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CDH13 is Frequently Inactivated by Promoter Hypermethylation in Pediatric Acute Myeloid Leukemia (AML)

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Abstract

There is growing evidence supporting a role for tumor suppressor as targets in aberrant mechanisms of DNA hypermethylation. Methylation in the promoter of tumor suppressor always plays important roles in pediatric Acute Myeloid Leukemia (AML). CDH13 gene is a tumor suppressor involved in tumorigenesis, metastasis and apoptosis in a variety of tumors. In this study, we are trying to investigate whether CDH13 was down regulated by promoter methylation in pediatric AML. MRNA transcriptional expression levels of CDH13 were evaluated by semi-quantitative PCR and real-time PCR. Methylation status of CDH13 prompter was investigated by Methylation Specific PCR (MSP) and Bisulfate Genomic Sequencing (BGS). CDH13 mRNA transcription was inactivated in AML cell lines. Promoter of CDH13 was aberrantly methylated in 55.6% (5/9) leukemia cell lines. Promoter aberrant methylation of CDH13 was detected in 34.2% (24/70) of the cases of pediatric AML. The methylation of CDH13 promoter could be detected in all FAB subtypes. There were no significant differences in clinical features between patients with and without *CDH13* methylation. Expression of *CDH13* was significantly lower in AML patients group compared to normal bone marrow (NBM) control samples .The expression of *CDH13* in thirty controls was significantly higher than pediatric AML patients. Both patients with *CDH13* methylation (n=24) and those without *CDH13* methylation (n=46) had significantly lower *CDH13* transcript than controls (p<0.001). *CDH13* transcript was significantly lower in patients with methylated *CDH13* than those without methylated *CDH13* (p=0.036). Inactivation of *CDH13* by promoter hypermethylation is frequent event in pediatric AML. Our results suggest that hypermethylation of *CDH13* promoter might be one of early events in the development of pediatric AML.

Keywords: CDH13; Methylation; AML; Genetics; Pediatrics

Introduction

Acute leukemia is the most common malignancy diagnosed in children, representing nearly one third of all pediatric cancers. Pediatric Acute Myeloid Leukemia (AML) comprises up to 20% of whole childhood leukemia. Pediatric AML is a heterogeneous clonal disorder of hematopoietic progenitor cells, is a complex and life-threatening disease which lose the ability to differentiate normally [1]. Recently, epigenetic and methylation disorders, such as aberrant promoter hypermethylation and abnormal histone modifications have been implicated in the pathogenesis of leukemia [2,3]. These include aberrations in methylation, which is a key epigenetic event responsible for enhanced proliferation and self-renewal, differentiation arrest, and impaired apoptosis of leukemic cells [4]. Inactivation of tumor suppressor genes by promoter hypermethylation has been recognized as key event in the development of pediatric AML. Compared to the incidences of DNA mutations and deletions, the frequency of aberrant DNA methylation of tumor suppressor genes is high in AML. This suggests that aberrant DNA methylation has important roles in this rare cancer. Identifying these methylated genes and deeply study of these genes may provide better understanding of many tumors , including pediatric AML [5].

CDH13 (also known as H-cadherin and T-cadherin) is a member of the cadherin gene super family which was isolated and mapped to 16q24. *CDH13* hypermethylation has been documented in breast [6-8] , lung cancers [9-11], pituitary adenomas [12,13], diffuse large B cell lymphoma [14], nasopharyngeal carcinoma [15-18] and cutaneous squamous cell carcinomas [19,20]. *CDH13* has been suggested as an early marker for lung cancers [21]. It is generally associated with poor prognosis of patients with lung cancers [22,23], ovarian cancers [24], basal cell cancers [20] and gallbladder carcinomas [25], cervical cancers [26-28] and prostate cancers [29-31].

There are several studies suggested that *CDH13* may functions as a tumor suppressor gene and possesses potent antitumor activity in several human cancers both *in vitro* and *in vivo*. Over-expression of *CDH13* in MDA-MB-435 (human breast cancer cells) can reduce their invasive and tumor formation potential *in vitro* and *in vivo* [32]. Loss of *CDH13* is associated with tumorigenicity of human non-small cell lung cancers. Over expression of *CDH13* in cutaneous squamous cell carcinoma cells can induce a delay in the G2/M cell cycle and inhibit the proliferation of cancer cells [20].

To date, there have been few reports in relation to the expression of CDH13 and the methylation status of its promoter in pediatric leukemia. In this study, we have provided the first evidence of CDH13 methylation in both AML cell lines and pediatric samples. These suggest that CDH13 may function as a tumor suppressor in pediatric AML.

Materials and Methods

Cell lines

Leukemia cell lines HL-60, MV4-11, SHI-1, U937, DAMI, K562 and SHI-1 were obtained from the American Type Culture Collection (ATCC). Jurkat and 697 cells lines (gifts from Professor Wang Jian-Rong, The Cyrus Tang Hematology center of Soochow University).

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The entire cell lines were maintained at 37°C in the RPMI 1640 supplemented with 10% fetal bovine serum.

Patients and samples

Bone marrow specimens were obtained at the time of diagnosis during routine clinical assessment of 70 patients with AML, who presented at the Department of Hematology and Oncology, Children's Hospital of Soochow University between 2000 and 2010. Ethical approval was provided by the Children's Hospital of Soochow University Ethics Committee (No. SUEC2000-021), and informed consent was obtained from the parents or guardians. AML diagnosis was made in accordance with the revised French–American–British (FAB) classification. Cytogenetic data were available in 64 patients. The main clinical and laboratory features of the patient cohort are summarized in table 1. Additionally, bone marrow samples from 12 healthy donors and 18 patients with Idiopathic Thrombocytopenic Purpura (ITP) were analyzed as controls. Bone marrow Mononuclear Cells (BMNCs) were isolated using Ficoll solution within 2 h after bone marrow samples harvested and immediately subjected for the extraction of total RNA and genomic DNA.

Semi-quantitative RT-PCR

RT-PCR was analyzed according to the manufacturer's instructions. Primer sequences for *CDH13* cDNA were designed according to Sun [18], generating a 203-bp PCR product: *CDH13*-RT-forward: TTCAGCAGAAAGTGTTCCATAT and *CDH13*-RTreverse: GTGCATGGACGAACAGAGT. PCR was carried out in a total volume of 20 μl system. Glyceraldehyde-3-Phosphate Dehydrogenase (*GAPDH*) was amplified from the same cDNA sample as the internal control. The primer sequences for *GAPDH* cDNA were: GAPDH-RT-forward: AAGCTCACTGGCATGGCCTT, and GAPDH -RT-reverse:CTCTCTTCCTCTTGTGCTCTTG, generating a 375-bp PCR product. PCR conditions were 94°C for 30 s, 58°C for 30 s (*CDH13*) or 60°C for 30 s (*GAPDH*), and 72°C for 30 s, 33 cycles for the *CDH13* gene and 24 cycles for the *GAPDH* gene. The amplified PCR products were then identified on 2% agarose gels. Images were acquired with a CCD camera (Bio-Rad, USA).

Quantitative Real-time PCR

RNA isolation and first-strand cDNA was synthesized as described above. Real-time PCR was performed according to the manufacturer's protocol (Light Cycler 480 system, Roche). In brief, PCR mixture contained 100 pmol of each primer, Light Cycler 480 SYBR Green I Master (04 887 352 001 Roche, USA) and 2 μl cDNA. PCR conditions were 94°C for 10 s, 60°C for 10 s and 72°C for 15 s, 45cycles for the *CDH13* and *GAPDH* gene.

Sodium bisulphite modification of genomic DNA

The sodium bisulphite modification procedure was according to the manufacturer's instructions of EZ DNA methylation Gold Kit (www.zymoresearch.com). Briefly 2 μg of extracted DNA was bisulphite-modified with the EZ DNA methylation Kit which converted all unmethylated cytosines to uracils and leaving methylcytosines unaltered. Modified DNA was resuspended in TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 7.5).

Methylation-specific PCR

The methylation status of the CDH13 promoter region was determined by methylation-specific PCR. Primers distinguishing unmethylated (U) and methylated (M) alleles were designed to amplify the sequence: CDH13-M-forward: 5-GTTTTTTTGGTGAGTTTTCGTTTTCGTTC-3; CDH13-M-reverse: 5-AATACCAAATCTCCCTATTCTCCGCG-3; CDH13-U-forward: 5-TTGTTTTTTTGGTGAGTTTTTGTTTTTGTTT-3; CDH13-U-reverse:5-AAAATACCAAATCTCCCTATTCTCCACA-3.

Each PCR reaction contained 20 ng of sodium bisulphite-modified DNA, 250 pmol of each primer, 250 pmol deoxynucleoside triphosphate, 1× PCR buffer, and one unit of ExTaq HS polymerase (Takara, Tokyo) in a final reaction volume of 20 μl. Cycling conditions were initial denaturation at 95°C for 3 min, 40 cycles of 94°C for 30 s, 58°C (M) or 56°C (U) for 30 s, and 72°C for 30 s. PCR products were separated on 2% agarose gels.

Bisulfite genomic sequencing

Bisulfite Genomic Sequencing (BGS) were performed as previously described [33]. BGS primers were CDH13-F1:5-AAAGAAGTAAATGGGATGTTATTTT-3 and CDH13-R1:5-ACCAAAACCAATAACTTTACAAAAC-3.

CDH13-F2: 5- GTGATGTTGTTGTTGATTTATTTGG -3 and

Patient's parameter	Status of CDH13 methylation			
	Methylated (n=24)	Unmethylated(n=46)	Total	p value
Age (median and range, year)	6.30(1-13)	6.72(1-11)	6.47(1-13)	0.94
Gender (male and female)	14/10	20/26	34/46	0.88
Laboratory parameters (median and range)				
WBC (10^9/L)	16.72(0.8-51.1)	16.31(0.8-43.6)	16.43(0.8-51.1)	0.90
Hemoglobin (g/L)	75.36(32-176)	72.11(32-107)	73.20(32-176)	0.83
Platelet count (10^9/L)	68.37(12-310)	64.12(23-273)	65.54(12-310)	0.75
FAB subtype, n				
M1	2	10	12	
M2	12	20	32	
M3	6	4	10	
M4	2	3	5	
M5	5	4	9	0.22
Cytogenetic, n				
Normal	10	13	23	
Abnormal	24	21	45	0.61
CDH13 transcript	0.53	1.72	1.31	0.04*

* p<0.05 FAB, French-American-British; WBC, white blood cells.

Table 1: Correlation of CDH13 methylation with clinical features in pediatric AML patients.

CDH13-R2: 5- AACCCCTCTTCCCTACCTAAAA-3. Amplified BGS products were TA-cloned and five to six randomly chosen colonies were sequenced. DNA sequences were analyzed with BiQ Analyzer (http://biq-analyzer.bioinf.mpi-inf.mpg.de). (Additional files 1 and 2).

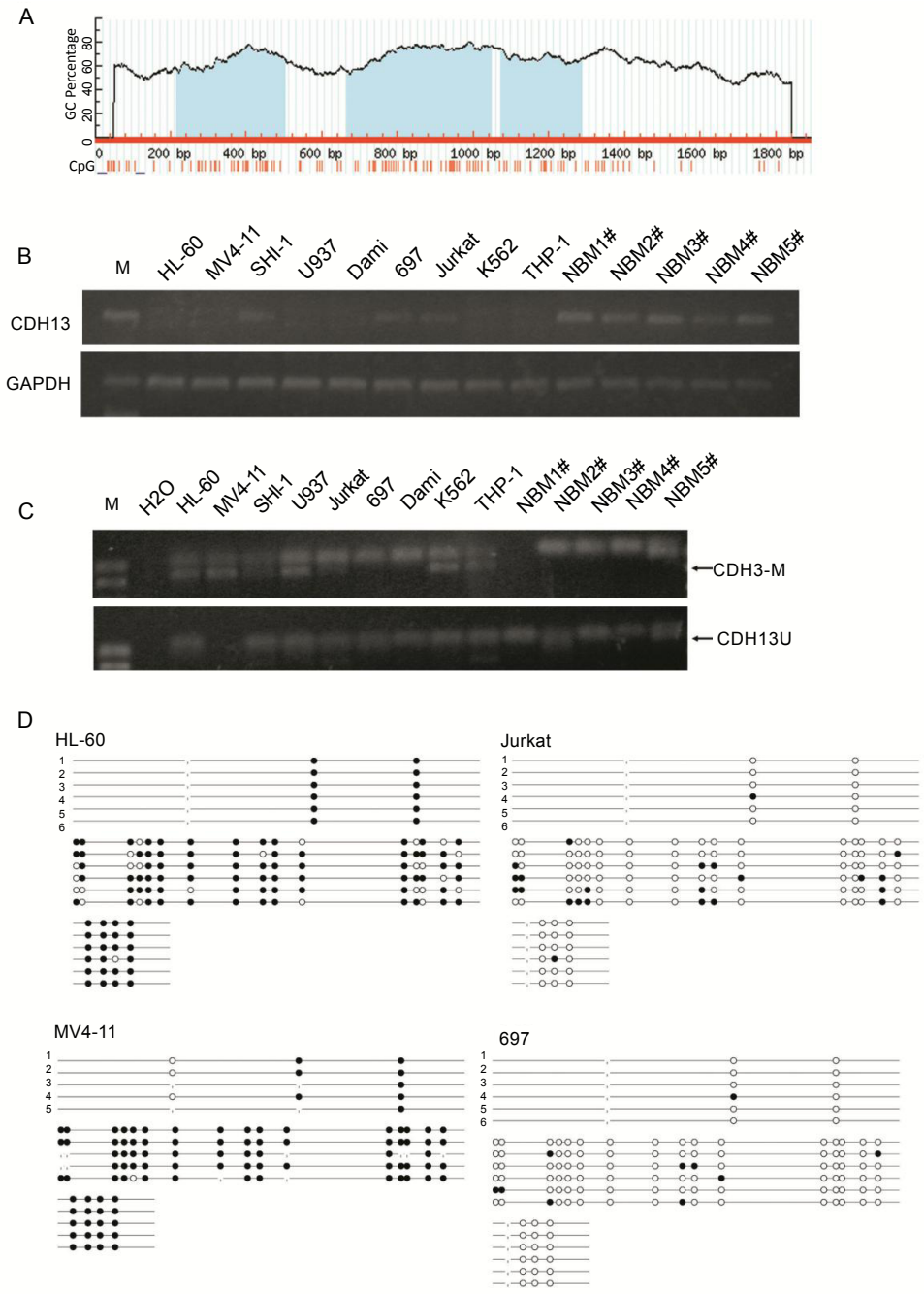
Statistical analysis

SPSS v11.5 (SPSS Inc., Chicago, IL) was used for statistical analysis. Association between methylated sample data and clinical pathological features of AML patients were analyzed by Pearson chi-square test or Fisher's exact test. $p<0.05$ was considered statistically significant.

Results

CpG islands in the promoter of *CDH13*

Previously, we have analyzed the expression profiles of two AML cells before and after treatment with 5-Aza and found that the *CDH13*



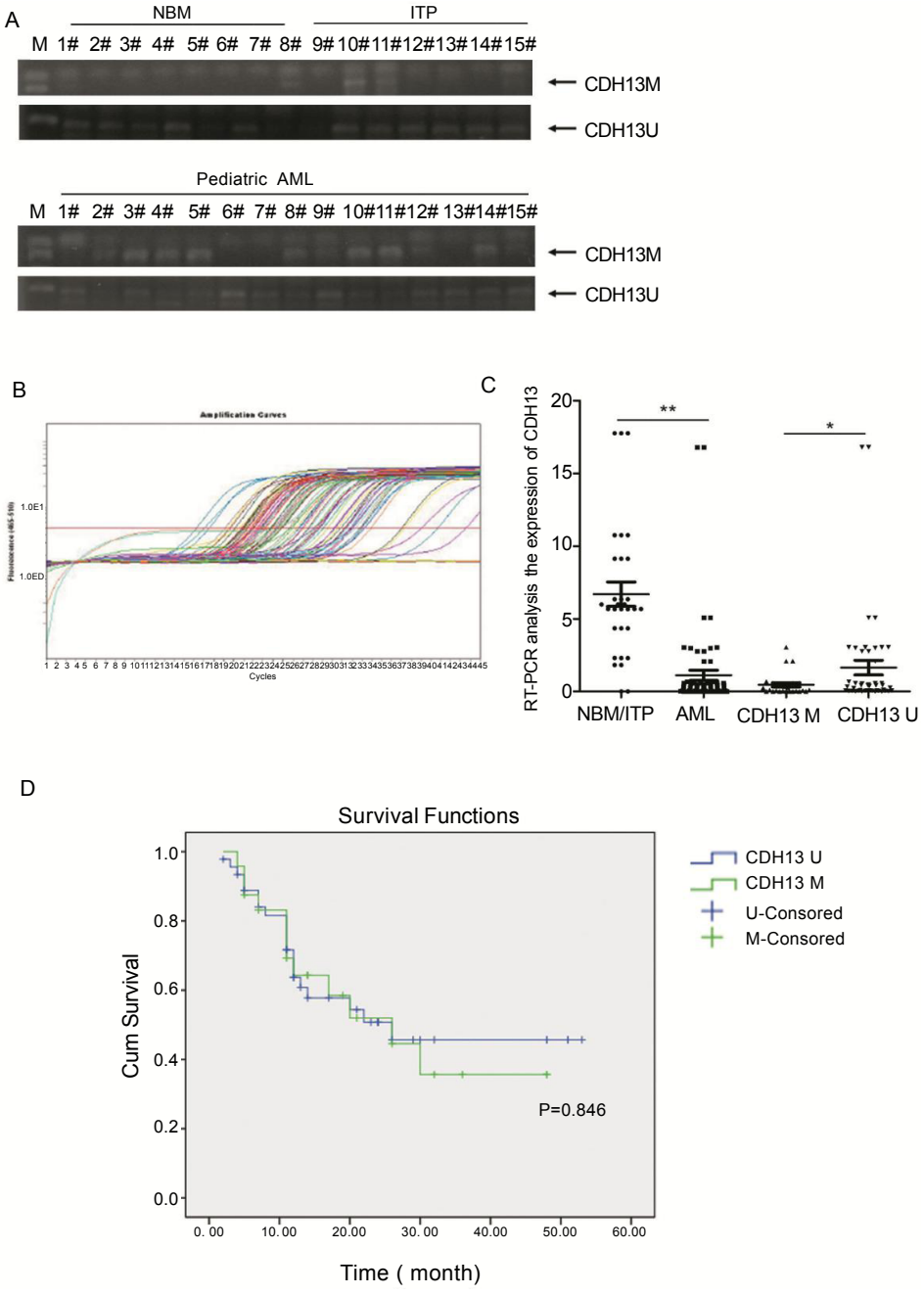
(A) Three CpG island regions can be identified in the promoter of *CDH13*. (B) Expression of *CDH13* transcript in leukemia cell lines. Semi-quantitative PCR analysis the expression of *CDH13* in nine leukemia cell lines and five NBM samples. (C) MSP analysis of the methylation status of *CDH13* in leukemia cell lines shows that the promoter is hypermethylated in 5/9 cell lines. M and U represent MSP results using primer sets for methylated and unmethylated *CDH13* genes, respectively. (D) Bisulfite genomic sequencing results of HL-60, MV411, Jurkat and 697 cell lines, ● methylated cytosines ,○ unmethylated sytosines.

Figure 1: *CDH13* is frequently inactivated by promoter hypermethylation in leukemia cell lines.

may be related with promoter methylation in AML cells. We analyzed the sequence of *CDH13* promoter and found there are three CpG island areas in the promoter of *CDH13* (Figure 1A). The correlation between aberrant methylation and downregulation of *CDH13* has been extensively documented in numerous cancers and cell lines, as lung caner, gastrointestinal system, reproductive system and numerous cell lines. There still rare repots about the methylation status of *CDH13* in blood system, especially in the pediatric leukemia.

Expression of *CDH13* transcript in leukemia cell lines

Semi-quantitative PCR analysis showed *CDH13* transcript is very low in nine leukemia cell lines. The expression of *CDH13* in only three cell lines can be detected (3/9). Mean while, the expression of *CDH13* in NBM group is significantly higher; the expression of *CDH13* in all of 5 NBM samples can be detected with PCR (Figure 1B).



(A) Methylation of *CDH13* promoter in Pediatric AML patients. MSP analysis of the methylation status of *CDH13* shows aberrant methylation in pediatric AML samples compared to NBM/ITP control samples. M and U represent MSP results using primer sets for methylated and unmethylated *CDH13* genes, respectively. (B) Real-time analysis the expression of *CDH13* transcript in pediatric AML patients. (C) Transcript level of *CDH13* gene in 70 AML patients and 30 control samples. *CDH13* transcript in pediatric AML patients was 1.31 ± 2.95 vs. 30 control samples 6.69 ± 4.67, p<0.001. Patients with *CDH13* methylation (n=24) and those without *CDH13* methylation (n=46) had significantly lower CDH13 transcript than controls (p<0.001) (D) Survival analysis the Pediatric AML patients with methylated and unmethylated *CDH13*.

Figure 2: *CDH13* is frequently inactivated by promoter hypermethylation in pediatric AML.

CDH13 promoter is hypermethylated in leukemia cell lines

Methylation-specific PCR (MSP) assays were performed to detect the methylation status of the *CDH13* promoter in 9 leukemia cell lines. The MSP primer was designed using MethPrimer (http://www.urogene.org/cgi-bin/methprimer/methprimer.cgi) to encompass the CpG islands of the *CDH13* promoter identified in figure 1A. The *CDH13* promoter was hypermethylated in 5 of 9 leukemia cell lines (HL-60, MV4-11, U937, K562 and THP-1). SHI-1, Jurkat, 697 and DAMI cells were unmethylated representative results of MSP were shown in figure 1C. Promoter methylation of HL-60, MV4-11, Jurkat and 697 cell lines was analyzed with bisulfite genomic sequencing. And the results consistent with the MSP assay (Figure 1D). In summary, these results showed that the *CDH13* promoter was consistently significantly methylated in leukemia cells, such as HL-60, MV4-11, U937, K562 and THP-1. In contrast the *CDH13* promoter was unmethylated in human lymphoblastic leukemia cells, such as Jurkat and 697. Based on these findings, we proposed that the promoter of *CDH13* may be methylated in pediatric AML patients.

The promoter of *CDH13* is methylated in Pediatric AML patients

We next examined the methylation status of the *CDH13* promoter in pediatric AML samples and NBM/ITP (normal bone marrow/idiopathicthrombocytopenic purpura) control samples. Aberrant methylation of *CDH13* was observed 2 (6.7%) in the 30 bone marrow samples from controls and 24 (34.3%) cases among70 pediatric AML samples (Figure 2A). Aberrant methylation of the *CDH13* gene could be detected in all FAB subtypes and in all cytogenetic risk groups. There were no significant differences in clinical features, such as sex, age, initial hemoglobin level, white blood cell counts, platelet counts, and chromosomal abnormalities between patients with and without *CDH13* methylation (Table 1). The survival time of the patients also has no relationship with the methylation status of *CDH13* (Figure 2D).

Expression of *CDH13* transcript in pediatric AML patients

The transcript level of *CDH13* gene was examined in 70 AML patients with available materials using Real-time PCR. As shown in table 1, *CDH13* expression was significantly decreased in AML patients (1.31 ± 2.95) compared to 30 NBM/ITP controls (6.69 ± 4.67, $p<0.001$); Both patients with *CDH13* methylation (n=24) and those without *CDH13* methylation (n=46) had significantly lower *CDH13* transcript than controls ($p<0.001$) (Figures 2B and 2C). Furthermore, *CDH13* transcript was significantly lower in patients with methylated *CDH13* than those without methylated *CDH13* ($p=0.036$) (Table 1).

Discussion

CDH13, instances of both hypermethylation and loss of function have been documented in numerous cancers. In our study, hypermethylation of the *CDH13* promoter was detected in 5 of 9 (55.6%) leukemia cell lines. A high frequency (34.3%) of *CDH13* promoter hypermethylation was also found in pediatric AML primary tumor cells, which implied that silencing of the *CDH13,* may be involved in the tumorigenesis of pediatric AML. Promoter hypermethylation of *CDH13* is frequently found in AML, but it is not associated with sex, age and patient's survival in our series. This is inconsistent with observations in non-small-cell lung cancer: a high methylation rate of *CDH13* is generally associated with poor prognosis in lung cancers [23], but consistent with colorectal cancers [34].

Several reports have implied the effects of *CDH13* gene on the proliferation and apoptosis of cancer cells. Melanoma cells that re-express *CDH13* show a reduction in the rate of tumor growth in a nu/nu mouse tumor model [35]. *CDH13* over-expression in hepatocellular carcinoma also increases sensitivity of tumor cells to TNFa-induced apoptosis [36]. Positive relationship between *CDH13* and Cyclin D2 methylation was reported in prostate cancer [37]. There still no report about the effects of *CDH13* in leukemia cells. Leukemia cells may share the same mechanism with solid tumor cells, or maybe there is totally different molecular mechanism in leukemia cells.

Besides the methylation of *CDH13*, gene mutation and spliced mRNAs may also play important role in pediatric AML. Two single nucleotide polymorphism in distinct introns of *CDH13* have been associated with greater sensitivity of lymphoblastoid cell lines to apoptosis induced by cisplatin and daunorubicin [38]. *CDH13* produces a lot of spliced mRNAs, many of these encode proteins are predicted to be secreted and thus, like proteolytic fragments of the major *CDH13* isoforms, might function as extracellular ligands. Until now little is known about these smaller proteins. Next step, we will explore the relationship between mutation/spliced mRNA of *CDH13* and pediatric leukemia.

Conclusions

This work demonstrated that inactivation of *CDH13* by promoter hypermethylation is a tumor specific and frequent event in pediatric AML.

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Declaration of Interest statement

The authors have no conflicts of interest to disclose.

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Additional file 1: Bisulfite genomic sequencing of HL-60 cells.

CDH13

1#

GGAAGTTGGCTGGCTGGCGAGGCAGAGCCTCTCCTCAAAGCCTGGCTCCCACGGAAAATA
TGCTCAGTGCAGCCGCGTGCATGAATGAAAACGCCGCCGGGCGCTTCTAGTCGGACAAAA
TGCAGCCGAGAACTCCGCTCGTTCTGTGCGTTCTCCTGTCCCAGGTAGGGAAGAGGGGCT
GCCGGGCGCGCTCTGCGCCCCGTTTCTGCATTCGGATCGCCCGGCACGGGCAGGGTGAGG

2#

GGAAGTTGGTTGGTTGGCGAGGTAGAGTTTTTTTTTAAAGTTTGGTTTTTACGGAAAATA
TGTTTAGTGTAGTCGCGTGTATGAATGAAAACGTCGTCGGGCGTTTTTAGTCGGATAAAA
TGTAGTCGAGAATTTCGTTCGTTTTGTGCGTTTTTTTGTTTTAGGTAGGGAAGAGGGGTT
GTCGGGCGCGTTTTGCGTTTCGTTTTTGTATTCGGATCGTTCGGTACGGGTAGGGTGAGG

3#

GGAAGTTGGTTGGTTGGCGAGGTAGAGTTTTTTTTTAAAGTTTGGTTTTTACGGAAAATA
TGTTTAGTGTAGTCGCGTGTATGAATGAAAACGTCGTCGGGCGTTTTTAGTCGGATAAAA
TGTAGTCGAGAATTTCGTTCGTTTTGTGCGTTTTTTTGTTTTAGGTAGGGAAGAGGGGTT
GTCGGGCGCGTTTTGCGTTTCGTTTTTGTATTCGGATCGTTCGGTACGGGTAGGGTGAGG

4#

GGAAGTTGGTTGGTTGGTTAGGTAGAGTTTTTTTTTAAAGTTTGGTTTTTACGGAAAATA
TGTTTAGTGTAGTTTTTTGTATGAATGAAAACGTCGTCGGGCGTTTTTAGTCGGATAAAA
TGTAGTCGAGAATTTCGTTCGTTTTGTGTTTTTTTTTTGTTTTAGGTAGGGAAGAGGGGTT
GTCGGGTTTTTTTTGCGTTTTTTTTTTTGTATTCGGATCGTTCGGTACGGGTAGGGTGAGG

5#

GGAAGTTGGTTGGTTGGCGAGGTAGAGTTTTTTTTTAAAGTTTGGTTTTTACGGAAAATA
TGTTTAGTGTAGTTTTTTGTATGAATGAAAACGTCGTCGGGCGTTTTTAGTCGGATAAAA
TGTAGTCGAGAATTTCGTTCGTTTTGTGCGTTTTTTTGTTTTAGGTAGGGAAGAGGGGTT
GTCGGGCGCGTTTTGCGTTTCGTTTTTGTATTCGGATCGTTCGGTACGGGTAGGGTGAGG

6#

GGAAGTTGGTTGGTTGGTTAGGTAGAGTTTTTTTTTAAAGTTTGGTTTTTACGGAAAATA
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TGTAGTTTAGAATTTCGTTCGTTTTGTGTTTTTTTTTTGTTTTAGGTAGGGAAGAGGGGTT
GTCGGGCGCGTTTTGCGTTTCGTTTTTGTATTCGGATCGTTCGGTACGGGTAGGGTGAGG

CDH13 Bisulfite genomic sequencing of MV4-11 cells

1#

GGAAGTTGGCTGGCTGGCGAGGCAGAGCCTCTCCTCAAAGCCTGGCTCCCACGGAAAATA
TGCTCAGTGCAGCCGCGTGCATGAATGAAAACGCTGCCGGGCGCTTCTAGTCGGACAAAA
TGCAGCCGAGAACTCCGCTCGTTCTGTGTGTTCTCCTGTCCCAGGTAGGGAAGAGGGGCT
GCCGGGTGCGCTCTGTGCCCCGTTTCTGCATTCGGATCGCCCGGCACGGGCAGGGTGAGG

2#

GGAAGTTGGCTGGCTGGCGAGGCAGAGCCTCTCCTCAAAGCCTGGCTCCCACGGAAAATA
TGCTCAGTGCAGCCGCGTGCATGAATGAAAATGCCGCCGGGCGCTTCTAGTCGGACAAAA
TGCAGCCGAGAACTCTGCTCGTTCTGTGCGTTCTCCTGTCCCAGGTAGGGAAGAGGGGCT
GCCGGGCGCGCTCTGCGCCCTGTTTCTGCATTCGGATCGCCCGGCACGGGCAGGGTGAGG

3#

GGAAGTTGGCTGGCTGGCGAGGCAGAGCCTCTCCTCAAAGCCTGGCTCCCACGGAAAATA
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TGCAGCCGAGAACTCCGCTCGTTCTGTGCGTTCTCCTGTCCCAGGTAGGGAAGAGGGGCT
GCCGGGTGTGCTCTGCGCCCCGTTTCTGCATTCGGATCGCCCGGCACGGGCAGGGTGAGG

4#

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TGCAGCCGAGAACTCCGCTCGTTCTGTGCGTTCTCCTGTCCCAGGTAGGGAAGAGGGGCT
GCCGGGCGCGCTCTGTGCCCCGTTTCTGCATTCGGATCGCCTGGCACGGGCAGGGTGAGG

5#

GGAAGTTGGCTGGCTGGCGAGGCAGAGCCTCTCCTCAAAGCCTGGCTCCCACGGAAAATA
TGCTCAGTGCAGCTGCGTGCATGAATGAAAACGCCGCCGGGCGCTTCTAGTCGGACAAAA
TGCAGCCGAGAACTCCGCTCGTTCTGTGCGTTCTCCTGTCCCAGGTAGGGAAGAGGGGCT
GCCGGGTGTGCTCTGCGCCCTGTTTCTGCATTCGGATCGCCCGGCACGGGCAGGGTGAGG

CDH13 Bisulfite genomic sequencing of Jurkat cells

1#

GGAAGTTGGCTGGCTGGTGAGGCAGAGCCTCTCCTCAAAGCCTGGCTCCCATGAAAATA
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TGCAGCTGAGAACTCTGCTTGTTCTGTGTGTTCTCCTGTCCCAGGTAGGGAAGAGGGGCT
GCTGGGTGTGCTCTGTGCCCTGTTTCTGCATTCAGATTGCCTGGCATGGGCAGGGTGAGG

2#

GGAAGTTGGCTGGCTGGTGAGGCAGAGCCTCTCCTCAAAGCCTGGCTCCCATGAAAATA
TGCTCAGTGCAGCTGTGTGCATGAATGAAAACGCTGCTGGGTGCTTCTAGTTGGACAAAA
TGCAGCTGAGAACTCTGCTTGTTCTGTGTGTTCTCCTGTCCCAGGTAGGGAAGAGGGGCT
GCTGGGTGTGCTCTGTGCCCCGTTTCTGCATTCAGATTGCCTGGCATGGGCAGGGTGAGG

3#

GGAAGTTGGCTGGCTGGTGAGGCAGAGCCTCTCCTCAAAGCCTGGCTCCCATGAAAATA
TGCTCAGTGCAGCTGTGTGCATGAATGAAAATGCTGCTGGGTGCTTCTAGTTGGACAAAA
TGCAGCTGAGAACTCCGCTCGTTCTGTGTGTTCTCCTGTCCCAGGTAGGGAAGAGGGGCT
GCTGGGTGTGCTCTGTGCCCTGTTTCTGCATTCAGATTGCCTGGCATGGGCAGGGTGAGG

4#

GGAAGTTGGCTGGCTGGCGAGGCAGAGCCTCTCCTCAAAGCCTGGCTCCCATGAAAATA
TGCTCAGTGCAGCTGTGTGCATGAATGAAAATGCTGCTGGGTGCTTCTAGTTGGACAAAA
TGCAGCTGAGAACTCTGCTTGTTCTGTGCGTTCTCCTGTCCCAGGTAGGGAAGAGGGGCT
GCTGGGTGTGCTCTGTGCCCTGTTTCTGCATTCAGATTGCCTGGCATGGGCAGGGTGAGG

5#
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GCTGGGTGTGCTCTGTGCCCTGTTTCTGCATTCAGATTGCCTGGCATGGGCAGGGTGAGG
6#
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TGCTCAGTGCAGCTGTGTGCATGAATGAAAACGCTGCTGGGTGCTTCTAGTTGGACAAAA
TGCAGCTGAGAACTCCGCTTGTTCTGTGTGTTCTCCTGTCCCAGGTAGGGAAGAGGGGCT
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CDH13 Bisulfite genomic sequencing of 697 cells

1#
GGAAGTTGGCTGGCTGGTGAGGCAGAGCCTCTCCTCAAAGCCTGGCTCCCATGAAAATA
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TGCAGCTGAGAACTCTGCTTGTTCTGTGTGTTCTCCTGTCCCAGGTAGGGAAGAGGGGCT
GCTGGGTGTGCTCTGTGCCCTGTTTCTGCATTCAGATTGCCTGGCATGGGCAGGGTGAGG
2#
GGAAGTTGGCTGGCTGGTGAGGCAGAGCCTCTCCTCAAAGCCTGGCTCCCATGAAAATA
TGCTCAGTGCAGCTGTGTGCATGAATGAAAATGCTGCTGGGTGCTTCTAGTTGGACAAAA
TGCAGCTGAGAACTCTGCTTGTTCTGTGTGTTCTCCTGTCCCAGGTAGGGAAGAGGGGCT
GCTGGGTGTGCTCTGTGCCCCGTTTCTGCATTCAGATTGCCTGGCATGGGCAGGGTGAGG
3#
GGAAGTTGGCTGGCTGGTGAGGCAGAGCCTCTCCTCAAAGCCTGGCTCCCATGAAAATA
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TGCAGCTGAGAACTCCGCTCGTTCTGTGTGTTCTCCTGTCCCAGGTAGGGAAGAGGGGCT
GCTGGGTGTGCTCTGTGCCCTGTTTCTGCATTCAGATTGCCTGGCATGGGCAGGGTGAGG
4#
GGAAGTTGGCTGGCTGGCGAGGCAGAGCCTCTCCTCAAAGCCTGGCTCCCATGAAAATA
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GCTGGGTGCGCTCTGCGCCCTGTTTCTGCATTCAGATTGCCCGGCATGGGCAGGGTGAGG
5#
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GCTGGGTGTGCTCTGCGCCCTGTTTCTGCATTCAGATTGCCTGGCATGGGCAGGGTGAGG
6#
GGAAGTTGGCTGGCTGGTGAGGCAGAGCCTCTCCTCAAAGCCTGGCTCCCATGAAAATA
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TGCAGCTGAGAACTCCGCTCGTTCTGTGTGTTCTCCTGTCCCAGGTAGGGAAGAGGGGCT
GCTGGGTGTGCTCTGCGCCCTGTTTCTGCATTCAGATTGCCTGGCATGGGCAGGGTGAGG

Additional file 2: Real-time PCR analysis the expression of *CDH13* gene in NBM and pediatric AML patients.

			Pediatric AML	
	NBM/ITP	**Pediatric AML**	**CDH13 methylated**	**CDH13 unmethylated**
1	5.985902	0.009562268	0.009562268	0.4312417
2	10.75226	0.008266937	0.008266937	16.81374
3	17.77242	0.5850244	0.5850244	2.773232
4	5.663008	3.045267	0.009562268	5.086131
5	4.351668	0.5850244	0.5850244	0.4312417
6	5.682669	0.2729235	0.2729235	2.094446
7	2.307881	0.3442596	0.3442596	0.05243117
8	0.000212032	0.0282926	0.0282926	0.05170934
9	9.136038	0.6743495	0.6743495	0.05170934
10	1.823321	0.008266937	0.008266937	0.07363669
11	6.347538	0.09615919	0.09615919	0.07363669
12	5.985902	0.3741189	0.3741189	3.045267
13	10.75226	2.992951	0.008266937	3.045267
14	17.77242	0.1143532	0.1143532	0.5850244
15	5.663008	0.06990641	0.06990641	3.045267
16	4.351668	0.003992667	0.003992667	0.5850244
17	5.682669	0.5850244	0.5850244	0.2729235
18	2.307881	0.01853722	0.01853722	0.3442596
19	0.000212032	0.04057032	0.04057032	0.0282926
20	9.136038	0.2351362	0.2351362	0.6743495
21	1.823321	3.045267	3.045267	0.008266937
22	6.347538	2.094446	2.094446	0.09615919
23	5.985902	0.5402011	0.5402011	0.3741189
24	10.75226	3.045267	3.045267	2.992951
25	17.77242	0.4312417		0.1143532
26	5.663008	16.81374		0.06990641

27	4.351668	2.773232		0.003992667
28	5.682669	5.086131		0.5850244
29	2.307881	0.4312417		0.01853722
30	9.136038	2.094446		0.04057032
31		0.05243117		0.2351362
32		0.05170934		0.002774741
33		0.05170934		0.0106838
34		0.07363669		0.5402011
35		0.07363669		0.4868565
36		0.009562268		0.4312417
37		0.008266937		16.81374
38		0.5850244		2.773232
39		3.045267		5.086131
40		0.5850244		0.4312417
41		0.2729235		2.094446
42		0.3442596		0.05243117
43		0.0282926		0.05170934
44		0.6743495		0.05170934
45		0.008266937		2.992951
46		0.09615919		3.045267
47		0.3741189		
48		2.992951		
49		0.1143532		
50		0.06990641		
51		0.003992667		
52		0.5850244		
53		0.01853722		
54		0.04057032		
55		0.2351362		
56		0.002774741		
57		0.0106838		
58		0.5402011		
59		0.4868565		
60		0.4312417		
61		16.81374		
62		2.773232		
63		5.086131		
64		0.4312417		
65		2.094446		
66		0.05243117		
67		0.05170934		
68		0.05170934		
69		2.992951		
70		3.045267		
AV	6.698232841	1.310098949	0.533199123	1.716575293
SD	4.672074863	2.959071041	0.892060633	3.540122523