

Chromosome 16q genes *CDH1*, *CDH13* and *ADAMTS18* are correlated and frequently methylated in human lymphoma

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Abstract. The products of the E-cadherin (*CDH1*), H-cadherin (*CDH13*) and a disintegrin and metalloproteinase with thrombospondin motif 18 (*ADAMTS18*) genes are proteins displaying structural features and functions on the cell surface membrane, and have been reported to be involved in cancer progression. Using reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and methylation-specific PCR (MSP) analysis, the promoter methylation status and messenger RNA (mRNA) expression levels of *CDH1*, *CDH13* and *ADAMTS18*, which are putative tumor-suppressor genes located on chromosome 16q, were evaluated. In addition, the mRNA expression levels of DNA methyltransferases (*DNMTs*) 1, 3A and 3B were examined, and the correlations among the different parameters analyzed were studied in 36 lymphomas and 16 non-malignant lymphoid tissue samples. A significant positive correlation was identified between the expression levels of *CDH1* and *CDH13* ($r=0.735$, $P<0.01$). *ADAMTS18* expression also exhibited a significant positive correlation with both *CDH1* and *CDH13* mRNA expression levels ($r=0.625$, $P<0.01$; and $r=0.720$, $P<0.01$, respectively). Our results indicated that *CDH1*, *CDH13* and *ADAMTS18*, which are localized on chromosome 16q, are remarkably correlated and frequently methylated in human lymphomas, and their methylation could not be explained solely by the mRNA expression level of *DNMTs*.

Introduction

Aberrant promoter hypermethylation contributes to the transcriptional inactivation of a number of genes in various malignant diseases (1). However, the precise mechanisms underlying this aberration remain unclear. DNA methylation is

established by the catalytic activity of a family of DNA methyltransferases (*DNMTs*), which includes *DNMT1*, *DNMT3A* and *DNMT3B* (2). The association between *DNMTs* expression and promoter hypermethylation of the tumor-suppressor gene (TSG) *P15*^{IN4B} has been reported in acute myeloid leukemia (3). In another study on diffuse large B-cell lymphoma (DLBCL), the overexpression of *DNMT3B* and *DNMT1* proteins was significantly correlated with the promoter hypermethylation of various genes, including *p16* and von Hippel-Lindau (4). However, a lack of association between deregulated *DNMTs* expression and aberrant promoter methylation has also been reported (5-8).

E-cadherin, the gene product of *CDH1*, is a calcium-dependent cell adhesion molecule that is essential for maintaining the integrity of cell-cell adhesions (9). Downregulation of E-cadherin has been identified in numerous human cancers, and a loss of E-cadherin function was demonstrated to be associated with *CDH1* promoter hypermethylation and increased invasiveness and metastasis in human tumors (9,10). However, in another study on DLBCL, *CDH1* promoter hypermethylation was observed not to be correlated with the expression levels of *DNMTs* (4).

H-cadherin, the gene product of *CDH13*, is a novel member of the cadherin family (11). H-cadherin has a unique feature, in that it is devoid of a transmembrane domain, and is anchored to the cell surface membrane via a glycosylphosphatidylinositol (GPI) moiety instead. In addition, it also lacks a cytoplasmic domain (11). *CDH13* downregulation has been associated with the tumorigenesis of multiple cancers (11). *CDH13* silencing due to promoter hypermethylation and/or loss of heterozygosity is associated with tumor progression in BCLs (12).

A disintegrin and metalloproteinase with thrombospondin motif 18 (*ADAMTS18*) belongs to the *ADAMTSs* family, a group of secreted proteases that control several cell functions (13). Porter *et al* (14) demonstrated that there was a downregulation of several members of the *ADAMTS* family, including *ADAMTS18*, in human breast cancer compared with non-neoplastic mammary tissues. In addition, silencing of *ADAMTS18* by methylation of promoter CpG islands has been reported in multiple carcinoma cell lines, particularly in cell lines derived from esophageal and nasopharyngeal carcinomas (15).

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Tumor cells are characterized by frequent deletions of chromosomal regions that encode multiple TSGs (16). *CDH1*, *CDH13* and *ADAMTS18* are all located on chromosome 16q (16q22.1, 16q24.2 and 16q23.1, respectively). Similar to epigenetic modifications, genetic changes that are caused mainly by chromosomal loss of heterozygosity or mutations within the genes have been demonstrated to be associated with the upregulation of oncogenes or the downregulation of TSGs (16,17).

To the best of our knowledge, the correlations among the expression levels of *CDH1*, *CDH13*, *ADAMTS18* and *DNMTs*, as well as their associations with the promoter methylation status of *CDH1*, *CDH13* and *ADAMTS18* in human lymphoma, have not been analyzed to date. Therefore, in the present study, the expression levels of *CDH1*, *CDH13* and *ADAMTS18* were investigated by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) in human lymphoma. In addition, the frequency of methylation of the *CDH1*, *CDH13* and *ADAMTS18* gene promoters was examined using methylation-specific PCR (MSP). It was observed that these chromosome 16-located TSGs are frequently methylated and correlated, but they are not associated with the *DNMTs* levels in human BCL.

Patients and methods

Patients. From the files of the Department of Hematology of Gunma University Hospital (Gunma, Japan), cases of surgically biopsied lymph nodes from patients that were collected between December 2006 and July 2012 were obtained upon receiving appropriate institutional review board approval from Gunma University and patients' written informed consent. For all cases, the diagnosis was based on a morphological and immunohistochemical analysis according to the World Health Organization classification (18). The criterion for the selection of these cases was the availability of fresh-frozen optimal cutting temperature compound-embedded tumor biopsy specimens collected prior to any treatment. The present study included 29 cases of DLBCL, 7 cases of mantle cell lymphoma and 16 samples of non-malignant lymphoid tissues (including necrotizing lymphadenitis, reactive lymph nodes, immunoglobulin G4-associated diseases, follicular hyperplasia and granulomatous lymphadenitis).

Purification of genomic DNA (gDNA) and total RNA. gDNA and total RNA were purified from each tumor tissue using the AllPrep DNA/RNA Mini kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's protocol.

RT-qPCR. Total RNA ($\leq 1 \mu\text{g}$) for qPCR assays was treated and reverse transcribed using PrimeScript RT Reagent kit with gDNA Eraser (Takara Biotechnology Co., Ltd., Dalian, China) according to the protocol provided by the manufacturer. RT-qPCR was performed using SYBR Green PCR Master Mix (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA) on a 7300 Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.). Complementary DNA (1 μl) was mixed with gene-specific primers (listed in Table I), along with SYBR Green PCR Master Mix in a final volume of 20 μl . PCR was performed under the following conditions:

Initial denaturation at 95°C for 10 min, followed by 45 cycles of denaturation at 95°C for 15 sec and annealing/extension at 60°C for 1 min. The relative level of expression of *DNMTs*, *CDH1*, *CDH13* and *ADAMTS18* were normalized using a reference gene (β -actin) and control cells (HL60), and calculations were performed using the $2^{-\Delta\Delta C_q}$ method (19).

Bisulfite modification and MSP. The extracted DNA was modified using the Methyl Easy Xceed Rapid DNA Bisulphite Modification kit (Genetic Signatures, Darlinghurst, Australia) according to the manufacturer's protocol. MSP was performed in a total volume of 20 μl , containing 2 μl sodium bisulfite-modified template DNA, using EpiTaq HS for bisulfite-treated DNA (Takara Biotechnology Co., Ltd.). Each MSP reaction was performed with the following conditions: Denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 30 sec, 30 sec at the specific annealing temperature for *CDH1* and *CDH13* [53°C for methylated (M) and 57°C for unmethylated (U) DNA] and *ADAMTS18* (57°C for M and U DNA) and extension at 72°C for 30 sec, followed by a final 4-min extension at 72°C.

The primer sequences were as follows: *CDH1* (*CDH1UM* sense, 5'-GGTTTGATTTGATTGTATTT-3' and *CDH1UM* antisense, 5'-AAATACATCCCTCACAAAT-3'; and *CDH1M* sense, 5'-GGTTCGATTCGATCGTATTC-3' and *CDH1M* antisense, 5'-GAATACGTCCCTCGCAAAT-3'); *CDH13* (*CDH13UM* sense, 5'-TTGTGGGGTGTGTTTTTGT-3' and *CDH13UM* antisense, 5'-AACTTTTCATTTCATACACACA-3'; and *CDH13M* sense, 5'-TCGCGGGGTTCGTTTTCGC-3' and *CDH13M* antisense, 5'-GACGTTTTCATTTCATACACGCG-3'); and *ADAMTS18* (*ADAMTS18UM* sense, 5'-AAATTGTAGTTTGGTAGGTTTGT-3' and *ADAMTS18UM* antisense, 5'-CAACTCCAAATAAAACCACCA-3'; and *ADAMTS18M* sense, 5'-TTGTATTTCGGTAGGTTTCGC-3' and *ADAMTS18M* antisense, 5'-ACTCCAAATAAAACCGCCG-3'). Primer sequences were described previously (15,20,21) and were purchased from Eurofins MWG Operon, Inc. (Huntsville, AL, USA). MSP products were visualized under ultraviolet illumination following electrophoresis in 2% agarose gels containing GelRed nucleic acid gel stain (Biotium, Fremont, CA, USA).

5-Aza-2'-deoxycytidine (5-aza-dC) treatment. Established lymphoma cell lines [Raji (22) (Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan), CTB-1 (23) and SLVL (24) (Riken, Saitama, Japan)] and one patient primary DLBCL cell line were cultured in RPMI-1640 medium (Wako Pure Chemical Industries, Ltd., Osaka, Japan) at 37°C in an atmosphere of 5% CO₂ and treated with the demethylating agent 5-aza-dC (Wako Pure Chemical Industries, Ltd.). Cells (5×10^4) were grown in the absence or presence of 5-aza-dC at a concentration of 3 μM . The demethylation treatment was performed three times to verify the results.

Statistical analysis. Using the SPSS 20 software (IBM SPSS, Armonk, NY, USA), differences in the mean rank values between groups were analyzed with the Mann-Whitney *U* test. The significance of associations between parameters were analyzed by Spearman's correlation coefficient. $P < 0.05$ was considered to indicate a statistically significant difference.

Table I. Primers, amplicon sizes and accession number of genes analyzed by RT-qPCR.

Target genes	Primer sequences (5'-3') ^a	Size (bp)	Accession number
<i>DNMT1</i>	GCCAACGAGTCTGGCTTTGAG (sense) GTGTCGATGGGACACAGGTGA (antisense)	101	NM001130823
<i>DNMT3A</i>	ACCCGACTTCATAATGGTGCTTTC (sense) CGCCATCTGCAAGCTGTCTC (antisense)	139	NM022552
<i>DNMT3B</i>	TAAATACAAGGGCTGGAGTCTGCAC (sense) TGACGTCATCCCTGCTGAAATC (antisense)	80	NM006892
<i>CDH1</i>	GAGTGCCAACTGGACCATTCAGTA (sense) AGTCACCCACCTCTAAGGCCATC (antisense)	86	NM0043603
<i>CDH13</i>	GACATTGTCACTGTTGTGTCACCTG (sense) CCGTGCCTGTTAATCCAACATC (antisense)	121	NM0012573
<i>ADAMTS18</i>	AAGTGACATAAACGTGGTTGTGGTG (sense) GAGACTGGTCTGCATGATGGTTG (antisense)	89	NM1993552
β -actin	TGGCACCAGCACAATGAA (sense) CTAAGTCATAGTCCGCCTAGAAGCA (antisense)	186	NM_0011013

^aPrimers were purchased from Takara Biotechnology Co., Ltd. and designed using One Step PrimeScript™ RT-PCR kit (Perfect Real Time), which is an online support system to search for optimized primers when using SYBR® Green I detection in RT-qPCR. *DNMT*, DNA methyltransferase; *CDH1*, E-cadherin; *CDH13*, H-cadherin; *ADAMTS18*, a disintegrin and metalloproteinase with thrombospondin motifs 18; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

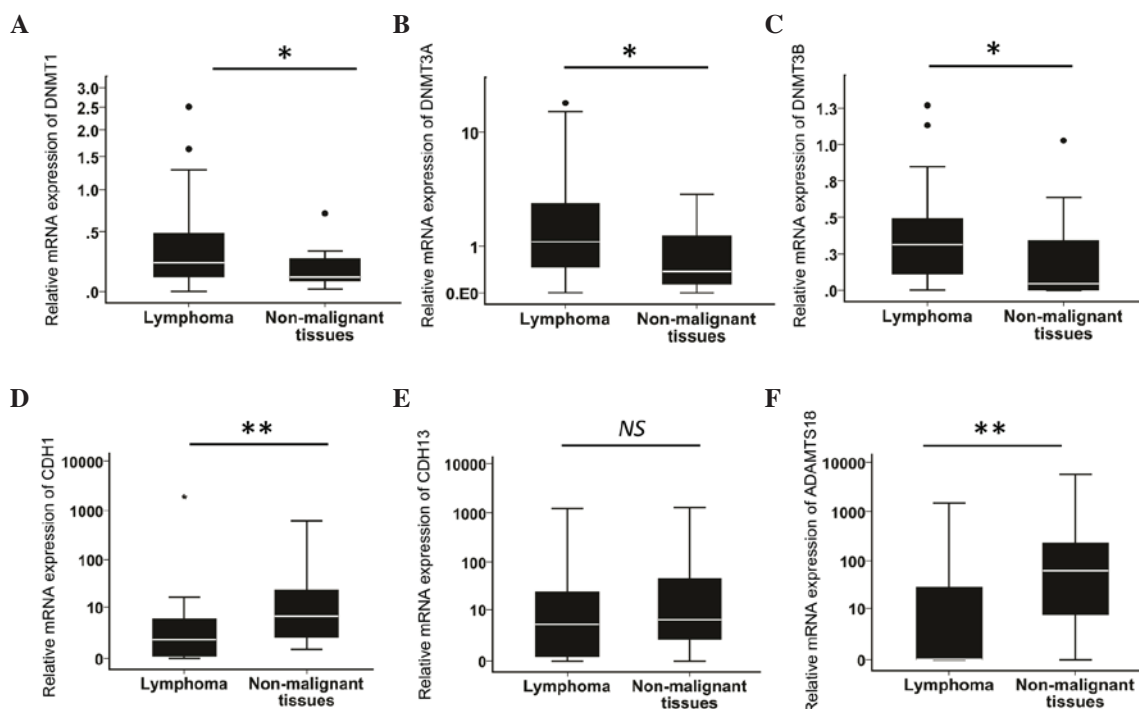


Figure 1. Relative messenger RNA expression levels of *DNMT1*, *DNMT3A*, *DNMT3B*, *CDH1*, *CDH13* and *ADAMTS18*. The expression levels were determined by reverse transcription-quantitative polymerase chain reaction in 36 cases of lymphoma (29 diffuse large B-cell lymphoma and 7 mantle cell lymphoma) and 16 samples of non-malignant lymphoid tissues. Relative (A) *DNMT1*, (B) *DNMT3A*, (C) *DNMT3B*, (D) *CDH1*, (E) *CDH13* and (F) *ADAMTS18* expression levels (* $P < 0.05$, ** $P < 0.01$ by the Mann-Whitney *U* test). *DNMT*, DNA methyltransferase; *CDH1*, E-cadherin; *CDH13*, H-cadherin; *ADAMTS18*, a disintegrin and metalloproteinase with thrombospondin motifs 18; NS, not significant.

Results

Expression of *DNMTs*, *CDH1*, *CDH13* and *ADAMTS18*.
The relative mRNA expression levels of *DNMT1*, *DNMT3A*, *DNMT3B*, *CDH1*, *CDH13* and *ADAMTS18* were detected

by RT-qPCR in 36 cases of lymphoma and in 16 samples of non-malignant lymphoid tissue. The mean rank expression levels of *DNMTs* (1, 3A and 3B) in the lymphomas were significantly higher ($P = 0.044$, $P = 0.036$ and $P = 0.013$) than those in the non-malignant tissues by 45.4, 45.9 and 60.4%, respectively

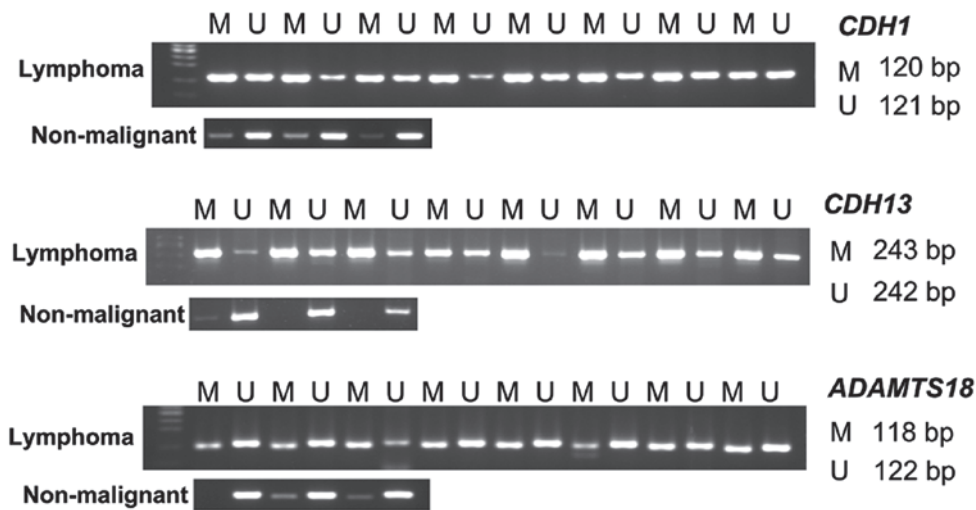


Figure 2. Methylation-specific PCR. The DNA methylation status of *CDH1*, *CDH13* and *ADAMTS18* was assessed for selected lymphoma and non-malignant samples that were amplified by PCR with primers specific for the U or M CpG islands of the target genes upon modification with sodium bisulfite. PCR, polymerase chain reaction; *CDH1*, E-cadherin; *CDH13*, H-cadherin; *ADAMTS18*, a disintegrin and metalloproteinase with thrombospondin motifs 18; M, methylated; U, unmethylated.

(Fig. 1A-C). The expression of *CDH1* and *ADAMTS18* were both significantly ($P<0.01$) reduced in lymphomas by 36.0% with respect to non-malignant tissues, while *CDH13* expression was non-significantly reduced by 14.4% in lymphomas compared with non-malignant tissues (Fig. 1D-F).

Methylation status of *CDH1*, *CDH13* and *ADAMTS18*, and their association with the mRNA expression levels of *DNMTs*, *CDH1*, *CDH13* and *ADAMTS18*. MSP was used (Fig. 2) to investigate the promoter methylation status of *CDH1*, *CDH13* and *ADAMTS18* in 36 lymphoma and 16 non-malignant lymphoid tissue samples. Promoter hypermethylation of *CDH1*, *CDH13* and *ADAMTS18* was detected in 31/36 (86.1%), 33/36 (91.7%) and 28/36 (77.8%) lymphomas, respectively, and in 4/16 (25.0%), 8/16 (50.0%) and 5/16 (31.3%) non-malignant tissue samples, respectively. The expression of *CDH1* and *ADAMTS18* was significantly ($P=0.008$ and $P=0.019$, respectively) reduced in samples with a hypermethylated promoter compared with those with an unmethylated promoter, while *CDH13* expression displayed a non-significant reduction (17.9%) in subjects with a methylated *CDH13* promoter (Fig. 3A). No associations were identified between the increased levels of *DNMTs* mRNA and the *CDH1*, *CDH13* or *ADAMTS18* promoter hypermethylation (Fig. 3B-D).

To confirm that promoter methylation was responsible for the silencing of *CDH1*, *CDH13* and *ADAMTS18* expression, methylated lymphoma cell lines were treated with 5-aza-dC, a demethylating agent, and *CDH1*, *CDH13* and *ADAMTS18* expression was examined by RT-qPCR. Upon treatment with 5-aza-dC, the promoter region of the *CDH1*, *CDH13* and *ADAMTS18* genes exhibited hypomethylation, and their mRNA expression levels were increased (Fig. 4).

Associations between the relative mRNA expression levels of *CDH1*, *CDH13* and *ADAMTS18*. The correlations between the different tested parameters were evaluated across the spectrum of both lymphoma and non-malignant tissues. The

CDH1 expression level exhibited a significantly positive correlation with the *CDH13* expression level ($r=0.735$, $P<0.01$) (Fig. 5A). Furthermore, the *ADAMTS18* expression level was positively correlated with the *CDH1* and *CDH13* expression levels ($r=0.625$, $P<0.01$; and $r=0.720$, $P<0.01$, respectively) (Figs. 5B and C).

Discussion

Promoter hypermethylation and loss of function of *CDH1*, *CDH13* and *ADAMTS18* have been reported in various cancers and cancer cell lines (10,11,13). In the present study, the promoter methylation of *CDH1*, *CDH13* and *ADAMTS18*, which are putative TSGs located on chromosome 16q (16q22.1, 16q24.2 and 16q23.1, respectively), was investigated using MSP, and the expression levels of *DNMTs*, *CDH1*, *CDH13* and *ADAMTS18* were examined using RT-qPCR to study whether these parameters are correlated and associated with *DNMTs* in human BCL.

Methylation of the *CDH1* promoter CpG islands and consequent loss of E-cadherin expression has been reported in multiple tumor tissues (25-31). *ADAMTS18* has been reported to be downregulated through promoter methylation in esophageal and nasopharyngeal cancer cell lines (15). In the present study, the relative mRNA expression levels of *CDH1* and *ADAMTS18* were both significantly reduced in lymphoma samples by 36.0% with respect to non-malignant tissues. In addition, promoter hypermethylation of *CDH1* and *ADAMTS18* was detected in 31/36 (86.1%) and 28/36 (77.8%) of lymphomas, respectively. Furthermore, the reduction of *CDH1* and *ADAMTS18* was significantly associated with their corresponding hypermethylated promoters when compared with their unmethylated promoters. Therefore, the current results suggest that the aberrant expression of *CDH1* and *ADAMTS18* and their promoter hypermethylation may be important in lymphomagenesis.

CDH13 downregulation due to promoter hypermethylation has been observed in various cancers (11), including

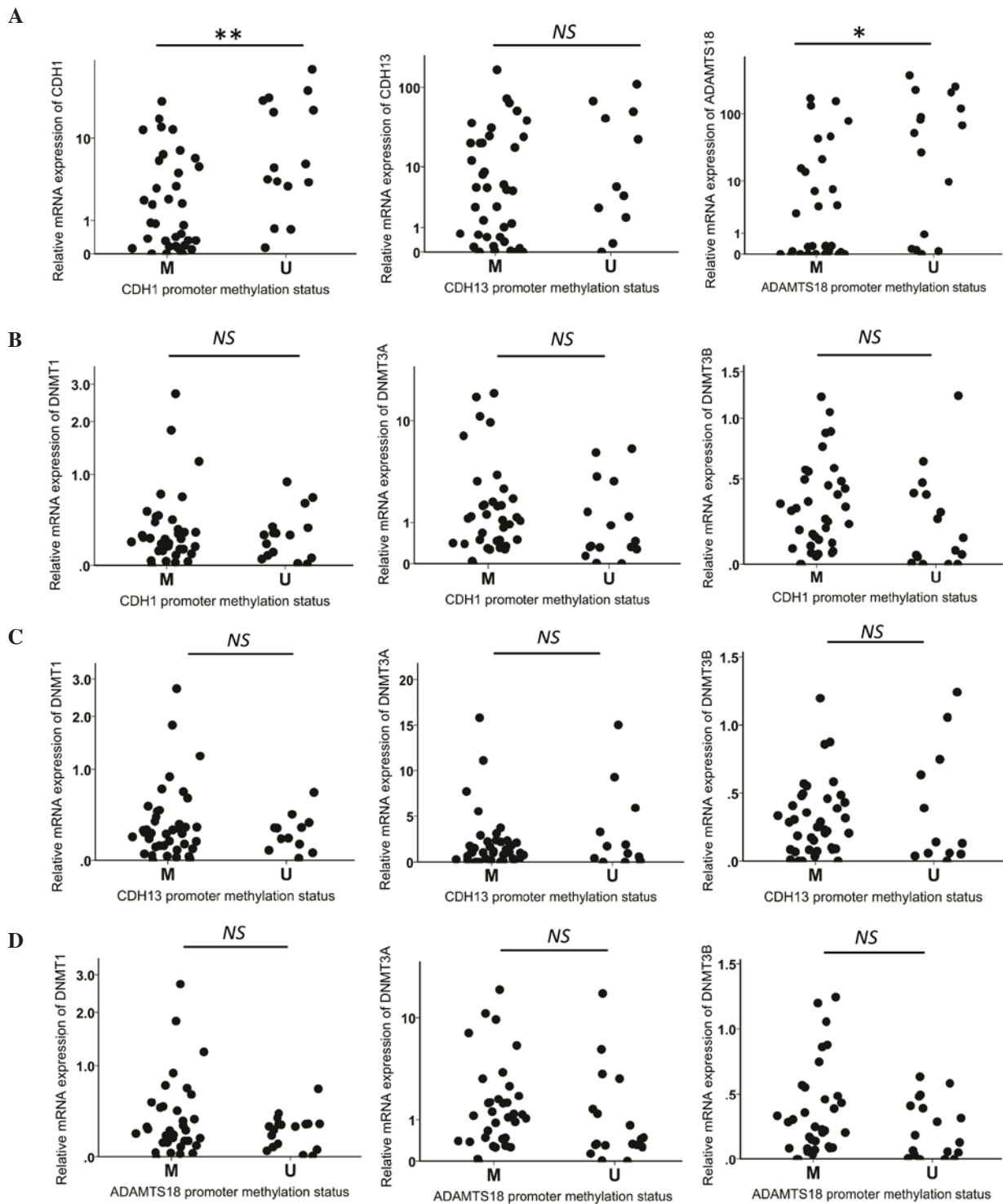


Figure 3. (A) Relative *CDH1*, *CDH13* and *ADAMTS18* mRNA expression levels and their corresponding promoter methylation status. (B-D) Correlations among the methylation status of (B) *CDH1*, (C) *CDH13* and (D) *ADAMTS18* and the mRNA expression levels of *DNMT1*, *DNMT3A* and *DNMT3B* (* $P<0.05$, ** $P<0.01$ by the Mann-Whitney *U* test). *DNMT*, DNA methyltransferase; *CDH1*, E-cadherin; *CDH13*, H-cadherin; *ADAMTS18*, a disintegrin and metalloproteinase with thrombospondin motifs 18; NS, not significant; M, methylated; U, unmethylated; mRNA, messenger RNA.

BCLs (12). In the present study, the relative mRNA expression level of *CDH13* in human lymphomas was reduced by 14.4% (not significant) compared with that in non-malignant tissues. Furthermore, methylation of *CDH13* was detected more frequently in lymphoma (91.7%) than in non-malignant tissues (50.0%), and there was a non-significant reduction in *CDH13* expression in subjects with a methylated *CDH13* promoter

(17.9%). No significant differences were observed among the expression levels of *DNMTs* in subjects with a methylated *CDH13* promoter compared with those with unmethylated promoters. These non-significant results may be due to the fact that human clinical samples are heterogeneous, with cytological diversity and different variants that may neutralize the aberrant expression of genes.

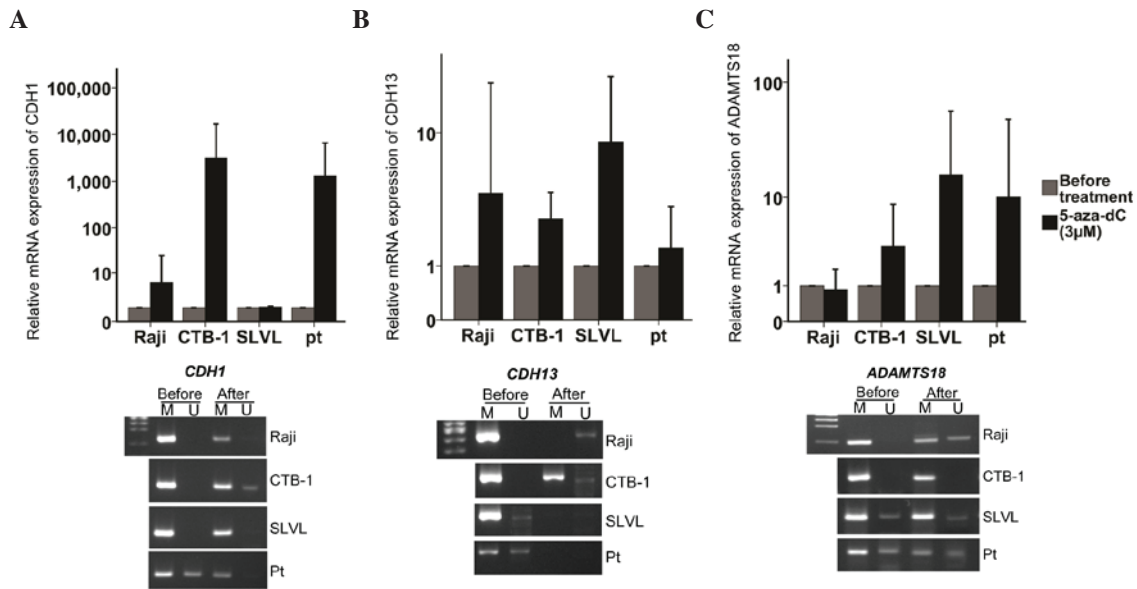


Figure 4. Treatment of lymphoma cell lines with 5-aza-dC. (A) *CDH1*, (B) *CDH13* and (C) *ADAMTS18* messenger RNA expression levels and their corresponding promoter methylation status prior and subsequent to the treatment of lymphoma cell lines (Raji, CTB-1 and SLVL) and one primary cell line from a patient with diffuse large B-cell lymphoma with 5-aza-dC at a concentration of 3 μ M for three times. *CDH1*, E-cadherin; *CDH13*, H-cadherin; *ADAMTS18*, a disintegrin and metalloproteinase with thrombospondin motifs 18; NS, not significant; M, methylated; U, unmethylated; pt, patient; 5-aza-dC, 5-aza-2'-deoxycytidine. Originally published in Blood 122: 4289, 2013.

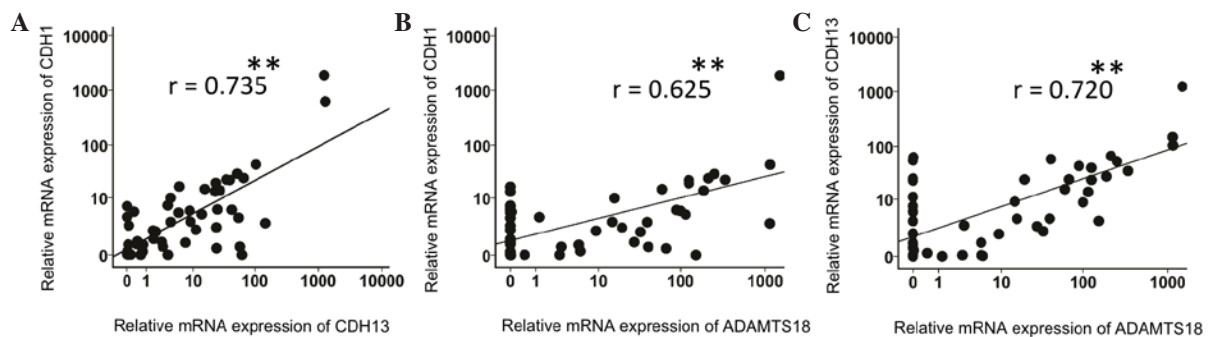


Figure 5. Correlations across the spectrum of both lymphoma and non-malignant tissues (n=52). (A) Correlation of the relative expression levels of *CDH1* and *CDH13*. (B and C) Correlation of the relative expression levels of *ADAMTS18* with (B) *CDH1* and (C) *CDH13* ($P < 0.01$ by Spearman's correlation coefficient). *CDH1*, E-cadherin; *CDH13*, H-cadherin; *ADAMTS18*, a disintegrin and metalloproteinase with thrombospondin motifs 18. Originally published in Blood 122: 4289, 2013.

Genes with transcriptional inactivation due to methylation are sensitive to DNA methylation inhibitors and can easily be reactivated (32). The methylation inhibitor 5-aza-dC, can be incorporated during DNA synthesis, and reduces the capacity for DNA methylation by DNMTs, thus reversing the methylation status of the promoters of genes (32,33). In the present study, methylated BCL cell lines (Raji, CTB-1, SLVL) and a primary patient cell line were treated with 5-aza-dC. Following demethylation treatment, the expression levels of *CDH1*, *CDH13* and *ADAMTS18* mRNA were upregulated ≥ 2 -fold, with corresponding complete or partial promoter hypomethylation.

By contrast, in the current surgically-resected tumor samples, not apparent significant correlation was identified between the methylation status of TSGs and the expression levels of *DNMTs*. These findings are in agreement with previous studies that failed to demonstrate any significant correlation between *DNMTs* expression and aberrant promoter

methylation of the tested genes (5,6,34,35). This finding can be explained by the fact that the overexpression of *DNMTs* is considered to be the primary mechanism responsible for the hypermethylation of TSGs in cancer cells, while a gain of methylation could also be secondary to the overexpression of transcriptional repressors or to the loss of transcriptional activators, as well as the result of an interallelic transfer of methylation via gene pairing (6,36). Another study (8) revealed that transgenic overexpression or complete depletion of *DNMT3B* did not affect the methylation status of HCT-116 colon cancer cells. Furthermore, in the present study, the expression levels of *DNMTs* mRNAs were significantly higher in lymphomas than in non-malignant tissue samples. This may be attributable to differences in the cell proliferation rate between lymphomas and non-malignant tissues (6). Taken together, since tumor cell lines are developed from single cells, and therefore consist of cells with a uniform genetic composition, it is logical that the expression of genes displayed a clearer association with their

corresponding promoter's methylation status than with that observed in the heterogeneous tumor clinical samples.

CDH1 and *CDH13* are important cell adhesion molecules, and alterations in their structure and function often cause a reduction in the adhesion between tumor cells, which may cause the detachment of cells from the primary tumors and the acquisition of invasive and metastatic properties (10,11). Notably, in the present study, a significant positive correlation between the expression levels of both *CDH1* and *CDH13* was observed. Regulation of VE-cadherin by N-cadherin was previously described in non-malignant human umbilical vein endothelial cells (37). It has also been demonstrated that N-cadherin and VE-cadherin are co-expressed in human breast cancer (38). N-cadherin controls the expression of VE-cadherin, and the latter regulates the subcellular localization of N-cadherin by causing its translocation from the cell surface (38). Since *CDH13* lacks the transmembrane and cytoplasmic regions of other cadherins, and instead uses a GPI anchor and the interactions with specific ligands (11), we suggest that *CDH1* may interact with *CDH13* and facilitate its signaling inside the cell.

In addition, the present study identified significant positive correlations between the expression level of *ADAMTS18* and the expression levels of *CDH1* and *CDH13*. *ADAMTS*s, including *ADAMTS18*, are important in mediating the degradation of extracellular matrix proteins, as well as the ectodomain shedding of growth factors, growth factor receptors and adhesion molecules (13). Interactions between metalloproteinases and adhesion molecules have been reported (10,39,40). For example, Maretzky *et al* (39) demonstrated that *ADAM10* contributes to E-cadherin shedding and to cell proliferation by modulating β -catenin signaling through E-cadherin shedding. Furthermore, *ADAM15* catalyzes soluble E-cadherin shedding, which in turn leads to its binding to the ErbB receptor and to the stimulation of ErbB receptor signaling via human epidermal growth factor (HER) 2 and HER3 in breast cancer cells (40). Therefore, the positive correlations between *ADAMTS18* and *CDH1* and *CDH13* mRNA in the current study may reflect the requirement of *ADAMTS* for cadherin processing in the cells, and also suggest the existence of a common factor regulating these genes, which are all located on chromosome 16q, thus further emphasizing the importance of this region.

Of note, as presented in Fig. 5B and C, the expression of *ADAMTS18* in replicate samples (16/52 samples, 30.8%) did not display any correlation with the expression of either *CDH1* or *CDH13*. The complete lack of *ADAMTS18* expression and the absence of correlation with *CDH1*, *CDH13* or *DNMTs* in these patients' samples may imply that there is a loss of the *ADAMTS18* gene locus.

Furthermore, to support our hypothesis that chromosome 16 and cell surface membrane-located TSGs are correlated with each other in human BCL, the present study examined the expression of another TSG located on the same chromosome, cyclin-dependent kinase inhibitor (*CDKN*) 2A (p16). It was observed that the expression levels of our tested TSGs did not exhibit any correlation with the *CDKN2A* expression level (data not shown).

In conclusion, our findings demonstrated that the expression levels of TSGs adjacently located at chromosome 16q (*CDH1*, *CDH13* and *ADAMTS18*) are positively correlated and frequently methylated, and that their methylation status is

not associated with the expression levels of *DNMTs* in human lymphoma. Our findings suggest that aberrantly methylated cell surface membrane TSGs located on chromosome 16q are correlated and may be important role in the pathogenesis of human BCL.

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