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Original Article

CTNNA1 promotor hypermethylation in chronic myeloid leukemia

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Abstract: Aberrant DNA methylation plays a significant role in the pathogenesis of chronic myeloid leukemia (CML). *CTNNA1* has been found to be low-expressed in some hematologic malignancies and various solid cancers. In the current study, we analyzed the methylation status of *CTNNA1* promoter in 70 Chinese patients with CML using methylation-specific PCR (MSP) and examined *CTNNA1* expression in 36 patients using real-time quantitative PCR (RQ-PCR). *CTNNA1* promoter hypermethylation was present in 21 (30.0%) CML cases. No significant differences were found in sex, white blood cells, hemoglobin concentration, platelet counts, chromosomal abnormalities and BCR-ABL transcript between *CTNNA1* hypermethylated and unmethylated groups (P>0.05). However, the age in methylated patients was significant higher than unmethylated patients (median 56 vs 41.5 years, P = 0.016). The frequency of *CTNNA1* promoter hypermethylation in chronic phase, in accelerated phase and in blast crisis were 30.9% (17/55), 33.3% (1/3) and 25.0% (3/12), respectively (P>0.05). Patients with *CTNNA1* hypermethylation had significantly lower level of *CTNNA1* expression (median 0.04) than those with *CTNNA1* unmethylation (median 0.51) (P = 0.020). Our data suggest that hypermethylation of *CTNNA1* promoter is a frequent molecular alteration in the CML patients.

Keywords: Chronic myeloid leukemia, *CTNNA1*, methylation

Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative stem cell neoplasm characterized by the presence of the cytogenetic abnormality of t(9;22) translocation. The t(9;22) gives rise to the bcr-abl fusion gene that encodes an oncoprotein $p210^{BCR\text{-}ABL1}$ with constitutive tyrosine kinase activity of inducing proliferation and reducing apoptosis of leukemic cells [1, 2]. Typically, CML course experiences the transition from the early chronic phase (CP) to acute phase (AP) and terminally to the invariably fatal blast crisis (BC) phase [2-4]. Although the pathogenesis of CML is well established, the underlying mechanism of the transition remains poorly defined. Recent studies have demonstrated that genetic changes and epigenetic abnormalities are responsible for this transition [2, 5-7].

Epigenetic events including DNA methylation, histone modification and post-transcriptional effects of microRNAs play a significant role in CML pathogenesis [1]. Aberrant promoter hypermethylation of quite a few tumor suppressor genes (TSG) has been determined as an important mechanism contributing to the pathogenesis and progression of CML [2, 8, 9].

Catenin (cadherin-associated protein) α 1 (*CTNNA1*) gene is mapped to chromosome band 5q31 and encodes a cytoplasmic 102-kD protein α-catenin, which consists of 906 amino acids and functions as an anchor connecting the E-cadherin/β-catenin complex to the filamentous actin cytoskeleton at adherent junctions [10, 11]. *CTNNA1* was considered as a candidate TSG because its reduced or absent expression was found in a large number of cancers with various types and was associated with disease progression, invasion, recurrence and poor prognosis [12]. Chromosome 5q deletion, promoter methylation and histone modification involved in regulating aberrant expression of *CTNNA1* in acute myeloid leukemia

Table 1. Correlations of the methylation of *CTNNA1* gene promoter with clinical features in CML patients

Patient's parameter	Status of *CTNNA1* methylation			*P* value
	Methylated (n = 21)	Hypomethylated (n = 49)	Total (n = 70)	
Sex (male/female)	16/5	30/19	46/24	0.227
Age (years)[a]	56 (20-70)	41.5 (15-83)	47 (15-83)	0.016
WBC (1×10^9/L)[a]	58.5 (2.5-183.8)	65.3 (0-219.6)	62.9 (0.0-219.6)	0.915
Haemoglobin (g/L)[a]	92 (57-152)	103 (58-180)	101(57-192)	0.227
Platelet (1×10^9/L)[a]	377 (57-990)	332 (20-2773)	341 (20-2773)	0.329
Staging				1.000
CP	17	38	55	
AP	1	2	3	
BC	3	9	12	
Cytogengtics				0.343
t(9;22)	14	35	49	
t(9;22) with additional alteration	3	6	9	
Normal karyotype	0	4	4	
No data	4	4	8	
bcr-abl[a]	275.26 (0.00-87794.34)	281.56 (21.42-6375.84)	278.41 (0.00-87794.34)	0.850
CTNNA1 thranscript (%)[a]	0.04 (0.00-2.62)	0.51 (0.00-6.73)	0.29 (0.00-6.73)	0.020

[a]Median (range). WBC, white blood cells; CP, chronic phase; AP, accelerated phase; BC, blast crisis.

(AML) and myelodysplastic syndrome (MDS) [13-16]. However, little is known about the methylation status of this gene in CML till date. In the present study, we aimed to investigate the methylation status of *CTNNA1* promoter and its clinical relevance in Chinese patients with CML for the first time.

Materials and methods

Patient samples, total RNA and genomic DNA isolation

The study was approved by Institutional Ethics Committee of the Affiliated People's Hospital of Jiangsu University. The bone marrow (BM) samples were collected from 70 patients with CML after the informed consent was signed. The diagnosis and clinical stages of CML patients were established in accordance with WHO criteria [17]. Karyotypes were analyzed by conventional R-banding method and karyotypic risk was classified according to the International System for Human Cytogenetic Nomenclature. Bcr-abl mRNA level detection was quantified using RQ-PCR established previously [18]. The main clinical features of all patients were provided in **Table 1**. The BM cells collected from 31 healthy donors were used as controls. Bone marrow mononuclear cells (BMNCs) were isolated by density-gradient centrifugation using Ficoll and washed twice with PBS. Total RNA was isolated using Trizol reagent (Invitrogen Life Technologies, USA) and genomic DNA was isolated from BMNCs using the DNA Purification Kit (Gentra, Minneapolis, MN, USA) according to the manufacturer's instructions.

Reverse transcription and RQ-PCR

2 μg total RNA was transcriptionally reversed into cDNA as reported previously [16]. After cDNA synthesis, real time quantitative PCR (RQ-PCR) was performed to detect mRNA expression of *CTNNA1* gene using of the specific primers 5'-ATGCCATAATCAGAACAC-3' (forward) and 5'-ACTGCCTTAGCAAACAC-3' (reverse) in a volume of 25 μL reaction system including 50 ng of cDNA, 4 mM of $MgCl_2$, 0.2 mM of dNTP, 1.2 μL of EvaGreen, 0.5 μ mol/L of primers, and 1.0 U of Taq DNA Polymerase (MBI Fermentas, Hanover, USA). The reaction was carried out at 94°C for 4 min, followed by 40 cycles at 94°C for 30 s, 58°C for 30 s, 72°C for 30 s and 82°C for 30 s to collect fluorescence data, eventually a melting program of one cycle at 95°C for 15 s, 60°C for 60 s, 95°C for 15 s, and 60°C for 15 s. Positive and negative controls were included in each assay. Relative *CTNNA1* expression levels were calculated using the following equation: $N_{CTNNA1} = (E_{CTNNA1})^{\Delta CT\ CTNNA1\ (control\text{-}sample)} \div (E_{ABL})^{\Delta CT\ ABL\ (control\text{-}sample)}$. The parame-

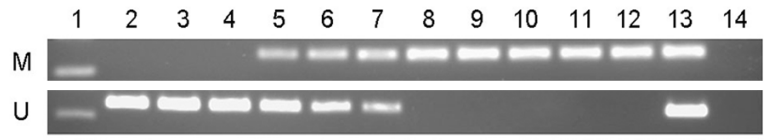

Figure 1. Electropheresis results of MSP products of *CTNNA1* gene in CML patients. 1: 100 bp DNA Ladder; 2: one normal control; 3-12: CML; 13: positive control; 14: negative control. M: methylation; U: unmethylation.

ter efficiency (E) was derived from the formula $E = 10^{(-1/slope)}$ (the slope referred to CT versus cDNA concentration plot).

Bisulfite modification and MSP

1 μg of genomic DNA was bisulfite modified using the CpGenome™ DNA Modification Kit (Chemicon, Ternecula, Canada) according to manufacture's protocol. The primers used for the methylated (M) were 5'-GTTTTAAGTTGGGTTTCGC-3' (forward) and 5'-CCATACTCGAACCCTACG-3' (reverse), and primers used for the unmethylated (U) were 5'-TTGGAGGGAGATAAAGTAGTGTTTG-3' (forward) and 5'-TCACAAAACCCAACTTAAAACAAC-3' (reverse). Methylation specific PCR (MSP) was performed to detect the status of *CTNNA1* methylation with a volume of 25 μL contained 1×PCR buffer, 0.5 μ mol/L primers, 0.25 mmol/L dNTP, 2.5 mmol/L $MgCl_2$, 50 ng bisulfite-treated DNA, and 1 U hot start DNA polymerase (Takara, Tokyo, Japan). PCR conditions were 95°C for 5 min, 40 cycles for 30 s at 94°C, 30 s at 62°C (M) or 59°C (U), 30 s at 72°C, eventually an extension step at 72°C for 7 min. MSP products were analyzed on 2% agarose gels. Placenta DNA processed with SssI methyltransferase (New England Biolabs, Beverly, MA, USA) and unprocessed in vitro were used as positive controls for methylated and unmethylated templates, respectively. A blank reaction with double sterilized water was also included as a negative control in all assays. It was defined as hypermethylation or unmethylation positive when the sample presented a visual band amplified with methylation or unmethylation-specific primers. Positive products of M and U reaction from three patients were cloned and sequenced (Shanghai GeneCore BioTechnologies, China).

Statistic analysis

All statistic analyses were carried out using the SPSS 17.0 software package (SPSS, Chicago, IL). Pearson Chi-square analysis or Fisher exact test was employed to compare the difference of categorical variables between patient groups. Mann-Whitney's U test was used to compare the difference of continuous variables between patient groups. For all analysis, two-tailed *P* values of 0.05 or less was considered having statistical significance.

Results

Association between CTNNA1 hypermethylation and clinical characteristics

CTNNA1 hypermethylation was present in 21 (30.0%, 21/70) CML cases but was not observed in all 31 (0%, 0/31) controls. The difference between these two groups was statistically significant (P<0.001). The representative results of MSP were shown in **Figure 1**. No significant differences were found in sex, white blood cells, hemoglobin concentration, platelet counts, chromosomal abnormalities and bcr-abl transcript between *CTNNA1* methylated and unmethylated groups (P> 0.05) (**Table 1**). However, the age in methylated patients was significant higher than that in unmethylated patients (median 56 vs 41.5 years, P = 0.016).

Associations of the hyermethylation of CTNNA1 promoter with the clinical stages in CML

The aberrant methylation of *CTNNA1* promoter was found in each stage of CML patients with the hypermethylation frequencies in CP, AP and BC of 30.9% (17/55), 33.3% (1/3) and 25.0% (3/12), respectively. There was not significant difference between these three groups (P>0.05). However, we analyzed the methylation status of *CTNNA1* promoter in two paired samples both in CP and in AP, the results exhibited that the two cases in CP lacked *CTNNA1* hypermethylation, but as the disease progressed into AP, both patients became *CTNNA1* hypermethylated.

Association between CTNNA1 hypermethylation and its expression

We determined *CTNNA1* expression in 36 CML patients as well as 21 controls with available

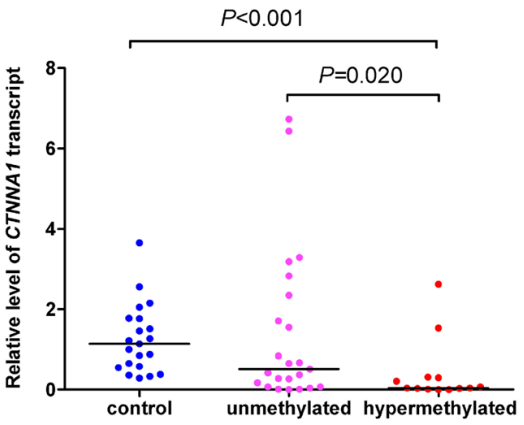

Figure 2. Levels of *CTNNA1* expression in controls and in CML patients with unmethylatin and hypermethylation.

mRNA. *CTNNA1* expression in the whole CML (median = 0.29) was significantly decreased compared to controls (median = 1.14) (P = 0.009). Moreover, patients with *CTNNA1* hypermethylation (n = 13) had significantly lower level of *CTNNA1* expression (median 0.04) than those with *CTNNA1* unmethylation (n = 23, median 0.51) (P = 0.020) and controls (median = 1.14) (P<0.001) (**Figure 2**).

Discussion

Aberrant DNA methylation is one of the most common epigenetic alterations in human cancers and is increasingly regarded as a key factor in the development of cancer. It is now well defined that gene-specific hypermethylation, global DNA hypomethylation, and regional hypomethylation occur during the generation of malignant cells [19]. Aberrant methylation of a large number of specific genes was present in CML and was associated with the development of CML [6, 18, 20-23]. Recently, Li et al disclosed the hypermethylation of SHP-1 promoter played a crucial role in the progression of CML by dysregulating MYC, AKT, MAPK and JAK2/STAT5 signaling [2]. In the present study, we provided the first evidence that 30% of the CML patients showed hypermethylation of *CTNNA1* gene promoter in comparison to only 0% of the controls, indicating that aberrant methylation of this gene is a frequent event in CML and may be correlated with the pathogenesis of this disease.

No significant correlation was observed in *CTNNA1* hypermethylation with the clinical features of CML patients except the age. So far, *CTNNA1* has been found to be low-expressed in hematopoietic malignancies (e.g., AML and MDS) and various kinds of solid cancers, such as colorectum, bladder, prostate, gastric, thyroid, breast, lung, and liver carcinomas [12-14, 24-33]. Genetic 5q deletion, epigenetic methylation and deacetylation were identified as underlying mechanisms of the regulation of *CTNNA1* expression in AML, MDS and prostate cancer [13-16, 27]. Our results first confirmed that DNA methylation was also involved in controlling the transcription of *CTNNA1* gene in CML. Although decreased *CTNNA1* expression was found to be associated with disease progression, invasion, recurrence and poor prognosis in various types of solid carcinomas and *CTNNA1* hypermethylation was identified to be associated with the progression of leukemia and higher IPSS risk in MDS [13-16, 24-33], we could not reveal the association of *CTNNA1* promoter hypermethylation with CML progression, which suggests that aberrant *CTNNA1* methylation might be an early molecular alteration in CML. However, it was interesting that the *CTNNA1* promoter changed into hypemethylated from unmethylated when the disease progressed into AP from CP in two CML patients. The result suggested the detection of methylation of *CTNNA1* promoter may be useful in monitoring the disease status of CML patients. Unfortunately, we could not analyze the effect of methylation on prognosis of the patients due to lack of complete clinical information of the investigated patients.

CTNNA1 counts for the generation of the junction complex and the polarized cell-cell association [11]. Functional studies indicate that *CTNNA1* has the ability of suppressing tumorigenicity in vivo and inhibiting cell proliferation and colony formation in vitro [24, 34, 35]. Moreover, reduced *CTNNA1* is implicated in myeloid development and transformation through the evolutionarily conserved PTEN-C/EBP-*CTNNA1* axis and restoration of *CTNNA1* in *CTNNA1*-decreased leukemic cells resulted in reduced proliferation and apoptotic cell death [13, 36]. These results suggest that the dysfunction of *CTNNA1* gene silenced by DNA hypermethylation may be essential to initiate leukemogenesis and development. Although

the treatment of CML is revolutionized by the tyrosine kinase inhibitor imatinib mesylate, there is a serious obstruction that patients are resistant to imatinib during the progression of CML. The new treatment is needed to explore in order to cure CML. Two phase II studies revealed a prospective method of the low-dose demethylating agent decitabine in imatinib refractory CML [37, 38]. Consequently, rescuing *CTNNA1* activity with decitabine may be a promising therapeutic strategy in the individuals with this molecular variant in CML.

In conclusion, our study first demonstrates *CTNNA1* promoter hypermethylation is a frequent molecular event and is associated with its expression in CML. The impact of *CTNNA1* promoter hypermethylation on the progression and prognosis of CML needs further study.

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Disclosure of conflict of interest

None.

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