MeCP2 and promoter methylation cooperatively regulate *E-cadherin* gene expression in colorectal carcinoma

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Reduced expression of E-cadherin (E-cad) owing to aberrant 5′CpG island hypermethylation has been regarded as one of the main molecular events involved in the dysfunction of the cell-cell adhesion system. The molecular mechanisms providing diversity and heterogeneity of E-cad expression in colorectal carcinoma were explored. In 29 cases of colorectal carcinoma in Indonesia, the expression of E-cad was analyzed by immunohistochemical staining, the methylation status of the E-cad promoter was determined by methylation-specific PCR, and the expression of methyl-CpG-binding protein (MeCP) 2 was studied by *in situ* hybridization. E-cad expression was strong, and no methylation was observed in normal colon mucosa and most of the well-differentiated adenocarcinoma. In contrast, both signet-ring cell carcinoma and mucinous adenocarcinoma showed fully methylated patterns and strong MeCP2 expression. In moderately- and poorly-differentiated adenocarcinomas, however, E-cad expression was rather heterogeneous, especially at the front of invasion and in the dissociated areas, where loss of MeCP2 expression correlated with E-cad reexpression even in the presence of E-cad promoter methylation. We conclude that both CpG methylation of the E-cad promoter and significant MeCP2 expression cooperatively and epigenetically regulate E-cad expression in colorectal cancer. (Cancer Sci 2003; 94: 442–447)

Cancer metastasis and invasion are closely associated with cellular properties including cell-to-cell adhesiveness.[1, 2] The *E-cadherin* (*E-cad*) gene encodes a cell-surface adhesion protein that plays a crucial role in homotypic cell-cell adhesion and maintenance of epithelial morphology. It also helps in controlling cell growth and differentiation.[3, 4] The expression of E-cad is lost during the progression of certain cancers,[5] and the loss of E-cad expression and function, besides causing loss of cell-cell adhesion, can also convey signals that actively induce tumor-cell invasion and metastasis[5, 6] and promote epithelial cells to a more malignant phenotype.[7]

The mechanism of gene silencing by methylated cytosine varies among promoters.[8, 9] The most general mechanism is repression of transcription by methyl-CpG-binding proteins (MeCPs), of which MeCP2 is most abundantly distributed as a chromosomal protein and requires a single methylated CpG site for preferential binding to DNA.[10–12] The general physiological role of MeCP2 is as a transcriptional repressor in the control of gene expression in mammalian cells, by non-specifically binding to methyl-CpG.[10] The binding of MeCPs prevents transcriptional factors such as Sp1 from DNA binding and results in alteration of the chromatin structure by histone deacetylase.[9, 13] Besides genetic alterations such as loss of chromosome 16,[14] aberrant hypermethylation of the CpG island in the E-cad promoter has also been postulated to be a major epigenetic mechanism for silencing *E-cad* gene expression.[15–19]

During tumor progression, loss of E-cad expression can be likened to dedifferentiation of human carcinomas *in vitro* and *in vivo*.[20] Generally, E-cad is strongly expressed in well-differentiated cancers which maintain tight cell-cell adhesion and show weak invasive properties, but the expression is lost or markedly reduced in undifferentiated cancers that lack cell-cell adhesion and show invasive tendencies.[21, 22] On the other hand, loss of E-cad expression by promoter methylation is evident even at early stages of cancer progression, and is thought to persist in invasive and metastatic lesions at advanced stages.[23] Throughout all stages of cancer progression, however, the pattern of E-cad expression exhibits a striking heterogeneity: sometimes cancer cells reexpress E-cad strongly at lymph node metastatic sites.[22, 23] To explore the molecular mechanisms providing such diversity and heterogeneity of E-cad expression, we investigated the expression of E-cad and MeCP2 and the methylation patterns of the E-cad promoter in cases of colorectal cancer in Indonesia.

Materials and Methods

Tissue samples. Colon cancer tissues from colon cancer cases in the Dr. Sutomo Hospital, Surabaya (Indonesia), were examined for pathological diagnosis. All tissue samples were fixed with 10% formalin and embedded in paraffin. Informed consent was obtained from all the patients or their families, and this joint Japan-Indonesia study was reviewed and approved by the local ethical committees at Kobe University and Airlangga University.

Immunohistochemistry. Paraffin-embedded tissues were cut and dewaxed through a graded alcohol series. After antigen retrieval by microwave irradiation (citrate, pH 6) for 10 min, endogenous peroxidase activity was blocked with 3% H_2O_2 in methanol for 10 min. Specimens were then incubated with 2% non-fat dry milk in phosphate-buffered saline (PBS) for 10 min and with primary antibodies against human, rat, and mouse E-cad (Transduction Laboratories A BD Co., Lexington, KY) for 15 min. After three 10-min washes with PBS, the specimens were incubated with rabbit anti-mouse IgG antibody preabsorbed with non-immunized serum. Finally, the E-cad protein was immunolocalized by the streptavidin-biotin-peroxidase complex method.

Preparation of digoxigenin-labeled single-stranded DNA MeCP2 probe. The single-stranded antisense DNA probe specific for human MeCP2 was prepared by PCR as described.[24] A 341-bp cDNA fragment from human MeCP2 was obtained by reverse transcriptase (RT)-PCR with rTth reverse transcriptase (ASTEC, PC-700, Fukuoka) using the following pairs of oligonucleotide primers: 5′-GCAGAGACATCAGAAGGGTC-3′ (sense), 5′-TTCTTAGGTGGTTTCTGCTC-3′ (antisense). The DNA sequence of purified PCR products was confirmed by the

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Table 1. Frequency of E-cad promoter methylation by MSP

Diagnosis	Unmethylated	Hemimethylated	Methylated
Normal mucosa	19/19 (100%)	0	0
WDA	13/17 (76.5%)	4/17 (23.5%)	0
MDA	9/12 (75%)	2/12 (16.7%)	1/12 (8.3%)
PDA	6/7 (85.7%)	1/7 (14.3%)	0
MA	1/2 (50%)	0	1/2 (50%)
SRCC	1/4 (25%)	1/4 (25%)	2/4 (50%)

WDA, well-differentiated adenocarcinoma; MDA, moderately-differentiated adenocarcinoma; PDA, poorly-differentiated adenocarcinoma; MA, mucinous adenocarcinoma; SRCC, signet-ring cell carcinoma.

Table 2. Methylation status of E-cad promoter and MeCP2 expression in tissues with no or nominal E-cad expression

Case number	Methylation status of E-cad	MeCP2 expression
6	Unmethylated	++
7	Hemimethylated	±
12	Unmethylated	+
13	Hemimethylated	++
15	Unmethylated	+
23	Methylated	+
27	Methylated	++

MeCP2 mRNA expression in colorectal carcinoma is designated as ± (expression 5–15%), + (expression 15–60%), and ++ (expression >60%).

Table 3. MeCP2 expression in cases with methylated E-cad promoter and positive E-cad immunostaining

Case number	Methylation status of E-cad	MeCP2 expression
14	Hemimethylated	–
17	Methylated	±
19	Hemimethylated	–
23	Methylated	±
24	Hemimethylated	–
26	Hemimethylated	±

MeCP2 mRNA expression in colorectal carcinoma is designated as – (expression <5%), ± (expression 5–15%).

dideoxy termination method (ABI PRISM-TM 310 Genetic Analyzer, Perkin-Elmer Applied Biosystems, Foster City, CA). To prepare the digoxigenin (DIG)-labeled single-stranded antisense DNA probe, the purified PCR product was subjected to unidirectional PCR with the antisense primer alone in the presence of DIG-dUTP (digoxigenin DNA labeling mixture, Boehringer Mannheim, Mannheim, Germany). For negative controls, DIG-labeled sense probe was prepared with sense primer-primed unidirectional PCR.

***In situ* hybridization (ISH).** After dewaxing and dehydration, tissue sections were treated with 2 μg/ml proteinase K (Sigma, St. Louis, MO) for 10 min at room temperature and refixed with 4% PFA for 10 min. Sections were then acetylated with 0.1 *M* triethanolamine (pH 8) for 3 min and with 0.1 *M* triethanolamine containing 0.25% acetic acid for 10 min, and dehydrated through a graded ethanol series. The sections were incubated in a hybridization medium [10 m*M* Tris-HCl (pH 7.3), 1 m*M* EDTA, 600 m*M* NaCl, 0.25% sodium dodecyl sulfate, 1× Denhardt's medium, 50% (v/v) deionized formamide/1 μg per ml probe DNA, 10% dextran sulfate] at 50°C in a moist chamber for 12 h. Negative controls were prepared with either a DIG-labeled sense DNA or by RNase predigestion. After hybridization, the slides were washed with 50% deionized formamide/2× SSC to remove the superfluous probe, and again with 2× SSC and 0.2× SSC. To visualize the hybridized probe, the slides were incubated with alkaline phosphatase-conjugated anti-DIG antibody (Boehringer Mannheim) for 60 min after blocking with 1.5% non-fat dry milk in PBS for 10 min. The specimens were then washed with 100 m*M* Tris-HCl (pH 7.5) containing 150 m*M* NaCl and immersed in 100 m*M* Tris-HCl (pH 9.5) containing 100 m*M* NaCl and 50 m*M* $MgCl_2$. The colorimetric reaction was done with nitro blue tetrazolium salt and 5-bromo-4-chloro-3-indolyl phosphate solution (Boehringer Mannheim) in the dark for 12 h, then stopped with 10 m*M* Tris-HCl (pH 8) containing 1 m*M* EDTA. Slides were finally mounted with Crystalmount (Biomeda, Foster City, CA) and analyzed under a light microscope without counterstaining.

ISH criteria of MeCP2 expression. When >60% of the carcinoma cells were positively stained, the case was regarded as uniformly positive, ++. When 15–60, 5–15 and <5% of carcinoma cells were positively stained, the cases were regarded as partially positive, +, ± and –, respectively. Any carcinoma showing very weak staining which was difficult to distinguish from the background level was regarded as negative even if all of the carcinoma cells were stained. These criteria were used for judgment of the expression of MeCP2 in Tables 2 and 3.

Laser capture microdissection. After deparaffinization, the sections were rapidly stained with hematoxylin for 30 s, washed with distilled H_2O for 30 s, dehydrated with an ethanol gradient, stained with eosin for 30 s, then dehydrated and air-dried. The sections were covered with LCM transfer film (CapSure LCM Transfer Film TF-100, Arcturus Engineering, Inc., Mountain View, CA) and the specific portions of the histologic section were affixed and dissected to the capture film using laser capture microdissection LM200 (Arcturus Engineering, Inc.).

DNA extraction. DNA was extracted from samples attached to the LCM transfer film by incubation with 25 μl of 1 mg/ml proteinase K (Sigma), 1% Tween 20, 1 *M* Tris-HCl pH 8.0 and 0.5 *M* EDTA pH 8.0 at 42°C overnight. The samples were centrifuged for 5 min at 5000 rpm to collect the DNA and then heated at 95°C for 10 min. DNA in a volume of 5 μl was mixed with an equal amount of preheated (80°C) and melted 3.2% low-temperature melting agarose (SeaPlaque, TaKaRa, Kyoto), and agarose beads were made by dropping 10 μl of the mixture into a 1.5 ml microcentrifuge tube containing 250 μl mineral oil at room temperature.[25, 26]

Bisulfite modification. The bisulfite reaction was carried out as described.[26, 27] One milliliter of freshly prepared bisulfite solution (3.5 *M* $NaHSO_3$, 1 m*M* hydroquinone, pH 5.0) was added to the microcentrifuge tubes, and the samples were incubated for 24 h at 50°C under a light-protected condition. The beads, washed twice with 1 ml of 10 m*M* Tris-HCl (pH 8.0) and 10 m*M* EDTA for 15 min each, were desulfonated by washing three times with 1 ml of 0.5 *N* NaOH for 15 min each. After neutralization with 200 μl of 1 *N* HCl for 5 min, the beads were washed twice with 1 ml of 10 m*M* Tris-HCl (pH 8.0) and 10

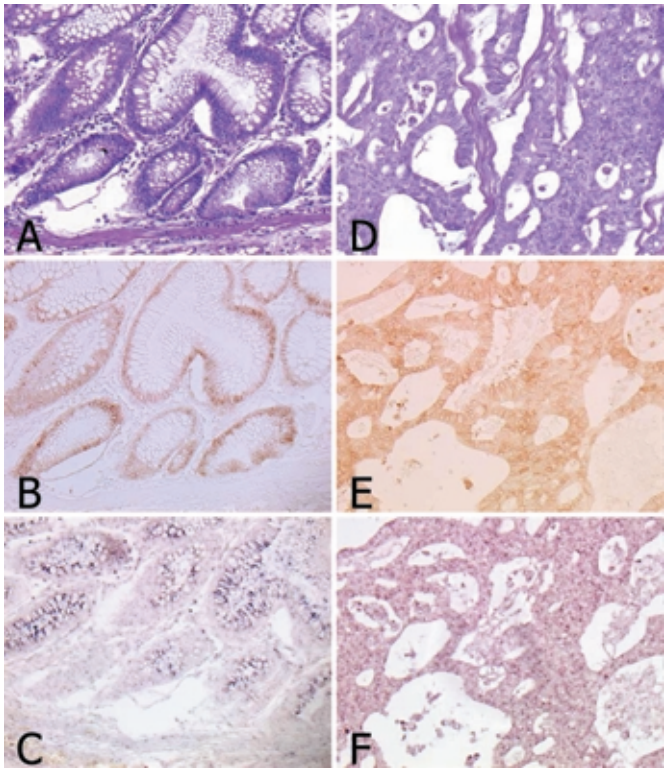

Fig. 1. Different patterns of E-cad and MeCP2 expression in normal and well-differentiated adenocarcinoma (WDA). (A) HE staining of normal colon mucosa. (B) Immunohistochemistry for E-cad. E-cad expression is uniformly observed on the cell membrane, particularly at areas of cell-cell adhesion. (C) *In situ* hybridization (ISH) for MeCP2 mRNA. Positive MeCP2 expression is observed in the cytoplasm throughout the normal epithelial cells. (D) HE staining of WDA. (E) Immunohistochemistry for E-cad. In WDA, strong and homogeneous E-cad expression comparable to that of normal mucosa is observed. (F) ISH for MeCP2 mRNA. Weak but definite MeCP2 expression is observed in the cytoplasm of WDA.

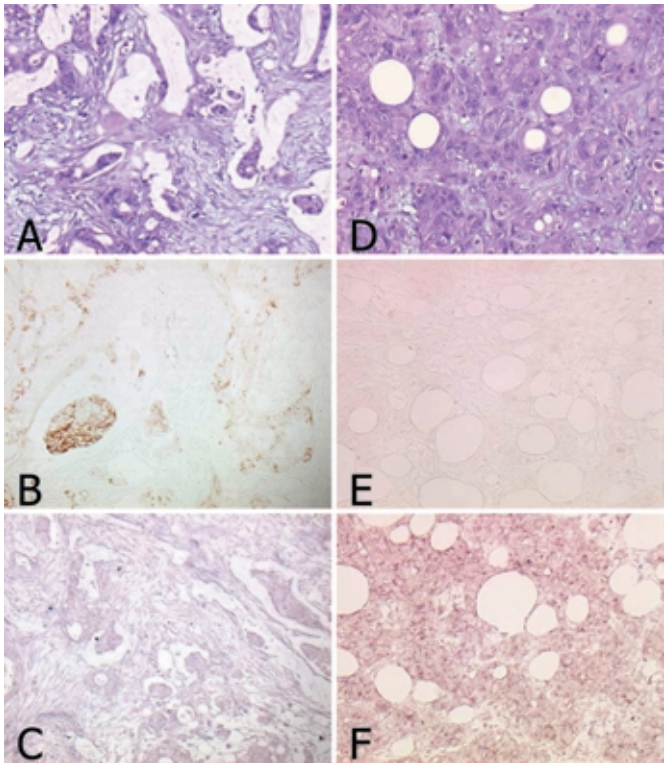

Fig. 2. Different patterns of E-cad and MeCP2 expression in moderately- (MDA) and poorly- (PDA) differentiated adenocarcinoma. (A) HE staining of MDA. (B) Immunohistochemistry for E-cad. Generally, albeit less strong than in normal or in WDA, positive E-cad expression is observed. In the invasive area of MDA, a weak and heterogeneous expression is observed. (C) ISH for MeCP2 mRNA. A moderately-weak MeCP2 expression is observed in the cytoplasm of MDA. (D) HE staining of PDA. (E) Immunohistochemistry for E-cad. Except for heterogeneous and scattered staining patterns with markedly reduced E-cad expression, most of the PDA samples were negative for E-cad expression. (F) ISH for MeCP2 mRNA. In PDA, moderate expression of MeCP2 mRNA is observed, especially where cancer cells scatter and invade the surrounding tissue.

m*M* EDTA, then sliced into several pieces, one of which was directly used as a template for PCR amplification.

Methylation-specific PCR (MSP) for E-cad. The DNA methylation pattern in the CpG island of the *E-cad* gene was determined by sodium bisulfite and subsequent MSP as described.[28] The primer sets used are described as island set 3 and span the transcription start site of E-cad[29]:5′-GGTGAATTTTTAGTTAATTAGCGGTAC-3′ (sense), 5′-CATAACTAACCGAAAACGCCG-3′ (antisense) for methylated DNA sequences of first MSP; 5′-GGTAGGTGAATTTTTAGTTAATTAGTGGTA-3′ (sense), 5′-ACCCATAACTAACCAAAAACACCA-3′ (antisense) for unmethylated DNA sequences of first MSP; 5′-TTTAGTTAATTAGCGGTACGGG-3′ (sense), 5′-ACTAACCGAAAACGCCGAACGA-3′ (antisense) for methylated DNA sequences of nested MSP; 5′-TGGTATGGGGGGTGGTGTTTTG-3′ (sense), 5′-CTAACCAAAAACACCAAACAA-3′ (antisense) for unmethylated DNA sequences of nested MSP. The conditions for the primary and nested MSP were as follows: 95°C for 3 min for denaturation, 38 cycles of 95°C for 1 min, 57°C for 1 min, and 72°C for 1 min, with a final elongation step of 5 min at 72°C. The PCR mixture contained 1× buffer (TaKaRa) with 1.5 m*M* $MgCl_2$, 10 pmol of each primer, 0.2 m*M* dNTPs and 100 ng of bisulfite-modified DNA in a final volume of 25 μl. PCR products were analyzed by 3% agarose gel electrophoresis.

Results

E-cad expression. Twenty-nine paraffin-embedded tissues from colorectal carcinoma examined immunohistochemically demonstrated strong E-cad expression in all non-neoplastic colon epithelia. The staining was localized mostly on the membrane, particularly at the areas of cell-cell contact (Fig. 1B). In contrast to normal epithelial cells, various staining patterns were observed in cancer tissues: all cases of well-differentiated adenocarcinoma (WDA) showed a strong and homogeneous E-cad expression pattern similar to that of the normal mucosa (Fig. 1E). Although the area of positive cells was smaller than that of normal mucosa, 9 of 12 moderately-differentiated adenocarcinomas (MDA), except at the site of invasion and the dissociated area, showed moderate staining (Fig. 2B), and 3 of 12 showed mixed or heterogeneous expression. On the other hand, 3 of 7 poorly-differentiated adenocarcinomas (PDA) exhibited weak E-cad expression in a small fraction of the tumor cells (two with heterogeneous and one with non heterogeneous expression) and 4 of 7 were negative (Fig. 2E). E-cad was not detected in any case of mucinous adenocarcinoma (MA), except for very faint staining in a small fraction of the cell nest (Fig. 3B). All cases of signet-ring cell carcinoma (SRCC) were also negative for E-cad expression (Fig. 3E) as compared with the strong expression in the surrounding normal colon mucosa.

Methylation status of E-cad promoter. The E-cad promoter exam-

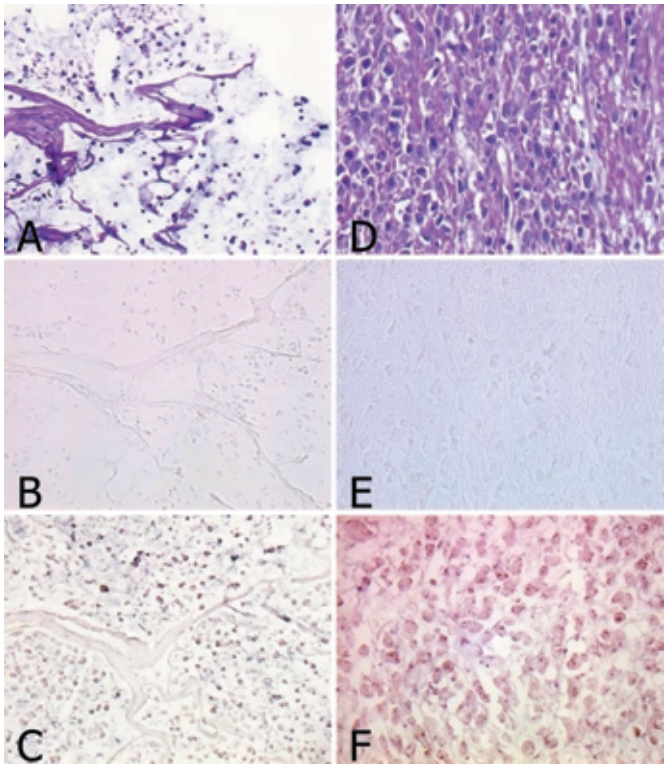

Fig. 3. Different patterns of E-cad and MeCP2 expression in mucinous adenocarcinoma (MA) and signet-ring cell carcinoma (SRCC). (A) HE staining of MA. (B) Immunohistochemistry for E-cad. In MA, E-cad expression is not detected in cancer cells except for a faint staining on the cell clusters in mucinous area. (C) ISH for MeCP2 mRNA. A strong signal due to MeCP2 mRNA expression is observed in cancer cells in MA. (D) HE staining of SRCC. (E) Immunohistochemistry for E-cad. In SRCC, E-cad expression is completely negative. (F) ISH for MeCP2 mRNA. Strong and homogeneous MeCP2 expression is observed in the cytoplasm of SRCC.

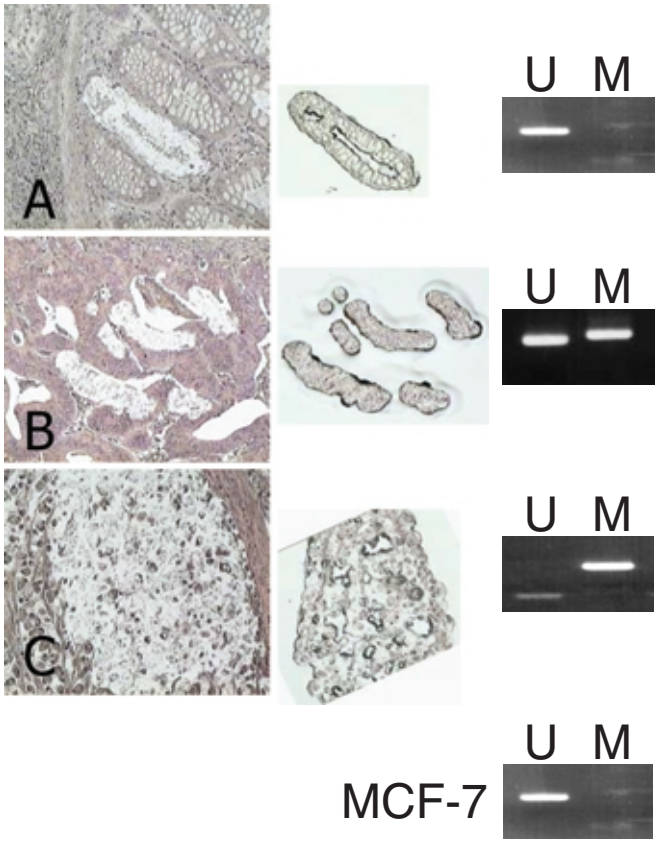

Fig. 4. Detection of methylated cytosine by methylation-specific PCR analysis of the E-cad CpG island region. Isolation of normal cells, moderately-differentiated adenocarcinoma (MDA) cells, and mucinous adenocarcinoma (MA) cells using laser-assisted microdissection. Tissue samples were stained with hematoxylin and eosin. After bisulfite modification, DNA from each microdissected sample was amplified by MSP. MSP products using primers that specifically amplify only unmethylated DNA are indicated by visible PCR products in lines U while visible PCR products in lines M indicate those amplified by primers specific for methylated DNA. (A) In normal colon tissues sections, MSP shows an unmethylated pattern (U). (B) Colon tissue section, an example of hemi-methylated pattern in MDA. MSP shows both unmethylated (U) and methylated (M) patterns. (C) An example of methylated pattern (M) in MA. MCF-7 DNA, in which the third E-cad CpG island is known to be unmethylated, used as control.

ined in microdissected samples (Fig. 4) was unmethylated in the mucosa of all the normal colon samples (n=19). In WDA only 4 hemimethylated cases among 17 informative cases (23.5%) were detected, all from the invasive area. Among 12 MDA cases, methylation was observed in 3 (25%), hemimethylation in 2 (16.7%) and full methylation in 1 (8.3%). Of 7 PDA cases, only 1 (14.3%) demonstrated a hemimethylation pattern, whereas full methylation was observed in 50% of MA cases. The incidence of methylation was markedly high in SRCC cases with 75% showing a methylation pattern: 50% fully methylated and 25% hemimethylated.

MeCP2 expression. MeCP2 mRNA was identified in the tissue section of paraffin-embedded colorectal carcinoma by ISH. In normal colon mucosa (Fig. 1C) the expression was observed clearly. In WDA (Fig. 1F), strong and homogeneous expression was observed. Moderately weak or no expression was observed in both MDA (Fig. 2C) and PDA (Fig. 2F). The staining pattern was rather heterogeneous, especially where E-cad expression was also heterogeneous. On the other hand, strong MeCP2 expression was observed in almost all cases of MA (Fig. 3C) and SRCC (Fig. 3F). The relationship between E-cad expression, E-cad promoter methylation and MeCP2 expression is summarized in Tables 2 and 3.

Discussion

The epigenetic mechanism regulating E-cad expression in 29 cases of colorectal carcinoma was analyzed. Examination of the methylation status of the E-cad promoter by MSP revealed that different histological phenotypes exhibited definite methylation patterns. Because transcriptional repression by methyl-CpG is mediated mainly by MeCPs in a sequence-independent process that involves changes in histone acetylation levels and chromatin structure,[11, 36, 37] we also studied the expression of MeCP2, the most abundant among chromatin MeCPs, by ISH.

E-cad is expressed in most human epithelial tissues[20] and is lost during the development of the breast, colon, prostate, stomach, liver, esophagus, skin, kidney and lung carcinomas.[15, 38] As shown in Table 4, E-cad expression was also observed in all WDA. MDA also showed positive E-cad staining, although the fractions of positive cells were always less than those in normal mucosa and WDA. On the other hand, half of the PDA samples showed weak E-cad expression in a small fraction of tumor cells. E-cad was not detected in most samples of MA or SRCC. Also in MDA and PDA, E-cad expression became weaker or was lost at the periphery, displaying a heterogeneous population at the invasive site. Indeed, highly metastatic ovarian tumor cell lines and high-grade prostate cancer have also been shown to be heterogeneous in E-cad expression.[21, 39] Taken together,

Table 4. Comparison between E-cad promoter CpG island methylation and histologic subtype among Indonesian cases and those of other countries

		Intestinal		Diffuse		
		WDA	MDA	PDA	MA	SRCC
Indonesia[31]	Number of cases	155 of 318 (48.7%)	63 of 318 (19.8%)	57 of 318 (17.9%)	39 of 318 (12.3%)	4 of 318 (1.3%)
	E-cad expression					
	Strong	17 of 17 (100%)	7 of 12 (58.3%)			
	Moderate		5 of 12 (41.7%)			
	Weak or negative			7 of 7 (100%)	2 of 2 (100%)	4 of 4 (100%)
	E-cad methylation	4 of 17 (23.5%)	3 of 12 (25%)	1 of 7 (14.3%)	1 of 2 (50%)	3 of 4 (75%)
Japan[30–32]	Number of cases	235 of 471 (49.9%)	197 of 471 (41.8%)	25 of 471 (5.3%)	10 of 471 (2.1%)	4 of 471 (0.9%)
	E-cad expression					
	Strong	17 of 17 (100%)			28 of 37 (75.7%)	
	Moderate				5 of 37 (13.5%)	
	Weak or negative				4 of 37 (10.8%)	
	E-cad methylation	12 of 35 (34.3%)		10 of 12 (83.3%)		8 of 14 (57.1%)
Europe[33–35]	Number of cases	263 of 372 (70.7%)		50 of 372 (13.4%)	51 of 372 (13.7%)	8 of 372 (2.2%)
	E-cad expression					
	Strong	7 of 8 (87.5%)	4 of 36 (11.1%)			
	Moderate	1 of 8 (12.5%)	24 of 36 (66.7%)	4 of 28 (14.3%)		
	Weak or negative		8 of 36 (22.2%)	24 of 28 (85.7%)		
	E-cad methylation	9 of 13 (69.2%)		4 of 15 (26.7%)		

these data show that mixed or heterogeneously composed tumor cells may have increased invasive potential.[21, 39]

Promoter hypermethylation, together with MeCPs and histone deacetylation, has been identified as a major epigenetic event associated with the loss of tumor suppressor gene expression during cancer progression.[2, 11, 40] Transcriptional regulation of the *E-cad* gene by CpG methylation within its promoter region has been widely investigated.[15–19] Although a significant correlation between CpG methylation around the promoter region and reduced E-cad expression in colorectal cancer was also noted in our study, 3 of 7 E-cad-negative cases showed an unmethylated pattern by MSP. As an alternative mechanism of *E-cad* gene expression, snail is reported as a good candidate that binds the E-boxes of the human E-cad promoter and represses the transcription of E-cad.[3] Also a novel glycoprotein, dysadherin, downregulates E-cad protein expression.[41] We therefore speculate that, albeit methylation being the major cause of the gene suppression, a mechanism other than promoter methylation would be involved in such cases. On the other hand, in six cases, which were E-cad-positive in spite of promoter methylation (Table 3), the ISH study showed that MeCP2 expression was negative or reduced. We therefore speculate that promoter methylation and a significant amount of MeCP2 are both requisite for silencing the gene.

Graff *et al*.[23] have reported that the heterogeneous loss of E-cad expression reflects a heterogeneous pattern of promoter region methylation; that in cultured human tumor cells, such heterogeneous methylation is dynamic, varying from allele to allele and shifting in relation to the tumor microenvironment; and that the mechanism controlling transient loss of E-cad expression during metastatic progression should, therefore, be potentially reversible.[23] In our study, because hemimethylation was observed in a number of tumor cells at the invasion site, passive demethylation by the binding of transcriptional factors to replicating DNA[9] may be involved in this process. At the same time, MeCP2, essential in suppressing the methylated *E-cad* gene, was also heterogeneously expressed, especially at the invasion site. We assume therefore that the heterogeneity of MeCP2 expression is another important epigenetic factor that controls reversible E-cad expression. Since numerous CpG loci cluster around the 5′-flanking region of the *MeCP2* gene,[10] such periodic plasticity of MeCP2 expression during cancer progression may also be regulated by an epigenetic event.

Finally, to assess whether or not geographic or etiologic variations influence E-cad promoter methylation, we compared our current results with those in the literature from Japan[30–32] and Europe.[33–35] The distribution pattern of the histologic subtype of colorectal cancer in Indonesia is much more like that in Europe (Table 4). The difference between Japan and Indonesia is probably based on diagnostic procedures and the background population that undergoes endoscopic examinations. Moreover, methylation itself is related to the histologic subtype, irrespective of geographic variations. Although the number of subjects studied is limited, we speculate that E-cad promoter methylation is seen mainly as a subset of the CpG island hypermethylation phenotype of colorectal carcinomas, rather than as an

etiologic factor.

In conclusion, we examined E-cad expression in colorectal cancers and found that both CpG methylation of E-cad promoter and MeCP2 expression cooperatively and epigenetically regulated E-cad expression in colorectal cancers.

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