


RESEARCH ARTICLE

Identification and validation of prognosis-related *DLX5* methylation as an epigenetic driver in myeloid neoplasms

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Abstract

The deregulated *DLX* gene family members *DLX1/2/3/4/5/6* (*DLXs*) caused by DNA methylation has been demonstrated in various cancers with therapeutic target value. However, the potential role of *DLXs* methylation in myeloid neoplasms such as acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) remains to be elucidated. Clinical significance of *DLXs* methylation/expression was analyzed in patient with AML and MDS. The functional roles of *DLXs* were determined in vitro. In the identification stage, we found that lower *DLX5* expression was correlated with prognosis in AML among all *DLXs* analyzed by The Cancer Genome Atlas datasets. In the validation stage, we revealed that reduced *DLX5* expression was frequently occurred, and was also correlated with promoter hypermethylation in AML evaluated by targeted bisulfite sequencing. Epigenetic studies also showed that *DLX5* promoter DNA methylation was associated with its expression. By quantitative polymerase chain reaction, we also validated that *DLX5* hypermethylation was frequent event in both AML and MDS, and also correlated with MDS transformation to leukemia. Moreover, *DLX5* hypermethylation was associated with lower rate of complete remission and shorter time of leukemia-free/overall survival, and was also confirmed by Logistic/Cox regression analysis. Functional studies revealed the antiproliferative and pro-apoptotic effects of *DLX5* in MDS-derived AML cell-line SKM-1. Finally, bioinformatics analysis demonstrated that *DLX5* functioned in leukemogenesis may be

Abbreviations: 5-aza-dC, 5-aza-2'-deoxycytidine; AML, acute myeloid leukemia; AUC, area under the ROC curve; BM, bone marrow; BMMNCs, BM mononuclear cells; CN-AML, cytogenetically normal AML; CR, complete remission; *DLXs*, *DLX* gene family members (*DLX1/2/3/4/5/6*); FAB, French-American-British; LFS, leukemia-free survival; MDS, myelodysplastic syndromes; OS, overall survival; ROC, receiver operating characteristic; RT-qMSP, real-time quantitative methylation-specific PCR; RT-qPCR, real-time quantitative polymerase chain reaction; TCGA, The Cancer Genome Atlas.

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through the association with PI3K/Akt signaling pathway. Collectively, our findings demonstrated that *DLX5* methylation, negatively correlated *DLX5* expression, was a potential prognostic and predictive indicator in patients with AML and MDS, which could also act as an epigenetic driver in myeloid neoplasms.

KEY WORDS

AML, *DLX5*, MDS, methylation, progression

1 | INTRODUCTION

Acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) are clonal diseases of myeloid hematopoietic stem cells, and seen as the most common aggressive diseases of myeloid neoplasms.^{1,2} AML is characterized by normal hematopoietic failure caused by clonal proliferation of unconscionable blasts,¹ whereas MDS is defined as invalid hematopoiesis and high risk of progression into AML, also regarded as a preleukemia disease.² Both AML and MDS are clinically and biologically heterogeneous disorders due to the genetically and molecular diverse.^{1,2} In spite of the recent advances made in the personalized and precision medicine, the outcome of AML and MDS remains poor particularly in high-risk MDS and AML including the MDS-derived AML.^{1,3} Consequently, the identification of molecular alterations as potential biomarkers associated with prognosis and progression in MDS and AML could give better insights into leukemogenesis that allow molecular-based therapies, and finally may improve clinical outcome of AML and MDS.

The *DLX* gene family members *DLX1/2/3/4/5/6* (*DLXs*), which correlated with *Drosophila* distal-less gene, belongs to the homeobox gene subfamily. *DLXs* encode transcription factors that are expressed in the development of embryonic including appendages, nervous system, branchial arches, and hematopoiesis by regulating cell growth and differentiation.^{4,5} To date, a total of six *DLXs* are identified, represented by three clusters, namely, *DLX1/2*, *DLX3/4*, and *DLX5/6*. Accumulating studies have showed the deregulated *DLXs* caused by DNA methylation in various cancers with therapeutic target value.^{6,7} For example, Tong et al revealed that *DLX1* and *DLX4* were frequently hypermethylated, which played a role in silencing gene expression by whole-genome wide methylation analysis and pyrosequencing in chronic lymphocytic leukemia.⁸ The locus-specific hypermethylation of oncogenic homeobox gene *DLX1* gene-body canyon can upregulate its gene expression by pan-cancer analysis.⁹ The homeobox genes (*IRX2*, *DLX2*, and *NKX2-2*) hypermethylation was identified correlated with Luminal A subtype in breast cancer.¹⁰ Additionally,

both *DLX1* and *DLX2* CpG island hypermethylation were shown in human astrocytomas.¹¹ As for *DLX3/4*, studies have reported that reduced *DLX3* expression was mediated by DNA hypermethylation at its promoter region in MLL-AF4 childhood acute lymphoblastic leukemia.¹² Notably, our previous studies have disclosed that *DLX4* hypermethylation was frequently occurred in myeloid neoplasms including AML, MDS as well as chronic myeloid leukemia, and predicted unfavorable prognosis or disease progression.¹³⁻¹⁵ In the cluster *DLX5/6*, the phenomena of *DLX5* hypermethylation was identified in breast cancer, neuroblastoma tumors, and colorectal cancer.¹⁶⁻¹⁸ Until now, the potential role of *DLXs* methylation in myeloid neoplasms remains to be elucidated.

In this investigation, (a) we first identified and validated that *DLX5* decreased expression, of all the *DLXs*, was correlated with prognosis in AML, and may be caused by promoter hypermethylation. (b) Next, by the targeted bisulfite sequencing and real-time quantitative methylation-specific polymerase chain reaction (RT-qMSP), we further validated and confirmed that *DLX5* hypermethylation was frequently occurred in AML and MDS, and observed that *DLX5* methylation was associated with leukemia transformation in MDS. Moreover, *DLX5* methylation predicted unfavorable prognosis in both MDS and AML. (c) Functional studies demonstrated the antiproliferative and pro-apoptotic effects of *DLX5* in MDS-derived AML cell-line SKM-1. (d) Finally, bioinformatics analysis revealed that *DLX5* functioned in leukemogenesis may be through the association with PI3K/Akt signaling pathway.

2 | MATERIALS AND METHODS

2.1 | Cases and samples

The investigation was approved by the Ethics Committee of Affiliated People's Hospital of Jiangsu University. First, we analyzed 200 AML patients (173 cases with *DLXs* expression) downloaded from The Cancer Genome Atlas (TCGA) databases from the Washington University as reported.¹⁹

Second, we analyzed two cohorts of AML and MDS patients as well as controls from our hospital. The first cohort included 111 de novo AML patients, 35 MDS patients, and 25 healthy volunteers, which was used for targeted bisulfite sequencing. The other cohort enrolled 159 AML cases (148 de novo AML and 11 MDS-derived AML), 61 MDS patients, and 46 healthy volunteers, which was used for RT-qMSP. Patients with antecedent hematological diseases (except for MDS-derived AML) or solid tumors or therapy-related diseases were excluded. The diagnosis of AML and MDS was based on the 2008 revised World Health Organization (WHO) criteria and the French-American-British (FAB) classifications.²⁰ The sex and age distribution in case and control group showed no statistical significance. The treatment regimens of these patients were as our previous work.²¹⁻²³ Common gene mutations of these patients were detected previously.²¹⁻²³ Bone marrow (BM) specimens were obtained from all participants at diagnosis time after signing informed consents. Total RNA specimens were extracted from BM mononuclear cells (BMMNCs), separating from BM samples by Lymphocyte Separation Medium (Solarbio, Beijing, China), using TRIzol reagent. Genomic DNA samples were collected from BMMNCs by Puregene Blood Core Kit B (QIAGEN, Duesseldorf, Germany).

2.2 | Targeted bisulfite sequencing

Targeted bisulfite sequencing (called as MethylTarget) was performed to detect *DLX5* methylation density (Genesky Biotechnologies Inc., Shanghai, China). The primers used for *DLX5* are shown in Table S1. A detailed description of the targeted bisulfite sequencing assay was reported previously.²⁴

2.3 | Reverse transcription and real-time quantitative polymerase chain reaction

Reverse transcription was performed using random primers.²¹⁻²³ The program of RT was conducted based on the manufacturer's instructions. Determination of *DLX5* mRNA expression level was evaluated by real-time quantitative polymerase chain reaction (RT-qPCR) using AceQ qPCR SYBR Green Master Mix (Vazyme, Piscataway, NJ). The program of RT-qPCR was performed on a Thermo cycler (ABI 7500, Foster City, CA, USA). Both positive controls (recombinant plasmid) and negative controls (ddH₂O) were added in each PCR reaction. *ABL1* was applied to evaluate relative *DLX5* mRNA expression. The primers used for *DLX5* are shown in Table S1. The relative expression of *DLX5* mRNA was calculated by $2^{-\Delta\Delta CT}$ method.²³

2.4 | Bisulfite modification and RT-qMSP

Genomic DNA was bisulfite converted as reported.²¹⁻²³ The *DLX5* methylation level was detected by RT-qMSP using the same agents as described in RT-qPCR with primers listed in Table S1. Both positive controls (recombinant plasmid) and negative controls (ddH₂O) were added in each PCR reaction. *ALU* was also detected to assess the relative level of *DLX5* methylation. Relative methylation level of *DLX5* was calculated by $2^{-\Delta\Delta CT}$ method.²³

2.5 | Cell-line and culture

The MDS-derived AML cell-line SKM-1 were cultured in 10% fetal calf serum of RPMI 1640 medium, which was grown at 37°C in 5% CO₂ humidified atmosphere.^{25,26}

2.6 | Demethylation studies

SKM-1 cells in 2 mL (5×10^5 cells/mL) were treated with 5-aza-2'-deoxycytidine (5-aza-dC) with a final concentration of 10 μM during 4 days.

2.7 | Cell transfection

Human full-length *DLX5* coding sequence was introduced into the BamHI/AgeI of GV569 vector (GENECHEM, Shanghai, China), and transfected with lentiviruses.

2.8 | Western blot analysis

The procedures of western blotting were conducted as described.^{23,27} The antibodies included in this investigation were rabbit anti-*DLX5* (Abcam, Cambridge, MA, USA) and mouse anti-GAPDH (BOSTER, Wuhan, China).

2.9 | Cell growth assays

Cells were distributed equally to at 96-well plates (5×10^3 per well). After culture for 0, 1, 2, 3, 4, and 5 days, CCK-8 (Dojindo, Kumamoto, Japan) reagent was used to analyze the cell proliferation. The rate of cell growth was calculated as OD (Optical Density) value, which was measured at 450 nm of the absorbance using a microplate reader.

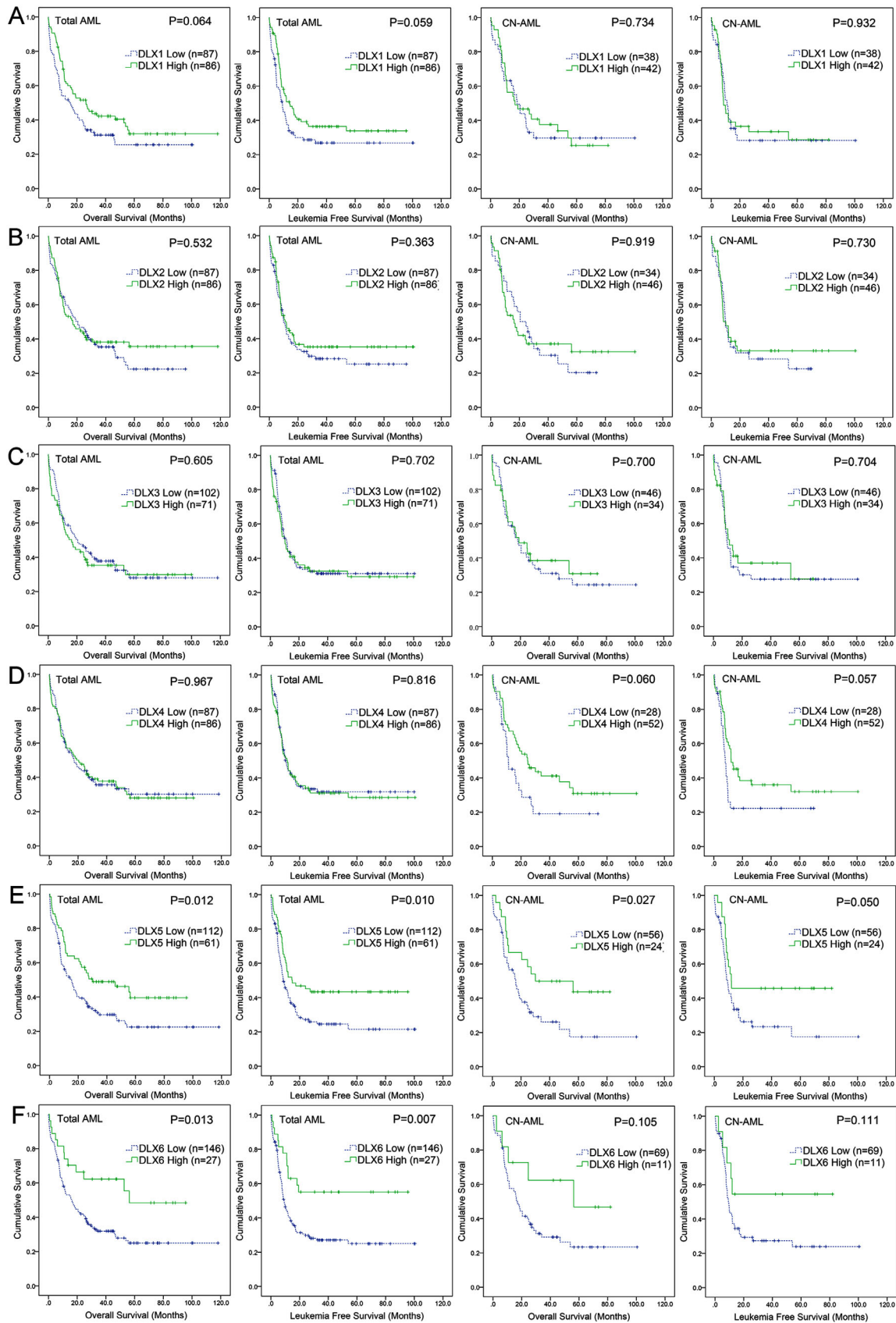


FIGURE 1 Identification of *DLXs* expression associated with prognosis in AML by public database. A-F, *DLX1-6*. The impact of *DLXs* expression on overall survival and leukemia-free survival was determined among whole-cohort AML and cytogenetically normal AML patients from TCGA databases. AML patients were divided into two groups by the median methylation level of each gene, respectively

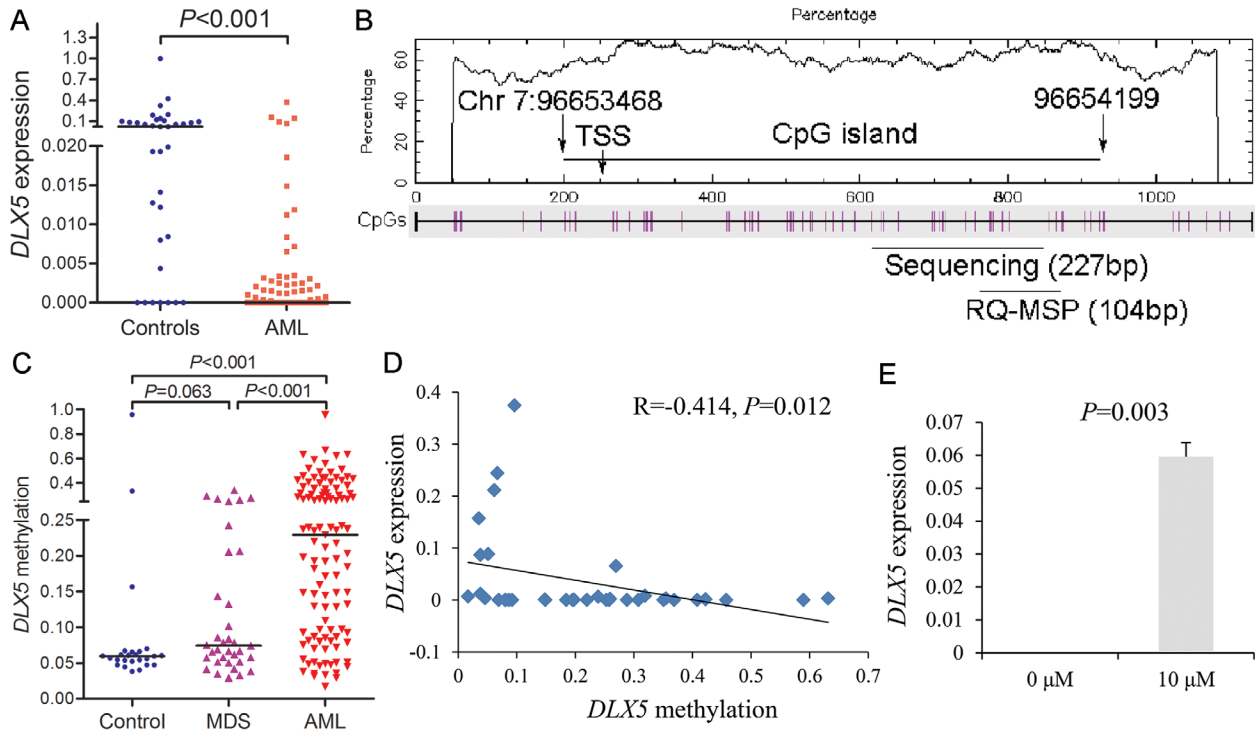


FIGURE 2 Validation of *DLX5* under-expression in AML associated with promoter methylation. A, *DLX5* expression level in controls and AML patients examined by real-time quantitative polymerase chain reaction (RT-qPCR). B, The genomic coordinates (GC) of *DLX5* promoter region CpG island and primer locations. The panel plots the GC content as a percentage of the total. Each vertical bar in the bottom panel represents the presence of a CpG dinucleotide. Black horizontal lines indicate regions amplified by sequencing primer pairs and real-time quantitative methylation-specific polymerase chain reaction (RT-qMSP) primer pairs. CpGplot (<http://emboss.bioinformatics.nl/cgi-bin/emboss/cpgplot>) and Methyl Primer Express v1.0 software were used for creating the figure (TSS, transcription start site). C, *DLX5* methylation density in controls, MDS, and AML patients detected by targeted bisulfite sequencing. D, Correlation between *DLX5* methylation (targeted bisulfite sequencing) and expression (RT-qPCR) in MDS and AML patients. The correlation analysis was conducted by Spearman test. E, *DLX5* expression in MDS-derived AML cell line SKM-1 before and after 10- μ M 5-aza-dC treatment

2.10 | Cell apoptosis analysis

Cells were distributed equally to at 96-well plates (5×10^5 per well). After culture for 2 days, Annexin V-APC Kit was applied to analyze the apoptosis rate based on the recommended protocols, and detected by flow cytometry.

2.11 | Cell cycle analysis

Cells were distributed equally to at 96-well plates (5×10^4 per well). After culture for 2 days, PI/RNase staining buffer Kit was applied to analyze the cell cycle distribution based on the recommended protocols, and detected by flow cytometry.

2.12 | Bioinformatics analysis

The details regarding the bioinformatics analysis of *DLX5* were as reported previously.²⁸

2.13 | Statistical analysis

SPSS software 20.0 and GraphPad Prism 5.0 were used in statistical analysis. The difference of continuous variables in two groups was compared by Mann-Whitney's *U*-test/Student *T*-test, whereas Pearson Chi-square analysis/Fisher exact test was applied to analyze the difference of categorical variables in two groups. The correlation analysis between *DLX5* methylation and expression or *DLX5* methylation density and methylation level was analyzed by Spearman test. The distinguishing value of *DLX5* methylation between AML and controls was analyzed by receiver operating characteristic (ROC) curve and area under the ROC curve (AUC). Complete remission (CR) was evaluated after one or two course of induction chemotherapy. Overall survival (OS) and leukemia-free survival (LFS) of AML and MDS patients were defined as our previous report.²¹ Kaplan-Meier analysis and Cox regression analysis (including univariate and multivariate analysis) were applied to evaluate the prognostic effect of *DLX5* methylation on OS and LFS. The definition of statistical significance was attached when a two-sided $P < .05$.

TABLE 1 Comparison of clinical and laboratory features between *DLX5* hypermethylated and nonhypermethylated AML patients

Patient's features	Nonhypermethylated (n = 80)	Hypermethylated (n = 79)	P-value
Sex, male/female	42/38	56/23	.022
Median age, years (range)	54 (18-85)	57 (18-86)	.367
Median WBC, $\times 10^9/L$ (range)	9.65 (0.3-528.0)	18.15 (0.8-185.4)	.325
Median hemoglobin, g/L (range)	79 (40-144)	77 (32-135)	.803
Median platelets, $\times 10^9/L$ (range)	43 (7-264)	33 (3-447)	.091
Median BM blasts, % (range)	53.5 (1-97.5)	50.5 (6.5-99)	.508
FAB classifications			.139
M0	1	1	
M1	4	4	
M2	27	35	
M3	21	9	
M4	12	18	
M5	12	7	
M6	3	3	
No data	0	2	
Karyotypes			.066
Normal	34	33	
t(8;21)	3	8	
inv(16)	1	0	
t(15;17)	20	8	
+8	2	3	
-5/5q-	0	0	
-7/7q-	0	1	
t(9;22)	1	1	
11q23	0	2	
Complex	10	7	
others	7	8	
No data	2	8	
Gene mutations			
<i>CEBPA</i> (+/-)	0/64	10/51	.001
<i>NPM1</i> (+/-)	7/57	4/57	.531
<i>FLT3</i> -ITD (+/-)	6/58	5/56	1.000
<i>C-KIT</i> (+/-)	1/63	6/55	.058
<i>N/K-RAS</i> (+/-)	3/61	8/53	.121
<i>IDH1/2</i> (+/-)	3/61	0/61	.244
<i>DNMT3A</i> (+/-)	3/61	4/57	.713
<i>U2AF1</i> (+/-)	1/63	2/59	.613
<i>SRSF2</i> (+/-)	2/62	2/59	1.000
<i>SETBP1</i> (+/-)	1/63	1/60	1.000
CR, total AML (+/-)	41/32	26/42	.043
CR, non-M3 AML (+/-)	27/30	20/40	.135
CR, CN-AML (+/-)	19/14	10/19	.081

Note. Patients' blasts less than 20% with t(15;17) cytogenetic aberrations.

Abbreviations: BM, bone marrow; CR, complete remission; FAB, French-American-British classification; WBC, white blood cells.

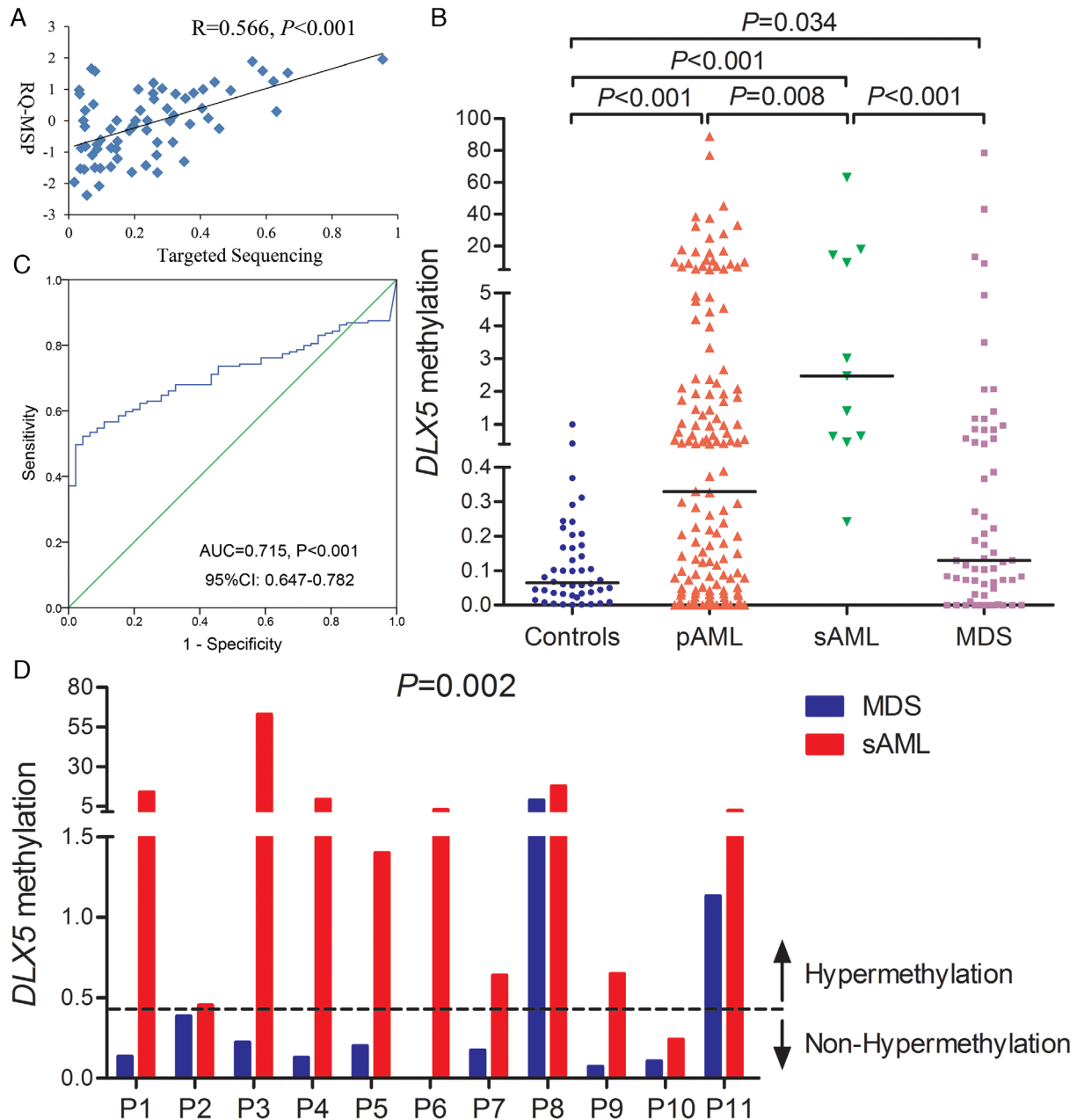


FIGURE 3 Further confirmation of *DLX5* methylation in AML and MDS patients together with its clinical significance. A, The correlation of the candidate gene methylation results between the targeted bisulfite sequencing and real-time quantitative methylation-specific polymerase chain reaction (RT-qMSP). The correlation was analyzed by Spearman correlation test. B, *DLX5* methylation level in larger samples of controls, MDS, pAML, and sAML patients analyzed by RT-qMSP. C, ROC curve analysis using *DLX5* methylation for discriminating AML patients from controls. D, *DLX5* methylation alterations in matched MDS/sAML patients examined by RT-qMSP (pAML, primary AML; sAML, secondary AML, indicated MDS-derived AML patients)

3 | RESULTS

3.1 | Identification of *DLXs* expression associated with prognosis in AML by public database

We first used the public TCGA data to identify the prognosis-related genes of *DLXs* (*DLX1/2/3/4/5/6*) in AML. Prognostic

significance of all *DLXs* was analyzed in two groups divided by the median level of each gene expression (*DLX1/2/3/4/5/6*), respectively. Kaplan-Meier analysis revealed that only *DLX5* expression was positively correlated with OS and LFS in both whole-cohort AML ($P = .012$ and $.010$; Figure 1) and cytogenetically normal AML (CN-AML) patients ($P = .027$ and $.050$; Figure 1), suggesting the negatively prognostic value of *DLX5* expression in AML.

TABLE 2 Comparison of clinical and laboratory features between *DLX5* hypermethylated and nonhypermethylated MDS patients

Patient's features	Nonhypermethylated (n = 50)	Hypermethylated (n = 11)	P-value
Sex (male/female)	26/24	8/3	.317
Median age, years (range)	59 (27-86)	58 (31-78)	.620
Median WBC, $\times 10^9/L$ (range)	2.8 (0.6-82.4)	2.5 (0.7-7.4)	.699
Median hemoglobin, g/L (range)	62 (35-140)	61 (40-107)	.656
Median platelets, $\times 10^9/L$ (range)	61 (0-505)	79 (20-654)	.240
Median BM blasts, % (range)	5 (0-19)	11 (0-18)	.159
WHO classifications			.258
RCUD/RARS	5	2	
RCMD/RCMD-RS	19	1	
RAEB-1	9	2	
RAEB-2	15	6	
MDS with isolated del(5q)	2	0	
MDS-U	0	0	
IPSS scores			.371
Low	6	0	
Int-1	24	5	
Int-2	9	1	
High	6	4	
No data	5	1	
Gene mutations			
<i>CEBPA</i> (+/-)	2/40	0/11	1.000
<i>IDH1/2</i> (+/-)	1/41	0/11	1.000
<i>DNMT3A</i> (+/-)	1/41	0/11	1.000
<i>U2AF1</i> (+/-)	4/38	1/10	1.000
<i>SRSF2</i> (+/-)	1/41	1/10	.375
<i>SF3B1</i> (+/-)	3/39	0/11	1.000

Abbreviations: BM, bone marrow; IPSS, International Prognostic Scoring System; MDS, myelodysplastic syndromes; WBC, white blood cells; WHO, World Health Organization.

3.2 | Validation of *DLX5* under-expression in AML associated with promoter methylation

To validate the expression pattern of *DLX5* in AML, we first performed RT-qPCR to detect *DLX5* mRNA level in 86 de novo AML patients and 35 controls. As showed in Figure 2A, *DLX5* expression was markedly decreased in de novo AML patients ($P < .001$). As reported previously, dysregulation of *DLXs* including *DLX5* caused by DNA methylation in various cancers potentially served as therapeutic targets. We further detected CpG island methylation pattern located at the *DLX5* promoter region (Figure 2B) by targeted bisulfite sequencing in samples of 111 de novo AML patients, 35 MDS patients, and 25 controls. The sequencing mean bait coverage attached 1694 \times , and Q30 was 75.56%. Although no statistical significance was showed of *DLX5* methylation in MDS patients and controls ($P = .063$; Figure 2C), the level of *DLX5* methylation in AML patients was markedly higher than in controls

and MDS patients (both $P < .001$; Figure 2C). Moreover, *DLX5* methylation was observed to be slightly negatively correlated with *DLX5* expression ($R = -.414$, $P = .012$; Figure 2D). In order to confirm the epigenetic mechanism, MDS-derived AML cell line SKM-1 were treated with demethylation agent 5-aza-dC. As a result, the level *DLX5* mRNA was markedly upregulated after 5-aza-dC treatment ($P = .003$; Figure 2E).

3.3 | Further confirmation of *DLX5* methylation by quantitative PCR in AML and MDS

We further expanded the clinical samples (46 controls, 148 primary AML [pAML] and 11 secondary AML [sAML]) to explore clinical implication of *DLX5* methylation using a more rapid and convenient methodology RT-qMSP. The

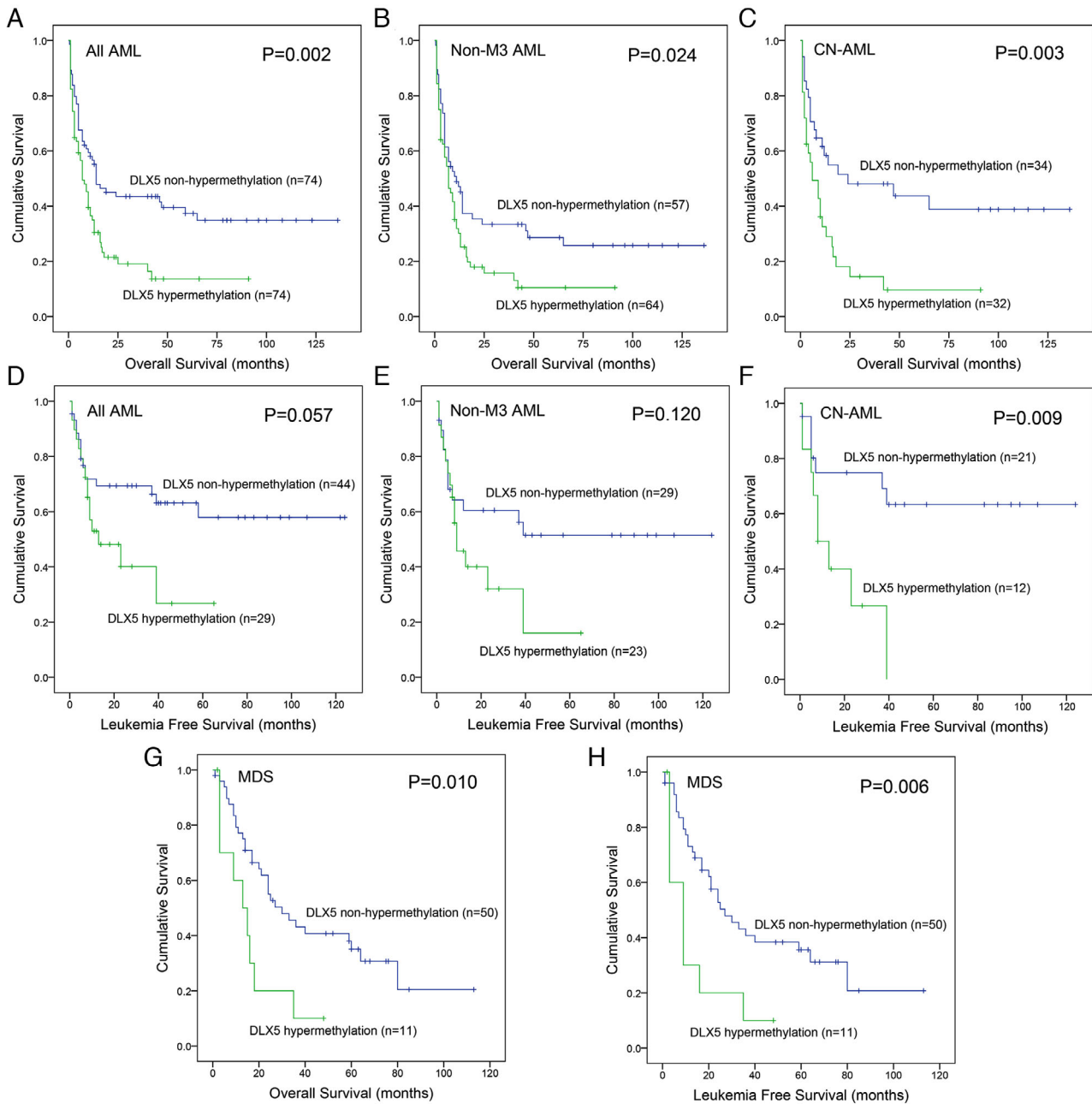


FIGURE 4 Prognostic value of *DLX5* methylation in AML and MDS patients. A-C, The impact of *DLX5* methylation on overall survival among whole-cohort AML, non-M3-AML, and cytogenetically normal AML (CN-AML) patients, respectively. D-F, The impact of *DLX5* methylation on leukemia-free survival among whole-cohort AML, non-M3-AML, and CN-AML patients, respectively. G-H, The impact of *DLX5* methylation on overall survival and leukemia-free survival among MDS patients

primer was designed located inside the sequencing primer (Figure 2B), and RT-qMSP results were positively associated with the results in targeted bisulfite sequencing ($R = .566$, $P < .001$; Figure 3A). According to RT-qMSP, *DLX5* promoter was markedly hypermethylated in MDS, pAML, and sAML patients ($P = .034$, $<.001$, and $<.001$; Figure 3B). Interestingly, sAML patients also showed markedly higher methylation level of *DLX5* than pAML and MDS patients ($P = .008$ and $<.001$; Figure 3B). In addition, *DLX5* methy-

lation may act as a potential marker for distinguishing AML from controls with an AUC of 0.715 analyzed by ROC analysis (95% CI, 0.647-0.782, $P < .001$; Figure 3C). Moreover, the sensitivity was 49.7% and the specificity was 97.8% when *DLX5* methylation set at the cutoff value of 0.425. To analyze the clinical implications of *DLX5* methylation, we divided the MDS and AML patients into two groups (*DLX5* hypermethylation and *DLX5* nonhypermethylation) according to the set cutoff value, respectively. For AML, no significant differ-

TABLE 3 Cox regression analyses of variables for overall survival in CN-AML patients

Variables	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value
<i>DLX5</i> methylation	2.389 (1.306-4.369)	.005	2.345 (1.113-4.942)	.025
Age	2.430 (1.340-4.408)	.003	2.601 (1.283-5.352)	.008
WBC	1.682 (0.930-3.041)	.085	1.878 (0.888-3.971)	.099
<i>CEBPA</i> mutations	6.498 (1.392-30.329)	.017	5.949 (1.132-31.279)	.035
<i>NPM1</i> mutations	0.375 (0.114-1.230)	.105	0.385 (0.082-1.812)	.227
<i>FLT3</i> -ITD mutations	0.664 (0.234-1.884)	.441		
<i>C-KIT</i> mutations	0.044 (0.000-15.404)	.296		
<i>NK-RAS</i> mutations	1.136 (0.398-3.241)	.811		
<i>DNMT3A</i> mutations	0.973 (0.342-2.768)	.960		
<i>U2AF1</i> mutations	2.797 (0.642-12.191)	.171	4.489 (0.935-21.559)	.061
<i>IDH1/2</i> mutations	0.693 (0.094-5.095)	.719		
<i>SRSF2</i> mutations	4.501 (0.995-20.367)	.051	3.105 (0.598-16.120)	.178
<i>SETBP1</i> mutations	8.208 (0.988-68.185)	.067	21.059 (2.179-203.556)	.008

Note. Variables including *DLX5* methylation (hypermethylation vs nonhypermethylation), age (≤ 60 vs > 60 years), WBC ($\geq 30 \times 10^9$ vs $< 30 \times 10^9$ /L), and gene mutations (mutant vs wild type). Multivariate analysis includes variables with $P < .200$ in univariate analysis.

ences were observed between two groups regarding age, WBC (white blood cells), HB (hemoglobin), PLT (platelets), as well as FAB and karyotype classifications ($P > .05$; Table 1). However, *DLX5* hypermethylation was associated with male patients ($P = .022$; Table 1). Among gene mutations, *DLX5* hypermethylation was associated *CEBPA* and *c-KIT* mutations ($P = .001$ and $.058$; Table 1). There were no association of *DLX5* hypermethylation with other gene mutations ($P > .05$; Table 1). For MDS patients, none of the clinical characteristics were found to be associated *DLX5* hypermethylation ($P > .05$; Table 2).

3.4 | *DLX5* methylation correlated with leukemia transformation in MDS

As presented above, sAML patients also showed markedly higher methylation level of *DLX5* than pAML and MDS patients ($P = .008$ and $< .001$; Figure 3B). We deduced that *DLX5* methylation may be correlated with disease evolution in MDS. To verify the hypothesis, we further detected *DLX5* methylation in 11 matched patients during evolution from MDS to AML (see patients' details in Table S2). As expected, the level of *DLX5* methylation was markedly upregulated at sAML stage than that at MDS stage in all matched patients ($P = .002$; Figure 3D).

3.5 | *DLX5* methylation was associated with prognosis in MDS and AML

For AML patients, we first observed the significant association of *DLX5* methylation with the rate of CR. Among whole-

cohort AML, the CR rate in *DLX5* hypermethylated patients was markedly lower than that in *DLX5* nonhypermethylated patients ($P = .043$; Table 1). In non-M3 AML and CN-AML, we did not observe the differences for CR between *DLX5* hypermethylated and nonhypermethylated patients ($P = .135$ and $.081$; Table 1). In addition, Logistic regression analysis including variables presented in Table S3 revealed that *DLX5* hypermethylation may be acted as a negative risk factor independently affecting CR in whole-cohort AML ($P = .067$; Table S3). We next analyzed the prognostic value of *DLX5* methylation on AML survival (including LFS and OS). Kaplan-Meier analysis demonstrated that *DLX5* hypermethylated cases had shorter OS time than *DLX5* nonhypermethylated cases among total AML, non-M3 AML, and CN-AML patients ($P = .002$, $.024$, and $.003$; Figure 4A-C). For LFS, due the limited cases, significant difference was only observed in CN-AML but not in total AML and non-M3 AML between two groups ($P = .009$, $.057$, and $.120$; Figure 4D-F). Furthermore, Cox regression analysis including variables presented in Tables 3 and S4 demonstrated that *DLX5* hypermethylation was a negatively prognostic factor independently affecting OS among whole-cohort AML with a trend ($P = .071$; Table S4) and CN-AML patients ($P = .025$; Table 3).

For MDS patients, we analyzed the prognostic value of *DLX5* methylation on MDS survival (also including OS and LFS). Kaplan-Meier analysis revealed that *DLX5* hypermethylated patients presented markedly shorter OS and LFS than *DLX5* nonhypermethylated patients ($P = .010$ and $.006$; Figures 4G and 4H). In addition, Cox regression analysis including variables presented in Table 4 showed that *DLX5* hypermethylation also acted as an independently prognostically poor indicator for OS and LFS in MDS patients ($P = .038$ and $.030$; Table 4).

TABLE 4 Cox regression analyses of variables for overall survival and leukemia-free survival in MDS patients

Variables	Univariate analyses		Multivariate analyses	
	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value
Overall survival				
<i>DLX5</i> methylation	2.602 (1.211-5.589)	.014	2.340 (1.050-5.217)	.038
Age	1.888 (1.000-3.564)	.050	1.862 (0.948-3.658)	.071
IPSS risks	1.523 (1.063-2.181)	.022	1.369 (0.949-1.976)	.093
<i>CEBPA</i> mutation	0.044 (0.000-14.199)	.289		
<i>IDH1/2</i> mutation	1.416 (0.192-10.469)	.733		
<i>DNMT3A</i> mutation	1.696 (0.228-12.601)	.606		
<i>U2AF1</i> mutation	1.107 (0.389-3.153)	.849		
<i>SF3B1</i> mutation	0.602 (0.082-4.424)	.618		
<i>SRSF2</i> mutations	2.712 (0.627-11.724)	.182		
Leukemia-free survival				
<i>DLX5</i> methylation	2.744 (1.280-5.884)	.009	2.394 (1.088-5.268)	.030
Age	2.014 (1.063-3.814)	.032	2.061 (1.037-4.093)	.039
IPSS risks	1.612 (1.113-2.336)	.012	1.518 (1.039-2.219)	.031
<i>CEBPA</i> mutation	0.044 (0.000-14.314)	.289		
<i>IDH1/2</i> mutation	1.427 (0.193-10.550)	.728		
<i>DNMT3A</i> mutation	2.251 (0.300-16.877)	.430		
<i>U2AF1</i> mutation	1.207 (0.424-3.436)	.724		
<i>SF3B1</i> mutation	0.554 (0.075-4.063)	.561		
<i>SRSF2</i> mutations	2.755 (0.642-11.826)	.173		

Note. Variables including age (≤ 60 vs > 60 years old), IPSS scores (low vs Int-1 vs Int-2 vs high), *DLX5* methylation (nonhypermethylated vs hypermethylated), and gene mutations (mutant vs wild type). Multivariate analysis includes variables with $P < .100$ in univariate analysis.

Abbreviation: IPSS, International Prognostic Scoring System.

3.6 | *DLX5* exhibited antiproliferative and pro-apoptotic effects in SKM-1 cells

To determine the potential role of *DLX5* in MDS progression, we performed gain-of-function experiments in vitro through MDS-derived AML cell-line SKM-1. We established SKM-1 cells stably overexpressing *DLX5* confirmed by RT-qPCR and Western blot (Figures 5A and 5B). The proliferation of SKM-1 cells was markedly inhibited by *DLX5* overexpression (Figure 5C), and caused G0/G1 arrest (Figures 5D and S1). Moreover, we also observed an increased ratio of apoptosis in SKM-1 cells when *DLX5* overexpressed (Figure 5E).

3.7 | Molecular insights of *DLX5* in AML

To gain better understanding of biological insights of *DLX5* in leukemogenesis, we first analyzed the differences of transcriptomes of low- and high-expression *DLX5* groups among 200 AML patients from TCGA databases. We identified 634 differentially expressed genes (FDR < 0.05 , $P < .05$, and \log_2 FCI > 1.5 ; Figures 6A and 6B; Supporting Information 1) to be associated with *DLX5* expression. A total of 515

positively correlated genes including *ID4*, *SLIT2/3*, *EBF3*, *WNT5A*, *DKK1*, and *SOX6* were reported with antileukemia effects.^{23,29-31} Furthermore, the analysis of gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment revealed that these genes were involved in system development, cell adhesion, growth factor binding, and PI3K-Akt signaling (Figure 6C).

Next, we compared the differences of noncoding small RNA microRNA expression signatures between lower and higher expression *DLX5* groups. We identified 45 differentially expressed microRNAs including 40 positively associated and five negatively associated (FDR < 0.05 , $P < .05$, and \log_2 FCI > 1.5 ; Figure 6D; Supporting Information 2). Negatively correlated microRNAs such as *miR-100*, *miR-134*, *miR-504*, *miR-495*, and *miR-494* were reported with antileukemia roles in previous works.³²⁻³⁷

4 | DISCUSSION

Epigenetic modifications especially in DNA methylation not only involved in leukemogenesis but also served as potential biological markers for AML and MDS patients' diagnosis and prognosis. Therefore, identifying more aber-

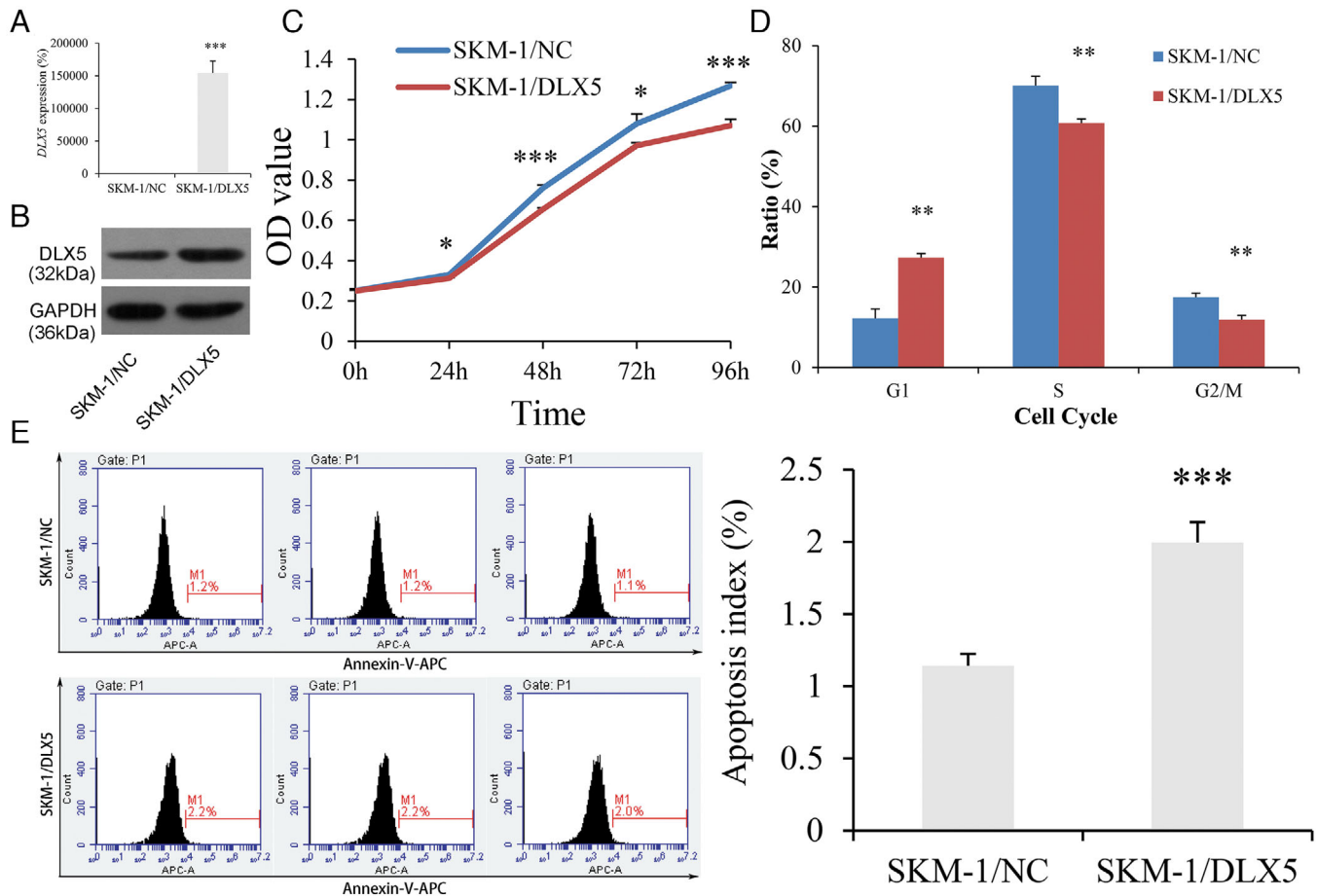


FIGURE 5 Biological functions of *DLX5* in MDS-derived AML cell line SKM-1. A, Confirmation of *DLX5* mRNA level in SKM-1 after transfection by real-time quantitative polymerase chain reaction (RT-qPCR). B, Confirmation of *DLX5* overexpression in SKM-1 after transfection by western blot. C, The proliferation ability in SKM-1 affected by *DLX5* overexpression. D, The cell cycle in SKM-1 affected by *DLX5* overexpression. E, The apoptosis ability in SKM-1 affected by *DLX5* overexpression. * $P < .05$; ** $P < .01$; *** $P < .001$

rant DNA methylation alterations in AML and MDS could make better understanding of leukemogenesis. In the current investigation, we for the first time identified and verified that prognosis-related *DLX5* expression, screened among all *DLXs* members by public data, was regulated by DNA methylation in AML. By targeted bisulfite sequencing and RT-qPCR, we further confirmed that *DLX5* hypermethylation was a common event in myeloid neoplasms, and could see as a potential biological marker used in predicting disease prognosis in AML and MDS. Although it is the first time to reveal *DLX5* hypermethylation in myeloid neoplasms, previous study has also proved the phenomena of *DLX5* methylation in breast cancer and neuroblastoma tumors by genome-wide methylation analysis.^{16,17} All the results suggested the crucial role of the DNA methylation-mediated *DLX5* silencing during oncogenesis. However, both AML and MDS are heterogeneous diseases, and *DLX5* methylation pattern were not analyzed in single tumor cell population such as CD34+ cell population, leading to a huge difference of *DLX5* methylation in patients with AML and MDS. Accordingly, further studies are

required to validate our results before we can use the potential biological markers for risk stratification and planning therapy in AML and MDS.

With regard to prognosis, another important finding in this study was that *DLX5* methylation was associated with MDS progression. Substantial progresses have been made in the understanding of the potential mechanism underlying MDS progression. Genetic alterations especially in gene mutations played vital roles in the disease progression of MDS.^{38,39} Mutations in *TP53*, *TET2*, *IDH1*, *IDH2*, *DNMT3A*, *EZH2*, *ASXL1*, and *ROBO1/2* were considered as progression-related drivers in MDS.^{29,38,39} Recently, epigenetic changes especially in DNA methylation also have been discovered to be involved in disease progression of MDS.⁴⁰⁻⁴² Jiang et al reported that abnormal DNA methylation, more frequently occurred than cytogenetic abnormalities, was the major mechanism for silencing tumor suppressor genes and clonal variation in MDS evolution to AML.⁴² Moreover, our study and other investigators also showed that *CDKN2B*, *SOCS1*, *NR4A2*, *ABAT*, *ID4*, *GPX3*,

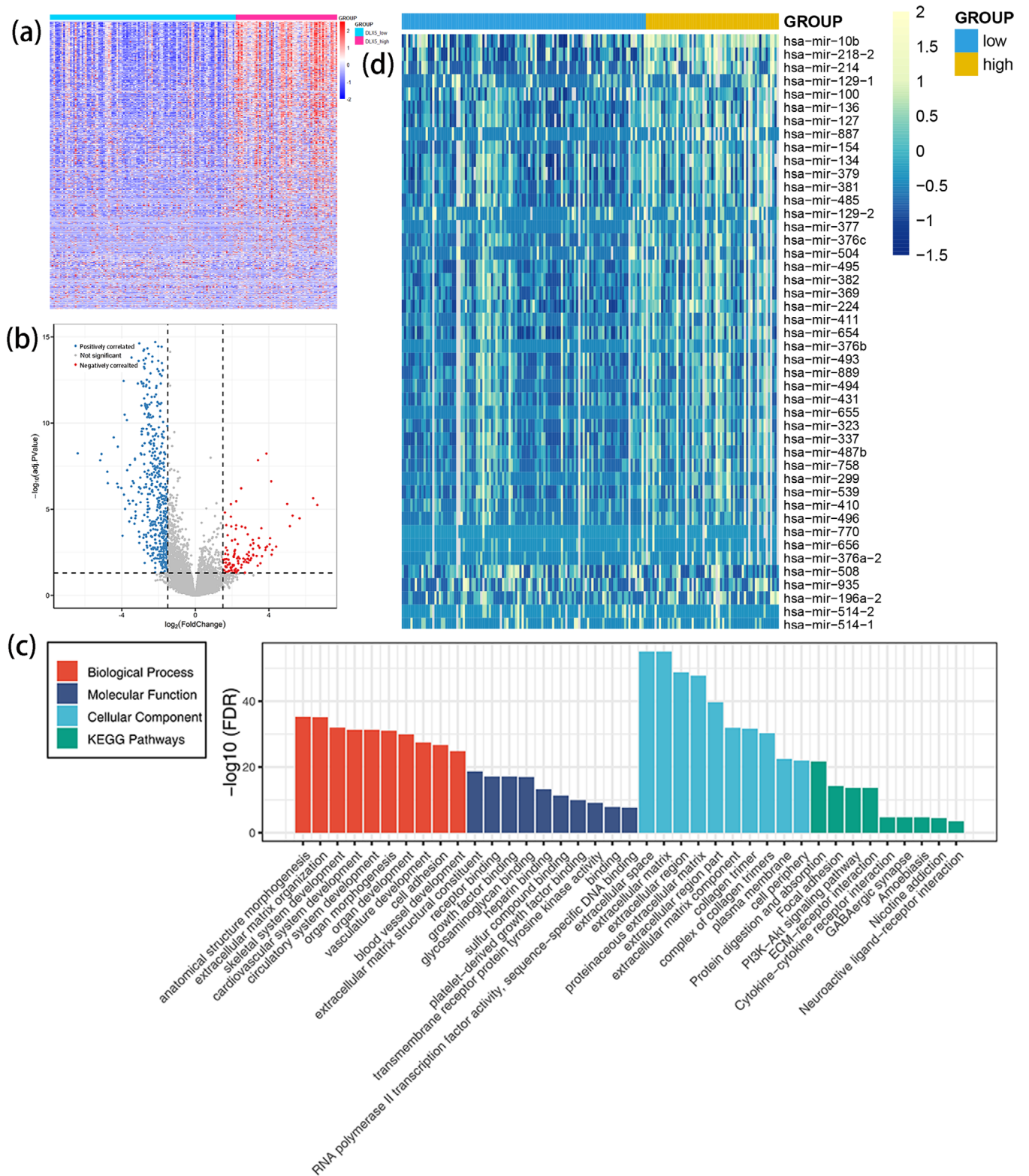


FIGURE 6 Molecular signatures associated with *DLX5* in AML. A, Expression heatmap of differentially expressed genes between lower and higher expressed *DLX5* in AML patients among TCGA (The Cancer Genome Atlas) datasets (FDR < 0.05, $P < .05$, and $\log_2 \text{FCI} > 1.5$). B, Volcano plot of differentially expressed genes between lower and higher expressed *DLX5* in AML patients among TCGA datasets (FDR < 0.05, $P < .05$, and $\log_2 \text{FCI} > 1.5$). C, Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of differentially expressed genes by using online website of STRING (<http://string-db.org>). D, Expression heatmap of differentially expressed microRNAs between lower and higher expressed *DLX5* in AML patients among TCGA datasets (FDR < 0.05, $P < .05$, and $\log_2 \text{FCI} > 1.5$)

and *SOX30* were associated with MDS progression by signal gene analysis.^{21,23,43-47} These results further give insights in the understanding of the epigenomes of MDS during disease progression, and may provide a theoretical basis for using the efficacy of DNMT inhibitors in MDS patients against disease progression.

In accordance with the clinical studies, the functional studies in vitro showed the antileukemia effects of *DLX5* by affecting cell proliferation and cell apoptosis in MDS-derived AML cell-line SKM-1. Bioinformatics analysis demonstrated that *DLX5* functioned in leukemogenesis may be through the association with PI3K/Akt signaling pathway. However, several studies indicated the oncogenic role of *DLX5* in other human cancers. Tan et al demonstrated that *DLX5* overexpression promoted cell proliferation by enhancing IRS-2-AKT signaling in ovarian cancer.⁴⁸ In T-cell lymphomas, Xu et al reported that *DLX5* could induce tumor cell proliferation by upregulating *MYC* by directly binding to the *MYC* promoter.⁴⁹ Also, Tan et al showed that *DLX5* by directly transactivating Notch signaling and upregulating Akt signaling derived murine T-cell lymphomagenesis.⁵⁰ All these results suggested that the function of *DLX5* may be specific in different cancer types, and further studies are required to evaluate the biological functions in diverse human cancers.

5 | CONCLUSION

In summary, our findings demonstrated that *DLX5* methylation, negatively correlated *DLX5* expression, was a potential prognostic and predictive indicator in patients with AML and MDS, which could also act as an epigenetic driver in myeloid neoplasms.

ETHICAL APPROVAL

The present study was approved by the Ethics Committee and Institutional Review Board of the Affiliated People's Hospital of Jiangsu University. Written informed consents were obtained from all enrolled individuals prior to their participation.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

Jing-dong Zhou and Jun Qian conceived and designed the experiments. Ting-juan Zhang and Yu Gu performed the experiments. Jing-dong Zhou and Zi-jun Xu analyzed the data. Wei Zhang collected the clinical data. Jun Qian, Jing-dong Zhou, Jiang Lin, Ji-chun Ma, Xiang-mei Wen, Zhao-qun Deng, Wei Zhang, and Jia-yan Leng offered the technical and funding support. Jing-dong Zhou wrote the paper. All authors read and approved the final manuscript.

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REFERENCES

- Döhner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. *N Engl J Med*. 2015;373(12):1136-1152.
- Adès L, Itzykson R, Fenaux P. Myelodysplastic syndromes. *Lancet*. 2014;383:2239-2252.
- Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood*. 2017;129(4):424-447.
- Takechi M, Adachi N, Hirai T, Kuratani S, Kuraku S. The DLX genes as clues to vertebrate genomics and craniofacial evolution. *Semin Cell Dev Biol*. 2013;24(2):110-118.
- Kraus P, Lufkin T. DLX homeobox gene control of mammalian limb and craniofacial development. *Am J Med Genet A*. 2006;140(13):1366-1374.
- Abate-Shen C. Deregulated homeobox gene expression in cancer: cause or consequence. *Nat Rev Cancer*. 2002;2(10):777-785.
- Samuel S, Naora H. Homeobox gene expression in cancer: insights from developmental regulation and deregulation. *Eur J Cancer*. 2005;41(16):2428-2437.
- Tong WG, Wierda WG, Lin E, et al. Genome-wide DNA methylation profiling of chronic lymphocytic leukemia allows identification of epigenetically repressed molecular pathways with clinical impact. *Epigenetics*. 2010;5(6):499-508.
- Su J, Huang YH, Cui X, et al. Homeobox oncogene activation by pan-cancer DNA hypermethylation. *Genome Biol*. 2018;19(1):108.
- Kamalakaran S, Varadan V, Giercksky Russnes HE, et al. DNA methylation patterns in luminal breast cancers differ from non-luminal subtypes and can identify relapse risk independent of other clinical variables. *Mol Oncol*. 2011;5(1):77-92.
- Wu X, Rauch TA, Zhong X, et al. CpG island hypermethylation in human astrocytomas. *Cancer Res*. 2010;70(7):2718-2727.

12. Campo Dell'Orto M, Banelli B, Giarin E, et al. Down-regulation of DLX3 expression in MLL-AF4 childhood lymphoblastic leukemias is mediated by promoter region hypermethylation. *Oncol Rep.* 2007;18(2):417-423.
13. Zhou JD, Zhang TJ, Wang YX, et al. DLX4 hypermethylation is a prognostically adverse indicator in de novo acute myeloid leukemia. *Tumour Biol.* 2016;37(7):8951-8960.
14. Zhang TJ, Zhou JD, Yang DQ, et al. Hypermethylation of DLX4 predicts poor clinical outcome in patients with myelodysplastic syndrome. *Clin Chem Lab Med.* 2016;54(5):865-871.
15. Zhou JD, Wang YX, Zhang TJ, et al. Epigenetic inactivation of DLX4 is associated with disease progression in chronic myeloid leukemia. *Biochem Biophys Res Commun.* 2015;463(4):1250-1256.
16. Karsli-Ceppioglu S, Dagdemir A, Judes G, et al. The epigenetic landscape of promoter genome-wide analysis in breast cancer. *Sci Rep.* 2017;7(1):6597.
17. Olsson M, Beck S, Kogner P, Martinsson T, Carén H. Genome-wide methylation profiling identifies novel methylated genes in neuroblastoma tumors. *Epigenetics.* 2016;11(1):74-84.
18. Mitchell SM, Ross JP, Drew HR, et al. A panel of genes methylated with high frequency in colorectal cancer. *BMC Cancer.* 2014;14:54.
19. Cancer Genome Atlas Research Network, Ley TJ, Miller C, et al. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N Engl J Med.* 2013;368(22):2059-2074.
20. Vardiman JW, Thiele J, Arber DA, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood.* 2009;114(5):937-951.
21. Zhou JD, Wang YX, Zhang TJ, et al. Identification and validation of SRY-box containing gene family member SOX30 methylation as a prognostic and predictive biomarker in myeloid neoplasms. *Clin Epigenetics.* 2018;10:92.
22. Zhang TJ, Zhou JD, Zhang W, et al. H19 overexpression promotes leukemogenesis and predicts unfavorable prognosis in acute myeloid leukemia. *Clin Epigenetics.* 2018;10:47.
23. Zhou JD, Zhang TJ, Li XX, et al. Epigenetic dysregulation of ID4 predicts disease progression and treatment outcome in myeloid neoplasms. *J Cell Mol Med.* 2017;21(8):1468-1481.
24. Xu ZJ, Ma JC, Zhou JD, et al. Reduced protocadherin17 expression in leukemia stem cells: the clinical and biological effect in acute myeloid leukemia. *J Transl Med.* 2019;17(1):102.
25. Nakagawa T, Matozaki S. The SKM-1 leukemic cell line established from a patient with progression to myelomonocytic leukemia in myelodysplastic syndrome (MDS)-contribution to better understanding of MDS. *Leuk Lymphoma.* 1995;17(3-4):335-339.
26. Nakagawa T, Matozaki S, Murayama T, et al. Establishment of a leukaemic cell line from a patient with acquisition of chromosomal abnormalities during disease progression in myelodysplastic syndrome. *Br J Haematol.* 1993;85(3):469-476.
27. Lin J, Ma JC, Yang J, et al. Arresting of miR-186 and releasing of H19 by DDX43 facilitate tumorigenesis and CML progression. *Oncogene.* 2018;37(18):2432-2443.
28. Zhou JD, Zhang TJ, Xu ZJ, et al. BCL2 overexpression: clinical implication and biological insights in acute myeloid leukemia. *Diagn Pathol.* 2019;14(1):68.
29. Xu F, Wu LY, Chang CK, et al. Whole-exome and targeted sequencing identify ROBO1 and ROBO2 mutations as progression-related drivers in myelodysplastic syndromes. *Nat Commun.* 2015;6:8806.
30. Tao YF, Xu LX, Lu J, et al. Early B-cell factor 3 (EBF3) is a novel tumor suppressor gene with promoter hypermethylation in pediatric acute myeloid leukemia. *J Exp Clin Cancer Res.* 2015;34:4.
31. Zhou HR, Fu HY, Wu DS, et al. Relationship between epigenetic changes in Wnt antagonists and acute leukemia. *Oncol Rep.* 2017;37(5):2663-2671.
32. Barbarani G, Fugazza C, Barabino SML, Ronchi AE. SOX6 blocks the proliferation of BCR-ABL1+ and JAK2V617F+ leukemic cells. *Sci Rep.* 2019;9(1):3388.
33. Zheng YS, Zhang H, Zhang XJ, et al. MiR-100 regulates cell differentiation and survival by targeting RBSP3, a phosphatase-like tumor suppressor in acute myeloid leukemia. *Oncogene.* 2012;31(1):80-92.
34. Chen K, Chen Y, Chen Z, et al. miR-134 increases the antitumor effects of cytarabine by targeting Mnks in acute myeloid leukemia cells. *Onco Targets Ther.* 2018;11:3141-3147.
35. Li SM, Zhao YQ, Hao YL, Liang YY. Upregulation of miR-504-3p is associated with favorable prognosis of acute myeloid leukemia and may serve as a tumor suppressor by targeting MTHFD2. *Eur Rev Med Pharmacol Sci.* 2019;23(3):1203-1213.
36. Jiang X, Huang H, Li Z, et al. MiR-495 is a tumor-suppressor microRNA down-regulated in MLL-rearranged leukemia. *Proc Natl Acad Sci USA.* 2012;109(47):19397-19402.
37. Tian C, Zheng G, Zhuang H, et al. MicroRNA-494 activation suppresses bone marrow stromal cell-mediated drug resistance in acute myeloid leukemia cells. *J Cell Physiol.* 2017;232(6):1387-1395.
38. Dan C, Chi J, Wang L. Molecular mechanisms of the progression of myelodysplastic syndrome to secondary acute myeloid leukaemia and implication for therapy. *Ann Med.* 2015;47(3):209-217.
39. Bravo GM, Lee E, Merchan B, Kantarjian HM, García-Manero G. Integrating genetics and epigenetics in myelodysplastic syndromes: advances in pathogenesis and disease evolution. *Br J Haematol.* 2014;166(5):646-659.
40. Qin T, Sotzen J, Rampal RK, et al. Risk of disease progression in low-risk MDS is linked to distinct epigenetic subtypes. *Leukemia.* 2019;33(11):2753-2757.
41. Figueroa ME, Skrabanek L, Li Y, et al. MDS and secondary AML display unique patterns and abundance of aberrant DNA methylation. *Blood.* 2009;114(16):3448-3458.
42. Jiang Y, Dunbar A, Gondek LP, et al. Aberrant DNA methylation is a dominant mechanism in MDS progression to AML. *Blood.* 2009;113(6):1315-1325.
43. Tien HF, Tang JH, Tsay W, et al. Methylation of the p15(INK4B) gene in myelodysplastic syndrome: it can be detected early at diagnosis or during disease progression and is highly associated with leukaemic transformation. *Br J Haematol.* 2001;112(1):148-154.
44. Wu SJ, Yao M, Chou WC, et al. Clinical implications of SOCS1 methylation in myelodysplastic syndrome. *Br J Haematol.* 2006;135(3):317-323.
45. Wong YF, Micklem CN, Taguchi M, et al. Longitudinal analysis of DNA methylation in CD34+ hematopoietic progenitors in myelodysplastic syndrome. *Stem Cells Transl Med.* 2014;3(10):1188-1198.
46. Zhao G, Li N, Li S, Wu W, Wang X, Gu J. High methylation of the 4-aminobutyrate aminotransferase gene predicts a poor prognosis in patients with myelodysplastic syndrome. *Int J Oncol.* 2019;54(2):491-504.

47. Zhou JD, Lin J, Zhang TJ, et al. GPX3 methylation in bone marrow predicts adverse prognosis and leukemia transformation in myelodysplastic syndrome. *Cancer Med.* 2017;6(1):267-274.
48. Tan Y, Cheung M, Pei J, Menges CW, Godwin AK, Testa JR. Upregulation of DLX5 promotes ovarian cancer cell proliferation by enhancing IRS-2-AKT signaling. *Cancer Res.* 2010;70(22):9197-9206.
49. Xu J, Testa JR. DLX5 (distal-less homeobox 5) promotes tumor cell proliferation by transcriptionally regulating MYC. *J Biol Chem.* 2009;284(31):20593-20601.
50. Tan Y, Sementino E, Xu J, et al. The homeoprotein DLX5 drives murine T-cell lymphomagenesis by directly transactivating Notch and upregulating Akt signaling. *Oncotarget.* 2017;8(9):14941-14956.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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