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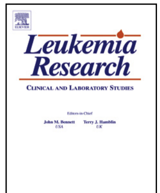

Methylation of *CTNNA1* promoter: Frequent but not an adverse prognostic factor in acute myeloid leukemia

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ABSTRACT

The reduced expression of *CTNNA1* gene, a putative tumor suppressor gene, has been found in several cancers including acute myeloid leukemia (AML). *CTNNA1* expression is regulated by methylation and histone deacetylation. However, the clinical significance of *CTNNA1* methylation in AML is rarely known. The present study was aimed to investigate the methylation status of *CTNNA1* promoter region using methylation-specific PCR (MSP) and its clinical relevance in Chinese AML patients. Patients with *CTNNA1* hypermethylation had significantly lower level of *CTNNA1* transcript than those without *CTNNA1* hypermethylation ($P = 0.031$). The relationship of *CTNNA1* methylation with clinical parameters was evaluated. Aberrant hypermethylation of *CTNNA1* gene was found in 23.9% (37/155) AML cases. The status of *CTNNA1* methylation was not correlated with the mutations of seven genes (*FLT3-ITD*, *NPM1*, *C-KIT*, *IDH1/IDH2*, *DNMT3A*, *N/K-RAS* and *C/EBPA*). There was no significant difference in the rates of complete remission (CR) between patients with and without *CTNNA1* methylation. Although the overall survival (OS) time of the *CTNNA1*-methylated AML was shorter than that of *CTNNA1*-unmethylated group (6 months vs 9 months), the difference was not statistically significant ($P = 0.681$). Our data suggest that *CTNNA1* methylation is a recurrent event but has no influence on prognosis in AML.

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1. Introduction

Acute myeloid leukemia (AML) is a group of heterogeneous malignant disorders characterized by clonal proliferation of myeloid progenitor cells and differentiation arrest [1]. A multi-step process involving several molecular events induces normal hematopoietic progenitor cell to lose the ability of self-renewal and differentiation to various mature cell lineages, to transform into a leukemic stem cell, and to accumulate in bone marrow [2,3]. The α-catenin protein (encoded by the *CTNNA1* gene) functioning as a connector links the E-cadherin/β-catenin complex to the filamentous actin cytoskeleton at adherens junctions [4]. *CTNNA1* gene, located on chromosome band 5q31, encodes a cytoplasmic 102-kD protein consisting of 906 amino acids [5]. It was proposed as a candidate tumor suppressor gene (TSG) because decreased or absent expression of *CTNNA1* was identified in many types of solid cancers and was associated with disease progression, metastasis and poor prognosis [6,7]. The nature of TSG has been confirmed that restoration of *CTNNA1* expression in *CTNNA1*-negative leukemic cells resulted in reduced proliferation and apoptotic cell death [8]. The abnormal expression of *CTNNA1* was regulated by promoter methylation and histone modification [8–10]. However, the precise relationship of *CTNNA1* hypermethylation with clinical parameters and prognostic significance is largely unknown. Our study was aimed to investigate the methylation status of *CTNNA1* promoter and reveal its clinical relevance in Chinese patients with AML.

2. Materials and methods

2.1. Patients and samples

The bone marrow (BM) samples from 155 primary patients were collected at the time of diagnosis and after the informed consent given at the Affiliated People' Hospital of Jiangsu University. The diagnosis and classification of AML patients were based on French–America–British (FAB) and World Health Organization (WHO) criteria (blast ≥ 20%) [11,12]. Karyotypes were analyzed using conventional R-banding method. Karyotype risk

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Table 1
Correlation between methylation of *CTNNA1* gene and patients parameters.

Patient's parameters	Status of *CTNNA1* methylation			
	Methylated ($n=37$)	Unmethylated ($n=118$)	Total ($n=155$)	*P*
Sex, male/female	17/20	77/41	155	0.053
Median age, years (range)	47 (17–86)	54 (10–85)	153	0.533
Median WBC, $\times 10^9$/L (range)	15.4 (0.5–160)	15.1 (0.8–528.0)	132	0.364
Median hemoglobin, g/L (range)	74.5 (33.0–133.0)	75 (32.0–147.0)	132	0.985
Median platelets, $\times 10^9$/L (range)	34.0 (3.0–447)	40 (4.0–399)	132	0.139
FAB				0.169
M0	1 (100%)	0 (0%)	1	
M1	2 (20%)	8 (80%)	10	
M2	15 (25%)	44 (75%)	59	
M3	11 (35%)	20 (65%)	31	
M4	3 (10%)	27 (90%)	30	
M5	4 (21%)	15 (79%)	19	
M6	1 (20%)	4 (80%)	5	
WHO				0.358
AML with t (8;21)	2 (20%)	8 (80%)	10	
APL with *t* (15;17)	11 (35%)	20 (65%)	31	
AML without maturation	3 (33%)	6 (67%)	9	
AML with maturation	13 (25%)	38 (75%)	51	
Acute myelomonocytic leukemia	3 (10%)	27 (90%)	30	
Acute monoblastic and monocytic leukemia	4 (21%)	15 (79%)	19	
Acute erythroid leukemia	1 (20%)	4 (80%)	5	
Karyotype classification				0.463
Favorable	13 (30%)	31 (70%)	44	
Intermediate	18 (21%)	66 (79%)	84	
Poor	5 (29%)	12 (71%)	17	
No data	1 (10%)	9 (90%)	10	
Karyotype				0.222
normal	14 (22%)	51 (78%)	65	
t (8;21)	2 (17%)	10 (83%)	12	
t (15;17)	11 (35%)	20 (65%)	31	
−5/5q−	0 (0%)	5 (100%)	5	
Others	9 (28%)	23 (72%)	32	
No data	1 (10%)	9 (90%)	10	
Gene Mutation*				
C/EBPA (±)	7/30 (19%)	11/105 (9%)	18/135 (12%)	0.144
NPM1 (±)	3/34 (8%)	19/97 (16%)	22/131 (14%)	0.286
FLT3 ITD (±)	3/34 (8%)	13/103 (11%)	16/137 (10%)	0.763
DNMT3A (±)	1/36 (3%)	8/108 (7%)	9/144 (6%)	0.688
IDH1/IDH2 (±)	3/34 (8%)	7/109 (6%)	10/143 (7%)	0.705
C-KIT (±)	1/36 (3%)	7/108 (6%)	8/144 (5%)	0.680
N/K-RAS (±)	5/32 (14%)	12/104 (10%)	17/136 (11%)	0.560
CR (±)	8/10 (44%)	42/49 (46%)	50/59 (46%)	1.000
CTNNA1 transcription	0.16 (0.08–0.62)	0.35 (0.02–7.15)	58	0.031

WBC, white blood cells; FAB, French–American–British classification; AML, acute myeloid leukaemia; CR, complete remission; (*) percentage was equal to the number of mutated patients divided by total cases in each group.

classification was identified according to the reported study [13]. The main clinical and laboratory features of the patient cohort were summarized in Table 1. The BM samples collected from 34 health donors were used as controls. The BM mononuclear cells were separated by density-gradient centrifugation using Ficoll.

2.2. Cell culture and treatment

Seven human leukemic cell lines (HL60, NB4, THP-1, SHI-1, U937, HEL and K562) were maintained in 90% IMDM with 10% fetal bovine serum. HL60 cell was treated with 50 μM 5-aza-2′-deoxycytidine (DAC) for 48 h with replacement of the medium and DAC every 24 h.

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

Total RNA was isolated using Trizol reagent (Invitrogen Life Technologies, USA) and reverse transcribed into cDNA [14]. The primers of *CTNNA1* gene were 5′-ATGCCATAATCAGAACAC-3′(forward) and 5′-ACTGCCTTAGCAAACAC-3′ (reverse) with expected PCR products of 142 bp. Real-time quantitative PCR (RQ-PCR) was performed on a 7300 Thermo cycler (Applied Biosystems, CA, USA) in a 25 μL reaction system including 50 ng of cDNA, 0.2 mM of dNTP, 4 mM of $MgCl_2$, 0.5 μM of primers, 1.2 μL of EvaGreen, and 1.0 U of Taq DNA Polymerase (MBI Fermentas, Hanover, USA). PCR program conditions were 94 °C for 4 min, followed by 40 cycles at 94 °C for 30 s, 58 °C for 30 s and 72 °C for 30 s as well as 82 °C for 30 s to collect fluorescence data. Each sample was detected twice. The mRNA abundance of *CTNNA1* gene was calculated relative to the expression of the housekeeping gene (ABL) according to the following equation: $N_{CTNNA1} = (E_{CTNNA1})^{\triangle CT\ CTNNA1(\text{control-sample})} / (E_{\text{ABL}})^{\triangle CT\ \text{ABL}\ (\text{control-sample})}$. The parameter efficiency (E) derived from the formula $E = 10^{(-1/\text{slope})}$ (the slope referred to CT versus cDNA concentration plot).

2.4. DNA isolation, bisulfite modification and methylation-specific PCR

Genomic DNA was isolated from BMNCs and from leukemic cell lines using genomic DNA purification kit (Gentra, Minneapolis, MN, USA) and quantified. 1 μg of genomic DNA was modified using the CpGenome™ DNA Modification Kit (Chemicon, Ternecula,

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