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DNMT3A intragenic hypomethylation is associated with adverse prognosis in acute myeloid leukemia

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ABSTRACT

DNA methyltransferase 3A (*DNMT3A*), a member of de novo methyltransferases, has been found with overexpression in several cancers including acute myeloid leukemia (AML). The present study was aimed to investigate the methylation status of *DNMT3A* intragenic differentially methylated region 2 (DMR2) using real-time quantitative methylation-specific PCR (RQ-MSP) and analyze its clinical significance in AML. Aberrant hypomethylation of *DNMT3A* gene was found in 55.3% (84/152) of AML cases, but the status of *DNMT3A* hypomethylation was not correlated with the expression of four *DNMT3A* isoforms as well as *DNMT3A* mutation. There was no significant difference in the rates of complete remission (CR) between patients with and without *DNMT3A* hypomethylation. However, the patients with *DNMT3A* hypomethylation had shorter overall survival (OS) time than those without *DNMT3A* hypomethylation (7 months vs 11 months, $P = 0.034$). Moreover, the patients with *DNMT3A* hypomethylation also showed significantly shorter OS than those without *DNMT3A* hypomethylation in cytogenetically normal AML (CN-AML) (7 months vs 25 months, $P = 0.011$). Multivariate analysis confirmed the independent adverse impact of *DNMT3A* hypomethylation in CN-AML. Our data suggest that *DNMT3A* DMR2 hypomethylation is a negative prognostic hallmark in CN-AML.

1. Introduction

Acute myeloid leukemia (AML) represents a clinically and biologically heterogeneous group of malignant disorders derived from uncontrolled proliferation of hematopoietic precursors [1,2]. Leukemogenesis involves complex molecular events triggered by diverse alterations of genomic DNA. Chromosome translocations, identified in specific subtypes of AML, generate fusion genes critical to the pathogenesis of AML [3–5]. The karyotype alterations provide the most helpful prognostic information for induction response and overall survival (OS) [6–8]. However, more than 40% of adults AML without abnormal karyotypes are a largely clinically and biologically heterogeneous population [9].

In recent years, many acquired gene mutations have been identified in AML, especially in cytogenetically normal AML (CN-AML), such as mutations in *NPM1*, *FLT3*, *C/KIT*, *C/EBPA*, *RUNX1*, *WT1*, *DNMT3A*, *ASXL1*, and *IDH1/2* genes [10–18]. Some of these mutations implicated into the risk stratification and treatment choice of AML [19]. *DNMT3A* is one of de novo methyltransferases which are essential for normal development, chromosome stability, maintaining gene expression states and proper telomere length [20–23]. DNA methylation plays a pivotal role in normal hematopoietic development and aberrant methylation is extensively discovered in hematopoietic neoplasms including AML [24,25]. In mice model, conditional ablation of *DNMT3A* has been shown to expand the hematopoietic stem cell (HSC) pool and to impair HSC differentiation [26]. Furthermore, in patients with AML, mutation in *DNMT3A* gene has been suggested to be an adverse prognostic factor [11,27]. Currently, *DNMT3A* intragenic differentially methylated region 2 (DMR2) hypermethylation was reported to be associated with significantly shorter event-free survival and shorter OS only in the data set of TCGA [28]. The current study was aimed to investigate the methylation status of *DNMT3A* DMR2 and further analyze its clinical relevance in Chinese AML patients.

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Table 1
Correlation between *DNMT3A* methylation and patients' parameters.

Patient's parameter	The status of *DNMT3A* methylation			
	Hypomethylated ($n=84$)	Hypermethylated ($n=68$)	Total ($n=152$)	*P* value
Age (years)[a]	56.0 (10–86)	54.0 (17–87)	55.0 (10–87)	0.631
Sex (male/female)	57/27	41/27	98/54	0.395
WBC ($\times10^9$/l)[a]	18.30 (0.9–244.4)	21.00 (0.8–528.0)	18.55 (0.8–528.0)	0.701
Hemoglobin (g/l)[a]	75.00 (32–147)	74.50 (34–138)	74.50 (32–147)	0.946
Platelets ($\times10^9$/l)[a]	38.50 (3–203)	40.00 (9–447)	40.00 (3–447)	0.567
FAB				0.622
M0	0 (0%)	1 (100%)	1	
M1	7 (50%)	7 (50%)	14	
M2	35 (58%)	25 (42%)	60	
M3	10 (63%)	6 (37%)	16	
M4	16 (44%)	20 (56%)	36	
M5	11 (61%)	7 (39%)	18	
M6	5 (71%)	2 (29%)	7	
Karyotyping				0.818
Normal	40 (55%)	33 (45%)	73	
t (8;21)	10 (63%)	6 (37%)	16	
t (15;17)	10 (63%)	6 (37%)	16	
11q23	1 (33%)	2 (67%)	3	
Complex	9 (64%)	5 (36%)	14	
Other	10 (43%)	13 (57%)	23	
No data	4 (57%)	3 (43%)	7	
Karyotype classification				0.741
Favorable	21 (64%)	12 (36%)	33	
Intermediate	49 (53%)	44 (47%)	93	
Poor	10 (53%)	9 (47%)	19	
No data	4 (57%)	3 (43%)	7	
Gene Mutation[*]				
DNMT3A (±)	7/69 (9%)	4/59 (6%)	11/128 (8%)	0.754
NPM1 (±)	10/66 (13%)	9/54 (14%)	19/120 (14%)	1.000
FLT3 (±)	9/67 (12%)	10/53 (16%)	19/120 (14%)	0.621
IDH1/2 (±)	5/71 (7%)	4/59 (6%)	9/120 (7%)	1.000
C/EBPA (±)	12/65 (16%)	7/59 (11%)	19/124 (14%)	0.463
C/KIT (±)	3/73 (4%)	4/59 (6%)	7/132 (5%)	0.701
U2AF1 (±)	1/75 (1%)	5/58 (8%)	6/133 (4%)	0.091
RAS (±)	10/66 (13%)	7/56 (11%)	17/132 (11%)	0.798
CR (±)	29/31 (48%)	31/28 (53%)	60/59 (47%)	0.715

[a] Median (range).
[*] Percentage was equal to the number of mutated patients divided by total cases in each group.

2. Materials and methods

2.1. Patients and samples

152 patients diagnosed with AML were enrolled in this study. The diagnosis and classification of AML patients were based on French-American-British (FAB) and World Health Organization (WHO) criteria [29,30]. The treatment protocol was previously reported [31]. Bone marrow (BM) samples from the patients were collected after signing written informed consent. The study was approved by the Ethics Committee Board of the Affiliated People' Hospital of Jiangsu University. The clinical characteristic of the patients is summarized in Table 1. BM samples from 18 healthy volunteers were collected as controls.

2.2. RNA isolation, reverse transcription and real-time quantitative PCR

Total RNA was isolated from BM mononuclear cells (BMNCs) using Trizol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's recommendations and reverse transcribed into cDNA by reverse transcription PCR. Real-time quantitative PCR (RQ-PCR) was performed on a 7300 Thermo cycler (Applied Biosystems, CA, USA). The primers of *DNMT3A* variant transcripts were shown in Table 2. The reaction for transcript 1, 3 and 4 consisted of 20 ng of cDNA, 0.8 μM of primers, 10 μM AceQ™qPCR SYBR Green Master Mix (Vazyme Biotech Co., Piscataway, NJ, USA), and 0.4 μM ROX Reference Dye1 (Invitrogen). The reaction for transcript 2 was composed of 20 ng of cDNA, 0.8 μM of primers, 10 μM SYBR Premix Ex Taq II and 0.4 μM ROX (TaKaRa, Japan). Relative *DNMT3A* expression levels were calculated using the following equation: $N_{DNMT3A} = (E_{DNMT3A})^{\Delta CT DNMT3A\,(\text{control-sample})} \div (E_{ABL})^{\Delta CT ABL(\text{control-sample})}$. The parameter PCR efficiency (E) was derived from the formula $E = 10^{(-1/\text{slope})}$ (the slope referred to CT vs cDNA concentration plot).

2.3. DNA isolation, bisulfite modification and real-time quantitative methylation-specific PCR

Genomic DNA (1 μg) was isolated from BMNCs, using Genomic DNA Purification Kit (Gentra, Minneapolis, MN, USA) and modified using the CpGenome DNA

Table 2
The sequences of primers used in RQ-PCR, RQ-MSP and BSP.

	Forward (5′→3′)	Reverse (5′→3′)	Product (bp)
RQ-PCR			
transcript 1	ACCCTGCCTGAAGCCTCAAG	CCATGCCGAGGCTCACCTT	200
transcript 2	GTGGATCGTAGCCTGAAAG	GGACAAGAATGCCACCA	220
transcript 3	GCGGCGAGAGCAGAGGA	TCCCCAAAGCCCAGCAG	219
transcript 4	AAGCGGGTGAGTCCTCAGC	GGATGCAGCCTGCGCATATG	177
RQ-MSP			
M	TTACGAATAAAGCGTTGGC	GTTTTCGGGTTTAGTTAGCG	169
U	GGTTATGAATAAAGTGTTGGT	GTTTTTGGGTTTAGTTAGTGGG	173
BSP	AGGGAGGTAGAGTTAGATAAGATGG	GGGGTTAGTTTAAAATTAGTTTAGG	298
ALU	TTAGGTATAGTGGTTTATATTTGTAATTTTAGTA	ATTAACTAAACTAATCTTAAACTCCTAACCTCA	110