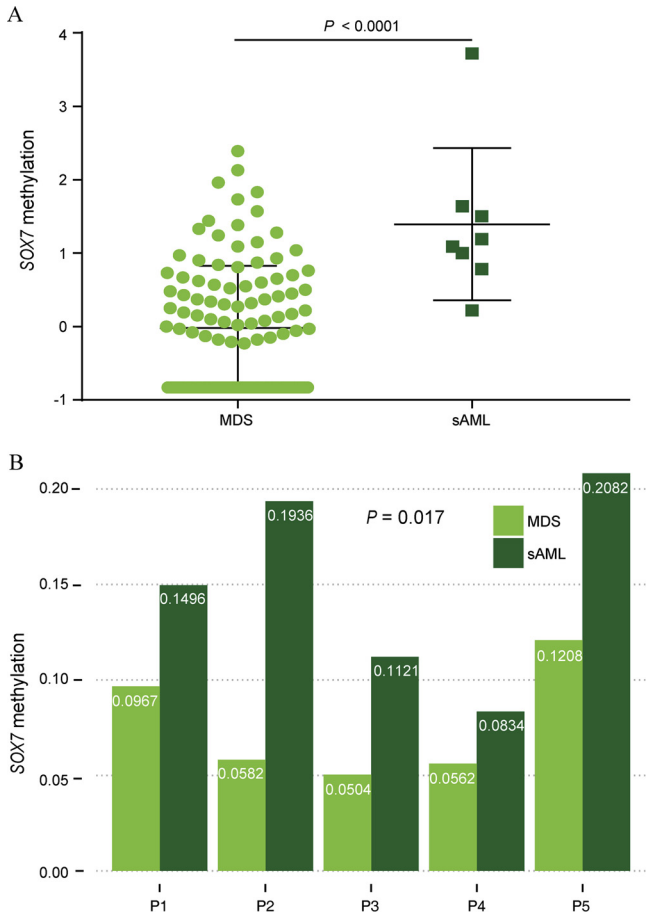

Fig. 5. Alterations in *SOX7* methylation during the progression from MDS to sAML. A: *SOX7* methylation levels in 99 MDS and 8 sAML patients as measured by RQ-MSP. For displaying purpose, SOX7 methylation levels were normalized using the Templeton's two-step method to reduce skewness. The methylation difference was calculated using non-parametric test; B: Dynamic changes of *SOX7* methylation level in 5 paired MDS/sAML patients as measured by RRBS. Comparisons were evaluated by paired Student's t-test. sAML: secondary AML. RRBS: reduced representation bisulfite sequencing.

specificity, not quantitative and false positive result [32,45]. We attempted to overcome these limitations by utilizing a real-time MSP approach combining qPCR with an additional melting step to increase the specificity. We found *SOX7* gene was methylated in 55.6% of our MDS cohort but in none of the healthy controls, which is very similar to the results reported by Fan et al (58.1%). We extended their findings by showing that *SOX7* methylation was significantly increased from MDS to MDS-derived AML, indicating possible association between *SOX7* methylation and MDS progression.

There is increasing evidence that *SOX7* may play as a tumor suppressor which has profound clinical implications [29]. For example, Wang et al. reported that *SOX7* downregulation was associated with pathological grade and poor prognosis in liver cancer [46]. Similarly, Cui et al. demonstrated that *SOX7* expression was significantly correlated with depth of invasion, lymph node metastasis, distant metastasis, and the TNM stage in gastric cancer, it also proved to be an independent predictor of patient survival [25]. While most studies have concentrated on the clinical role of *SOX7* expression, few have explored whether methylation of this gene, a frequent event in several cancers, could be used as a prognosis marker. During the last decades, abnormal promoter CpG island DNA hypermethylation has been widely perceived as a potential biomarker for cancer risk detection, as well as a useful predictor of prognosis and response to chemotherapy in cancer patients [47]. Also, methylation of DNA represents a stable DNA alteration, which could seldom degrade during sample preparation, thus provides a significant advantage over RNA-based methods. To date, there is only one study by Fan et al. regarding the clinical relevance of *SOX7* promoter methylation in MDS patients, which identified that methylation of *SOX7* was associated with age, marrow blast levels, IPSS risk as well as an advanced stage in MDS patients [31]. However, in the present study, only *U2AF1* mutation was found to be significantly associated with *SOX7* methylation. More studies are needed to determine the role of *SOX7* methylation in the progression of MDS.

Many efforts have been made to predict the prognostic impact of the epigenetic alternations, especially DNA methylation in MDS [48]. For instance, Shen et al developed a DNA methylation prognosis model with a combination of 10 genes, which confirmed that patients with a high methylation score had a significantly worse prognosis than those with a low score [13]. With regard to *SOX7* methylation, the previous study demonstrated its prognostic value in univariate analysis, but it had no significant impact on OS in multivariate analysis. Our results, however, found that *SOX7* methylation was an independent prognostic factor both as dichotomous and as continuous variable. Importantly, our final model was selected by the Akaike information criterion (AIC) method, a measure of goodness of fit that has been successfully used by Metze K, et al. [49] to compare predictive performance of different MDS classifications. Furthermore, the model stability was confirmed by Harrell's concordance index after bootstrapping with 1000 resamples of the original data. Interestingly, we observed the adverse impact of *SOX7* methylation on OS in male patients and patients older than 60 years. Since patients with MDS were older (86% of MDS cases were diagnosed in individuals aged 60 years) and over-represented by males-both were associated with worse outcome in MDS [2]. Thus, *SOX7* may provide additional prognostic information for these two groups. Collectively, these results implicate that *SOX7* methylation might play an important role in MDS pathogenesis. Recently, Man et al. discovered that *SOX7* was a negative regulator of the Wnt/β-catenin pathway in AML, and its expression was regulated by DNA hypermethylation; they also found that restoration of *SOX7* expression had antileukemic effects by inhibiting proliferation and reducing the clonogenic activity of leukemia cells [30]. It has been widely accepted that deregulation of Wnt pathway is a hallmark of several tumors, including hematological malignancies [50]. Hypermethylation of Wnt antagonist genes were frequently observed in AML and MDS patients, which proved to be useful prognostic markers [51–54]. Our previous studies have disclosed that SFRPs were hypermethylated in AML and hypermethylation of SFRPs was an independent prognostic factor in both non-M3 AML and CN-AML [55]. Our group also found that NKD2, which proved to be a Wnt antagonist gene, was repressed by promoter hypermethylation and its repression was associated with adverse outcome in AML [56]. These results together indicate that *SOX7* methylation is also involved in the pathogenesis of MDS and may lead to the Wnt pathway dysregulation.

In summary, our results suggest *SOX7* methylation is an independent prognostic factor in MDS patients and could be used as a potential marker to predict outcome in MDS. However, our study was based on a relatively small number of cases, further studies on the prognostic and biological role of *SOX7* methylation in MDS are warranted.

Conflict of interest

The authors declare that there are no conflicts of interest.

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