E-Cadherin Expression Is Silenced by 5′ CpG Island Methylation in Acute Leukemia[1]

**Paul G. Corn,[2] B. Douglas Smith,
Emily S. Ruckdeschel, Donna Douglas,
Stephen B. Baylin, and James G. Herman[3]**

The Johns Hopkins Oncology Center, Baltimore, Maryland 21231

ABSTRACT

E-Cadherin is a transmembrane glycoprotein that mediates Ca^{2+}-dependent intercellular adhesion in normal epithelium. In tumors of epithelial origin, E-cadherin expression frequently is reduced, an event that contributes to tumor invasion and metastasis. The role of E-cadherin in hematopoietic tissues is less clear. In normal bone marrow, E-cadherin is expressed on erythroid progenitors, CD34+ stem cells, and stromal cells, where it likely contributes to intercellular interactions during hematopoiesis. In this study, we used a nested-PCR approach to examine the methylation status of the E-cadherin 5′ CpG island in blood and bone marrow samples from normal donors and in bone marrow from patients with acute leukemia. In normal peripheral blood mononuclear cells and bone marrow, E-cadherin was completely unmethylated. In peripheral blood mononuclear cells, expression was evident by reverse transcription-PCR. Immunoblotting confirmed E-cadherin protein expression in two lymphoblastoid cell lines derived from normal donors. In contrast, E-cadherin was aberrantly methylated in 4 of 4 (100%) leukemia cell lines, 14 of 44 (32%) acute myelogenous leukemias, and 18 of 33 (53%) acute lymphoblastic leukemias. Genomic bisulfite sequencing of primary leukemias confirmed dense methylation across the CpG island. Methylation was associated with loss of E-cadherin RNA and protein in leukemia cell lines and primary leukemias. Following treatment with 5-aza-2′-deoxycytidine, a methylated leukemia cell line expressed both E-cadherin transcript and protein. Our results show that methylation of E-cadherin occurs commonly in acute leukemia and suggests a hypothesis for E-cadherin downregulation in leukemogenesis.

INTRODUCTION

Cadherins are a family of transmembrane glycoproteins that mediate Ca^{2+}-dependent intercellular adhesion. One member of this family, epithelial (E)-cadherin (120 kDa), is expressed predominantly on the surface of epithelial cells, where it plays a major role in the establishment and maintenance of normal tissue architecture (1). Whereas the extracellular domain of E-cadherin is responsible for both homophilic (E-cadherin/E-cadherin) and heterophilic binding (E-cadherin/non-E-cadherin) specificities, the adhesive function of the molecule is ultimately dependent on its interaction with the actin cytoskeleton through the intracellular catenins (1). β-Catenin (94 kDa) and γ-catenin (82 kDa) bind directly to the cytoplasmic domain of E-cadherin, whereas α-catenin (102 kDa) binds to β-catenin and links the complex to a network of actin microfilaments. β-Catenin also functions in signal transduction by binding to T-cell factor/lymphoid enhancer factor (Tcf/Lef) proteins, forming a complex that translocates to the nucleus and transactivates specific genes in the wingless/*wnt* signaling pathway (2). Recently, E-cadherin was shown to inhibit β-catenin-mediated transactivation by binding to β-catenin and blocking its interaction with Lef-1 (3). Thus, E-cadherin appears to have a dual purpose in the mammalian cell, participating in both intercellular adhesion and as part of a complex signaling pathway.

E-cadherin expression frequently is reduced or absent in a variety of epithelial cancers, and loss of normal intercellular junctions is thought to precede tumor invasion and metastasis (4–7). There are several mechanisms for abnormal E-cadherin expression in tumors. It has recently been shown that germ-line mutations of the gene (*CDH1*), although rare, predispose to familial gastric and colorectal cancer (8, 9). Similarly, although allelic loss of the E-cadherin locus at 16q22.1 occurs in a variety of different solid tumors, somatic mutations generally are infrequent, with the exception of diffuse gastric carcinomas and lobular breast carcinomas (10). In contrast, a common mechanism for E-cadherin loss is epigenetic silencing via DNA hypermethylation, occurring in 15–80% of oral, hepatocellular, breast, and prostate cancers (11–13). Methylation of the cytosines in CpG dinucleotides within promoter CpG islands is associated with transcriptional repression, an event that normally occurs during genomic imprinting and X-chromosome inactivation (14). In neoplasia, however, aberrant methylation of CpG islands results in inactivation of tumor suppressor genes and genes that maintain normal cell growth, such as *p16* and *MLH1* (14, 15).

Although the importance of E-cadherin expression to epithelium is well established, its role in hematopoietic tissues has been studied only recently. During normal hematopoiesis, erythroid, myeloid, and lymphoid progenitor cells adhere selectively to stromal cells and the extracellular matrix, allowing them to receive maturation influences from the bone marrow microenvironment (16). These influences include chemokines, growth

factors, and intercellular interactions mediated by adhesion molecules such as the integrins (17). In addition to regulating the physical interactions of progenitor cells, cell adhesion molecules are believed to influence differentiation by generating intracellular signals (18). E-Cadherin is in fact one of the adhesion molecules expressed by stromal cells and mononuclear cells in the bone marrow (19). Furthermore, during erythropoiesis, E-cadherin is selectively expressed by erythroid progenitors at specific stages of maturation, and the addition of anti-E-cadherin antibody to bone marrow mononuclear cells inhibited erythropoiesis *in vitro* (20, 21). Taken together, these data suggest that E-cadherin may contribute to intercellular interactions important for hematopoiesis.

In this study, we investigated the methylation status of the 5′ CpG island of E-cadherin in peripheral blood mononuclear cells from normal individuals and in leukemic blasts from patients with *de novo* acute leukemia.

MATERIALS AND METHODS

Patient Samples, Cell Lines, and Culture Conditions. The leukemia samples were diagnostic bone marrows obtained from patients undergoing evaluation and treatment at the Johns Hopkins Oncology Center (22). All patients gave informed consent for the bone marrow biopsy according to standard protocol. Lymphoblastoid cell lines were available from the Johns Hopkins University Genetic Resource Core Facility. The leukemia cell lines KG1A (AML),[4] U937 (AML), HL-60 (AML), and A301 (T-ALL) were analyzed in this study. Cell lines were maintained in the appropriate media and treated with 5-Aza-dC (Sigma) at a concentration of 1 μM for 3–5 days to achieve demethylation.

DNA Extraction. Genomic DNA was isolated from cell lines and primary leukemias by standard phenol and chloroform extraction with ethanol precipitation. DNA was isolated from bone marrow slides as described previously (23).

Methylation Analysis. Methylation patterns within the E-cadherin CpG island in exon 1 (sequence, −126 bp to +144 bp relative to transcription start; GenBank Accession No. D49685) were determined after the chemical modification of genomic DNA with sodium bisulfite (Sigma) as described (24). A nested-PCR approach was used. In the first round of PCR, 100 ng of bisulfite-treated DNA was amplified using sequencing primers (24). The sequencing primers were 5′-GTTTAGTTTT-GGGGAGGGGTT-3′ (sense) and 5′-ACTACTACTCCAAA-AACCCATAACTAA-3′ (antisense), and the cycling conditions consisted of an initial denaturation step at 95°C for 5 min, followed by the addition of 1 unit of *Taq* polymerase and then 30 cycles of 95°C for 30 s, 50°C for 30 s, and 72°C for 30 s. The size of the product following this initial PCR reaction was 270 bp. For the second round of PCR, this product was diluted 1:50 in water, and 2 μl of the dilution were used for MSP (25).

Nested primer sequences for E-cadherin were 5′-TGTAGT-TACGTATTTATTTTTAGTGGCGTC-3′ (sense) and 5′-CG-AATACGATCGAATCGAACCG-3′ (antisense) for the methy-lated reaction and 5′-TGGTTGTAGTTATGTATTTATTTT-TAGTGGTGTT-3′ (sense) and 5′-ACACCAAATACAATC-AAATCAAACCAAA-3′ (antisense) for the unmethylated reaction. PCR parameters were as above, except that the annealing temperatures for the methylated and unmethylated reactions were 64 and 62°C, respectively. The product sizes of the methylated and unmethylated reactions were 112 and 120 bp, respectively.

Immunoblotting. For analysis of cell lines, whole-cell lysates were prepared from cultured cells using lysis buffer (1× PBS, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) with complete protease inhibitors added (Boehringer Mannheim). Protein concentrations were determined using the BIO-RAD assay (Bio-Rad). Between 10 and 100 μg of protein were separated by SDS-PAGE, electrotransferred onto Immobilon-P membrane (Millipore) and incubated with the anti-E-cadherin antibody HECD-1 (Zymed laboratories, Inc). Immunoreactive bands on blots were visualized by the ECL method (Amersham). For analysis of primary leukemias, approximately $1–2 \times 10^6$ cells were lysed in NOVEX NuPAGE sample buffer and re-solved using a 4–12% Bid-Tris gel (NOVEX, San Diego, CA). HECD-1 antibody was used, and immunoreactive bands were visualized using ECL, as above. The ECL system has a detection limit in the nanogram range. To further maximize the sensitivity of detecting E-cadherin, Western blots of the primary leukemias were subsequently developed with the SuperSignal West Femto Maximum Sensitivity Substrate (Pierce), which can detect proteins in the picogram range.

Sodium Bisulfite DNA Sequencing. Sodium bisulfite-modified DNA was amplified using the E-cadherin sequencing primers described above. PCR products were cloned into the TA cloning vector pCR2.1 (Invitrogen), and individual clones were sequenced (ABI automated sequencing).

E-Cadherin Transcript Analysis. Cytoplasmic RNA was isolated using TriZOL Reagent (Life Technologies, Inc.) and reverse-transcribed using SuperScript II (Life Technologies). The RT-PCR primers for E-cadherin were 5′-GGAAGT-CAGTTCAGACTCCAGCC-3′ (sense, exon 1) and 5′-AGGC-CTTTTGACTGTAATCACACC-3′ (antisense, exon 3). The size of the RT-PCR product was 352 bp. Glyceraldehyde-3-phosphate dehydrogenase expression was analyzed as described previously (26).

RESULTS

In this study, primary leukemia samples were available from two main sources: bone marrow specimens that had been Ficoll-enriched for blasts and banked at −80°C, and bone marrow biopsy slides. Whereas genomic DNA isolated from banked samples was consistently suitable for our molecular studies, we found it difficult to reproducibly generate PCR products from material obtained from slides, most likely because of a problem in the quantity and/or quality of DNA substrate (data not shown). Subsequently, in an effort to facilitate the analysis of DNA samples isolated from slides, we developed a nested-PCR approach to study methylation of the E-cadherin promoter. In

---

[4] The abbreviations used are: AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; 5-Aza-dC, 5-aza-2′-deoxycytidine; MSP, methylation-specific PCR; ECL, enhanced chemiluminescence; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; RT-PCR, reverse transcription-PCR; PBMC, peripheral blood mononuclear cell.
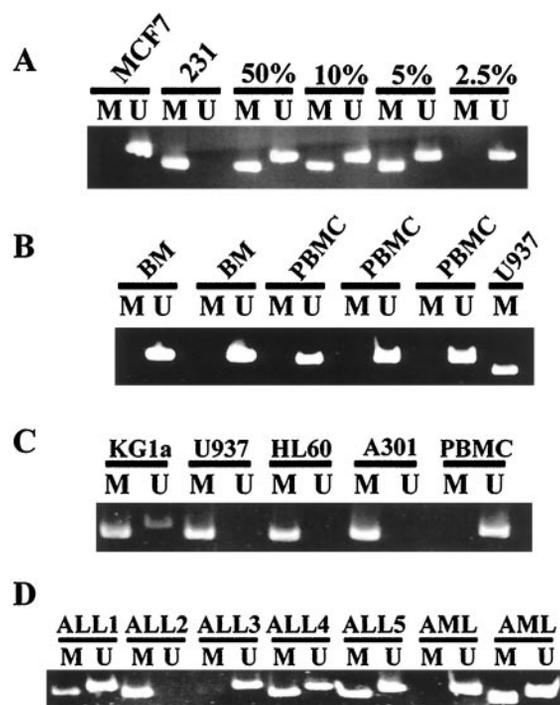
Fig. 1 Methylation of the E-cadherin promoter in cell lines and primary human samples. Visible PCR product in *Lanes U* indicates the presence of unmethylated alleles; visible PCR product in *Lanes M* indicates the presence of methylated alleles. *A*, representative mixing experiment. After bisulfite modification, MB-MDA-231 DNA, in which the E-cadherin promoter is extensively methylated, was mixed with MCF7 DNA, which is unmethylated, and assayed. Percentages indicate the proportion of methylated DNA used in the input DNA for the PCR. Note that methylated alleles of E-cadherin are no longer detectable when they comprise <5% of the total sample. *B*, MSP of PBMCs and normal bone marrow (*BM*) from healthy donors. All of the samples are unmethylated. The leukemia cell line U937 serves as a positive control for methylation. The unmethylated product for U937 is shown in *B*. *C*, MSP of leukemia cell lines. U937, A301 and HL-60 are fully methylated, whereas KG1a is predominantly methylated. *D*, MSP of primary ALLs and AMLs. The presence of unmethylated E-cadherin in all of the methylated tumors likely indicates the presence of contaminating normal lymphocytes.

the first round of PCR, the genomic region encompassing the transcription start site of E-cadherin was amplified using bisulfite-sequencing primers that avoid any CpG dinucleotides, so that methylated and unmethylated DNA are amplified with equal efficiency (27). This product was then used as a substrate for a second PCR, in which MSP primers were used to distinguish methylated *versus* unmethylated E-cadherin.

To estimate the sensitivity of nested-MSP for detecting methylation, DNA from a completely methylated breast cancer cell line, MB-MDA-231, was mixed at various ratios with DNA from a completely unmethylated cell line, MCF7, and assayed. With nested-MSP, methylated alleles were detectable when they comprised as little as 5% of the total DNA sample (Fig. 1*A*), a sensitivity comparable to previously published results using standard MSP (27). In additional experiments, the sensitivity of nested-MSP could be greatly enhanced by increasing the cycle number of the two-step PCR, but with a corresponding loss in

specificity (data not shown). Therefore, the cycling parameters for nested-MSP that we used were carefully chosen to permit the analysis of DNA collected from banked samples and slides with equal efficiency and accuracy (see below).

We initially studied the methylation status of E-cadherin in PBMCs collected from seven healthy donors. These normal cells are composed of both mature lymphoid and myeloid cells. E-Cadherin was unmethylated in all cases and also in normal bone marrow (*n* = 3; Fig. 1*B*). In addition, we studied two lymphoblastoid cell lines that were derived by taking normal lymphocytes and immortalizing them with EBV. Both of these lines were unmethylated at E-cadherin (data not shown).

To determine whether E-cadherin methylation occurred in leukemia, we next studied a series of leukemia cell lines that included both myeloid (*n* = 3) and lymphoblastic (*n* = 1) phenotypes. By nested-MSP, three of the cell lines (HL-60, U937, and A301) were fully methylated, and one (KG1a) was predominantly methylated (Fig. 1*C*). Methylation was also observed in primary acute leukemias of both myeloid and lymphoblastic phenotypes. In the AML group, 32% (14 of 44) of the samples were methylated at E-cadherin (Fig. 1*D*). The French-American-British classifications were available for 15 samples, and although all subtypes (M0–M7) were represented, the sample size was not large enough to evaluate for any differences in the frequency of E-cadherin methylation between groups. In the ALL group, 53% (18 of 33) of the samples were methylated at E-cadherin (Fig. 1*D*). The frequency of methylation was similar between B- and T-cell phenotypes. Ten of 17 (59%) B-cell leukemias were methylated, whereas 7 of 13 (54%) T-cell leukemias were methylated. Within the mixed-lineage ALLs, one of three (33%) was methylated. Unlike leukemia cell lines, primary leukemias always displayed evidence of unmethylated E-cadherin. This likely reflects the small proportion of normal PBMCs that are invariably present in primary leukemia samples when they are collected, and the high sensitivity of MSP for detecting such alleles.

Methylation of E-cadherin correlated with transcriptional silencing of the gene. The fully methylated cell lines U937 (AML) and A301 (T-ALL) had undetectable levels of E-cadherin transcript by RT-PCR (Fig. 2*A*). E-Cadherin was detectable only after treatment with the demethylating agent 5-Aza-dC. Western blot analysis of U937 confirmed that after 5-Aza-dC treatment, E-cadherin transcription was associated with E-cadherin protein expression (Fig. 2*B*). These data indicate that methylation is responsible for transcriptional repression of the gene. After several days, the treated cell lines demonstrated a slower growth rate and evidence of cell death, likely because of the general cytotoxic effect of 5-Aza-dC. Because other genes are methylated and silenced in U937 [for example, *p16* (28) and *p73* (29)], we cannot attribute these effects solely to demethylation of E-cadherin.

As in the cell lines, methylation of E-cadherin was associated with transcriptional silencing in primary leukemias. Compared with an unmethylated leukemia, two methylated leukemias did not express E-cadherin by RT-PCR, whereas a third had barely detectable levels (Fig. 2*C*). The low level of E-cadherin transcript detected in the methylated sample could theoretically reflect either transcription from contaminating normal PBMCs or minimal transcription from methylated leukemic
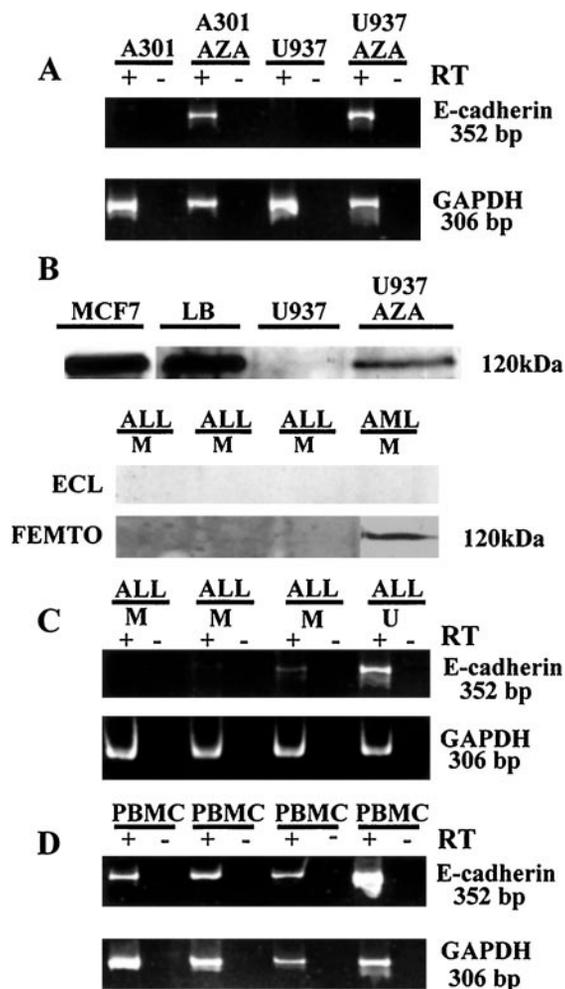
Fig. 2 Expression analysis of E-cadherin. *A*, E-cadherin expression in leukemia cell lines. The presence or absence of reverse transcriptase is indicated by + and −, respectively. Expression of glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was monitored in each sample to ensure the integrity of the RNA preparations. Note that for U937 and A301, expression is evident only after 5-Aza-dC treatment. *B*, immunoblot of E-cadherin. For MCF7, 10 μg of protein were loaded *versus* 100 μg each for the lymphoblastoid cell line (*LB*), U937, and U937 treated with 5-Aza-dC. MCF7, the breast cancer cell line that expresses high levels of E-cadherin, was used as a positive control. For primary leukemias, approximately 1–2 million cells were lysed and loaded directly onto the gel. *ECL*, blot was developed with a chemiluminescent reagent with the sensitivity to detect proteins in the nanogram range. *FEMTO*, blot was developed with a supersensitive chemiluminescent reagent that can detect proteins in the picogram range. *C*, E-cadherin RNA expression in primary ALLs. Three are methylated (*M*) at E-cadherin, whereas one is unmethylated (*U*). E-Cadherin expression is greatly reduced or absent in each of the methylated leukemias compared with the unmethylated leukemia. *D*, E-cadherin expression in PBMCs. Each expresses E-cadherin by RT-PCR.

alleles. In contrast, expression of E-cadherin transcript was uniformly evident in normal PBMCs (Fig. 2*D*). These data underscore the fact that methylation-associated silencing of E-cadherin is a leukemia-specific event.

By Western blot analysis, E-cadherin protein was expressed by normal lymphoblastoid cell lines, although at rela-

tively lower levels than the breast cancer epithelial cell line, MCF7 (Fig. 2*B*). To detect E-cadherin bands of comparable intensity, the amount of total protein loaded for the lymphoblastoid cell lines was roughly 10-fold greater than for MCF7. Among the primary leukemias, five methylated samples (three ALLs and two AMLs) initially showed no evidence of E-cadherin expression (Fig. 2*B*). Because our first analysis of E-cadherin expression had used RT-PCR, which is an exquisitely sensitive technique for detecting RNA, we sought to increase the sensitivity of the Western blot to detect minute quantities of E-cadherin protein. To do this, Western blots of the methylated primary leukemias were redeveloped with a supersensitive chemiluminescent reagent that can detect proteins in the picogram range (see "Materials and Methods"). Under these conditions, we could detect low-level expression of E-cadherin in the two methylated AMLs (Fig. 2*B*). Again, this band could reflect E-cadherin expression from normal PBMCs contaminating the primary samples or, alternatively, minimal expression from methylated leukemic alleles.

To further characterize E-cadherin methylation in primary leukemias and to correlate the density of methylation with expression, genomic bisulfite sequencing was performed on PBMCs and primary ALLs. Normal PBMCs were almost completely unmethylated, except for isolated CpG sites at the 5′ and 3′ ends of the region sequenced (Fig. 3, *bottom panel*). In contrast, two methylated ALLs (ALL1 and ALL2) demonstrated dense patterns of methylation in almost every allele, especially toward the 3′ end of the region sequenced. In ALL2, which had undetectable levels of E-cadherin transcript, there was near complete methylation of three CpG dinucleotides (8–10) immediately flanking the transcription starting point. In ALL1, which expressed very low levels of E-cadherin transcript, there was relatively less methylation at these same sites. Thus, CpG methylation observed with genomic sequencing correlated with transcriptional silencing of E-cadherin.

DISCUSSION

In this report, we found that aberrant methylation of the E-cadherin promoter is a frequent event in both AML and ALL. We used a nested-MSP approach that allowed us to analyze DNA samples obtained from banked leukemia samples and bone marrow slides with excellent reproducibility. Although one potential problem with nested-PCR would be the generation of false positives, the cycling parameters we chose were designed to demonstrate a high degree of sensitivity and specificity for the presence of significant methylation changes. As proof of this, methylation of cell lines and primary leukemias was associated with transcriptional silencing of the gene by RT-PCR. In addition, genomic bisulfite sequencing confirmed dense CpG methylation in those samples detected as methylated by nested-MSP. Because clinical material often is a scarce resource for molecular studies, the ability to analyze methylation changes using DNA from a single bone marrow slide represents a powerful tool.

The biological significance of E-cadherin methylation in acute leukemia is unclear. E-Cadherin has been shown to function during normal erythropoiesis, but its role during myeloid and lymphoid differentiation has not been studied (21). Al-
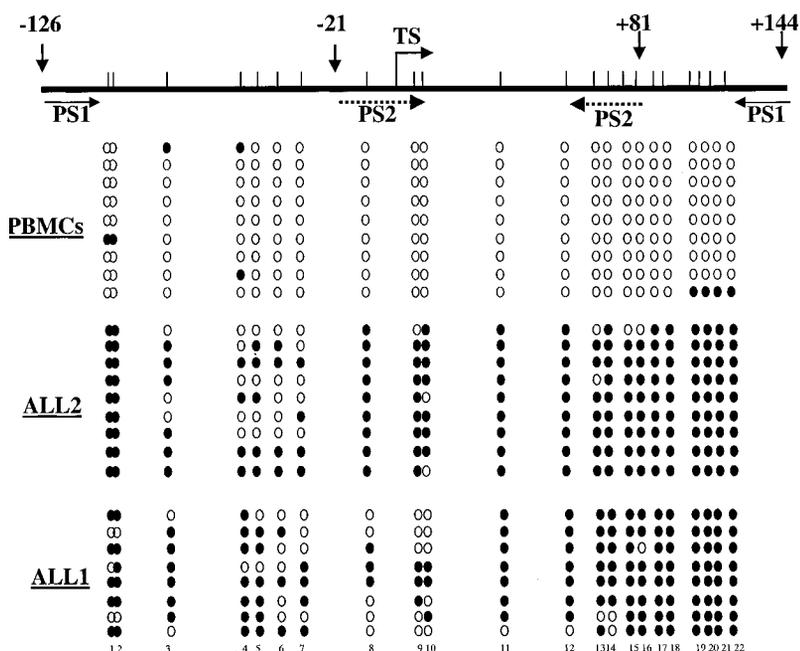
Fig. 3 Bisulfite sequencing of the E-cadherin CpG island. *Top*, map of the CpG island. Twenty-two individual CpG dinucleotides are indicated as *vertical lines*. *TS*, transcription start. Primer set 1 (*PS1*) indicates location of bisulfite sequencing primers. Primer set 2 (*PS2*) indicates location of nested-MSP primers. *Numbers* indicate positions relative to transcription start. *Bottom*, each *circle* indicates a CpG site in the primary sequence, and *each line of circles* represents analysis of a single cloned allele. ○, unmethylated CpG sites; ●, methylated CpG sites.

though analysis of E-cadherin expression on myeloid and lymphoid precursors was not performed in this study, we did find evidence of E-cadherin expression by mature PBMCs and normal lymphoblastoid cell lines. Our data suggest that E-cadherin expression is not limited to the erythrocyte lineage, but rather is a feature of multi-lineage hematopoiesis. In support of this, it was recently shown that E-cadherin is expressed on CD34+ cells, the pluripotent stem cell that is thought to give rise to erythroid, lymphoid, and myeloid progenitors (19).

The importance of adhesion mechanisms in the marrow is known from the extensive study of integrins, a family of adhesion molecules expressed by hematopoietic progenitors. During hematopoiesis, β1- (VLA-4, VLA-5) and β2-integrins (LFA-1) mediate interactions between progenitors and the bone marrow microenvironment that are thought to be important for normal proliferation, differentiation, and migration (30, 31). Interestingly, defects in integrin-mediated adhesion have been reported in B-lineage ALL and chronic myeloid leukemia (32). One possibility, then, is that E-cadherin represents another type of adhesion molecule that mediates interactions between progenitors and the bone marrow microenvironment. Loss of E-cadherin would contribute to the overall breakdown in adhesion that is a principal feature of leukemia.

In a recent study looking at multiple genes in AML, E-cadherin methylation was also observed (33). Together with our results, these data raise some intriguing possibilities for the role of epigenetic inactivation of E-cadherin during leukemogenesis. Further studies characterizing E-cadherin expression on myeloid and lymphoid cells at defined stages of maturation should begin to elucidate this role.

REFERENCES

1. Wijnhoven, B. P., and Pignatelli, M. E-Cadherin-catenin: more than a "sticky" molecular complex. Lancet, *354:* 356–357, 1999.

2. Behrens, J., von Kries, J. P., Kuhl, M., Bruhn, L., Wedlich, D., Grosschedl, R., and Birchmeier, W. Functional interaction of β-catenin with the transcription factor LEF-1. Nature (Lond.), *382:* 638–642, 1996.

3. Sadot, E., Simcha, I., Shtutman, M., Ben Ze'ev, A., and Geiger, B. Inhibition of β-catenin-mediated transactivation by cadherin derivatives. Proc. Natl. Acad. Sci. USA, *95:* 15339–15344, 1998.

4. Karayiannakis, A. J., Syrigos, K. N., Chatzigianni, E., Papanikolaou, S., Alexiou, D., Kalahanis, N., Rosenberg, T., and Bastounis, E. Aberrant E-cadherin expression associated with loss of differentiation and advanced stage in human pancreatic cancer. Anticancer Res., *18:* 4177–4180, 1998.

5. Sulzer, M. A., Leers, M. P., van Noord, J. A., Bollen, E. C., and Theunissen, P. H. Reduced E-cadherin expression is associated with increased lymph node metastasis and unfavorable prognosis in non-small cell lung cancer. Am. J. Respir. Crit. Care Med., *157:* 1319–1323, 1998.

6. Zheng, Z., Pan, J., Chu, B., Wong, Y. C., Cheung, A. L., and Tsao, S. W. Downregulation and abnormal expression of E-cadherin and β-catenin in nasopharyngeal carcinoma: close association with advanced disease stage and lymph node metastasis. Hum. Pathol., *30:* 458–466, 1999.

7. Oka, H., Shiozaki, H., Kobayashi, K., Inoue, M., Tahara, H., Kobayashi, T., Takatsuka, Y., Matsuyoshi, N., Hirano, S., and Takeichi, M. Expression of E-cadherin cell adhesion molecules in human breast cancer tissues and its relationship to metastasis. Cancer Res., *53:* 1696–1701, 1993.

8. Guilford, P., Hopkins, J., Harraway, J., McLeod, M., McLeod, N., Harawira, P., Taite, H., Scoular, R., Miller, A., and Reeve, A. E. E-Cadherin germline mutations in familial gastric cancer. Nature (Lond.), *392:* 402–405, 1998.

9. Richards, F. M., McKee, S. A., Rajpar, M. H., Cole, T. R., Evans, D. G., Jankowski, J. A., McKeown, C., Sanders, D. S., and Maher, E. R. Germline E-cadherin gene (CDH1) mutations predispose to familial gastric cancer and colorectal cancer. Hum. Mol. Genet., *8:* 607–610, 1999.

10. Berx, G., Becker, K. F., Hofler, H., and van Roy, F. Mutations of the human E-cadherin (CDH1) gene. Hum. Mutat., *12:* 226–237, 1998.

11. Saito, Y., Takazawa, H., Uzawa, K., Tanzawa, H., and Sato, K. Reduced expression of E-cadherin in oral squamous cell carcinoma: relationship with DNA methylation of 5′ CpG island. Int. J. Oncol., *12:* 293–298, 1998.

12. Kanai, Y., Ushijima, S., Hui, A. M., Ochiai, A., Tsuda, H., Sakamoto, M., and Hirohashi, S. The E-cadherin gene is silenced by CpG methylation in human hepatocellular carcinomas. Int. J. Cancer, *71:* 355–359, 1997.

13. Graff, J. R., Herman, J. G., Lapidus, R. G., Chopra, H., Xu, R., Jarrard, D. F., Isaacs, W. B., Pitha, P. M., Davidson, N. E., and Baylin, S. B. E-Cadherin expression is silenced by DNA hypermethylation in human breast and prostate carcinomas. Cancer Res., *55:* 5195–5199, 1995.

14. Baylin, S. B., Herman, J. G., Graff, J. R., Vertino, P. M., and Issa, J. P. Alterations in DNA methylation: a fundamental aspect of neoplasia. Adv. Cancer Res., *72:* 141–196, 1998.

15. Esteller, M., Levine, R., Baylin, S. B., Ellenson, L. H., and Herman, J. G. MLH1 promoter hypermethylation is associated with the microsatellite instability phenotype in sporadic endometrial carcinomas. Oncogene, *17:* 2413–2417, 1998.

16. Dorshkind, K. Regulation of hemopoiesis by bone marrow stromal cells and their products. Annu. Rev. Immunol., *8:* 111–137, 1990.

17. Soligo, D., Schiro, R., Luksch, R., Manara, G., Quirici, N., Parravicini, C., and Lambertenghi, D. G. Expression of integrins in human bone marrow. Br. J. Haematol., *76:* 323–332, 1990.

18. Clark, E. A., and Brugge, J. S. Integrins and signal transduction pathways: the road taken. Science (Washington DC), *268:* 233–239, 1995.

19. Turel, K. R., and Rao, S. G. Expression of the cell adhesion molecule E-cadherin by the human bone marrow stromal cells and its probable role in CD34(+) stem cell adhesion. Cell Biol. Int., *22:* 641–648, 1998.

20. Buhring, H. J., Muller, T., Herbst, R., Cole, S., Rappold, I., Schuller, W., Zhu, X., Fritzsch, U., Faul, C., Armeanu, S., Ullrich, A., Klein, G., and Schmidt, H. The adhesion molecule E-cadherin and a surface antigen recognized by the antibody 9C4 are selectively expressed on erythroid cells of defined maturational stages. Leukemia, *10:* 106–116, 1996.

21. Armeanu, S., Buhring, H. J., Reuss-Borst, M., Muller, C. A., and Klein, G. E-Cadherin is functionally involved in the maturation of the erythroid lineage. J. Cell Biol., *131:* 243–249, 1995.

22. Herman, J. G., Civin, C. I., Issa, J. P., Collector, M. I., Sharkis, S. J., and Baylin, S. B. Distinct patterns of inactivation of p15INK4B and p16INK4A characterize the major types of hematological malignancies. Cancer Res., *57:* 837–841, 1997.

23. Fujii, H., Marsh, C., Cairns, P., Sidransky, D., and Gabrielson, E. Genetic divergence in the clonal evolution of breast cancer. Cancer Res., *56:* 1493–1497, 1996.

24. Graff, J. R., Gabrielson, E., Fujii, H., Baylin, S. B., and Herman, J. G. Methylation patterns of the E-cadherin 5′ CpG island are unstable and reflect the dynamic, heterogeneous loss of E-cadherin expression during metastatic progression. J. Biol. Chem., *275:* 2727–2732, 2000.

25. Herman, J. G., Graff, J. R., Myohanen, S., Nelkin, B. D., and Baylin, S. B. Methylation-specific, P. C. R. a novel PCR assay for methylation status of CpG islands. Proc. Natl. Acad. Sci. USA, *93:* 9821–9826, 1996.

26. Wong, H., Anderson, W. D., Cheng, T., and Riabowol, K. T. Monitoring mRNA expression by polymerase chain reaction: the "primer-dropping" method. Anal. Biochem., *223:* 251–258, 1994.

27. Graff, J. R., Herman, J. G., Myohanen, S., Baylin, S. B., and Vertino, P. M. Mapping patterns of CpG island methylation in normal and neoplastic cells implicates both upstream and downstream regions in *de novo* methylation. J. Biol. Chem., *272:* 22322–22329, 1997.

28. Herman, J. G., Jen, J., Merlo, A., and Baylin, S. B. Hypermethylation-associated inactivation indicates a tumor suppressor role for p15INK4B. Cancer Res., *56:* 722–727, 1996.

29. Corn, P.G., Kuerbitz, S. J., van Noesel, M. M., Esteller, M., Compitello, N., Baylin, S. B., and Herman, J. G. Transcriptional silencing of the p73 gene in acute lymphoblastic leukemia and Burkitt's lymphoma is associated with 5′ CpG island methylation. Cancer Res., *59:* 3352–3356, 1999.

30. Ryan, D. H., Nuccie, B. L., Abboud, C. N., and Winslow, J. M. Vascular cell adhesion molecule-1 and the integrin VLA-4 mediate adhesion of human B cell precursors to cultured bone marrow adherent cells. J. Clin. Investig., *88:* 995–1004, 1991.

31. Kansas, G. S., Muirhead, M. J., and Dailey, M. O. Expression of the CD11/CD18, leukocyte adhesion molecule 1, and CD44 adhesion molecules during normal myeloid and erythroid differentiation in humans. Blood, *76:* 2483–2492, 1990.

32. Geijtenbeek, T. B., van Kooy, Y., van Vliet, S. J., Renes, M. H., Raymakers, R. A., and Figdor, C. G. High frequency of adhesion defects in B-lineage acute lymphoblastic leukemia. Blood, *94:* 754–764, 1999.

33. Melki, J. R., Vincent, P. C., and Clark, S. J. Concurrent DNA hypermethylation of multiple genes in acute myeloid leukemia. Cancer Res., *59:* 3730–3740, 1999.

Clinical Cancer Research

E-Cadherin Expression Is Silenced by 5′ CpG Island Methylation in Acute Leukemia

Paul G. Corn, B. Douglas Smith, Emily S. Ruckdeschel, et al.

Updated version	Access the most recent version of this article at: http://clincancerres.aacrjournals.org/content/6/11/4243

Cited articles	This article cites 32 articles, 15 of which you can access for free at: http://clincancerres.aacrjournals.org/content/6/11/4243.full#ref-list-1
Citing articles	This article has been cited by 23 HighWire-hosted articles. Access the articles at: http://clincancerres.aacrjournals.org/content/6/11/4243.full#related-urls

E-mail alerts	Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions	To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions	To request permission to re-use all or part of this article, use this link http://clincancerres.aacrjournals.org/content/6/11/4243. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.