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Genetic Alterations and Expression Pattern of CEACAM1 in Colorectal Adenomas and Cancers

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Abstract Carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) is expressed on epithelial cells throughout the intestinal tract and is a negative regulator of tumor cell growth, suggesting that it may function as a tumor suppressor. In this study, to determine whether the CEACAM1 is involved in colorectal tumorigenesis, we have investigated the genetic alterations, including mutations and allelic loss, of the CEACAM1 gene in 17 colonic adenomas and 123 sporadic colorectal cancers. In addition, the expression pattern of the CEACAM1 protein was examined in 60 colonic adenomas and 123 sporadic colorectal adenocarcinomas. No mutation was found in colonic adenomas, but four somatic missense mutations, L36F, T312I, V398I and A445V, were detected in colorectal cancers. Interestingly, all of the mutations were found in left-side colon cancers of the patients with clinical stage III. In LOH analysis, nine adenomas were informative for at least one of the markers and five (55.6%) showed allelic loss. Thirty-eight cancers were informative at D19S211 and D19S872 markers and 21 (56.3%) showed LOH at these markers. Statistically, the frequency of allelic loss at the CEACAM1 locus was not associated with clinicopathologic parameters ($P>0.05$). In immunohistochemical analysis, loss of expression of CEACAM1 protein was detected in nine (15.0%) and 30 (24.4%) of 60 colorectal adenomas and 123 colorectal cancers. Statistically, there was no significant relationship between loss of CEACAM1 expression and clinicopathologic parameters, including clinical stage, tumor location, tumor size, lymph node metastasis and 5-year survival ($P>0.05$). These data suggest that genetic alteration and loss of expression of the CEACAM1 may contribute to the development of colorectal cancers, as an early event.

Keywords CEACAM1 · Colorectal cancer · Somatic mutation · Expression · Immunohistochemistry

Introduction

Colorectal cancer is the second leading cause of cancer-related death in the western world [1]. In Korea, it accounts for an estimated 11.2% of all malignancies, with 11.6% in the male population and 10.7% in the female population [2]. Thus, colorectal cancer remains a significant contributor to the world's health burden. At a molecular level, much progress has been made in the last two decades in the identification and characterization of the genetic changes involved in the malignant colorectal transformation process. Thus, the concept of multistage carcinogenesis is now widely accepted as being a consequence of multiple genetic alterations accumulated in cancer cells [3]. However, the molecular mechanisms underlying dysregulated cell growth in the colorectal cancer remain the subject of intensive investigation.

Carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1), formerly known as biliary glycoprotein is a member of the CEA subfamily on chromosome 19q13 and has transmembrane and cytoplasmic domains [4–7]. CEACAM1 is expressed on epithelial cells throughout the gastrointestinal tract and in other lumen-forming organs such as the liver, breast, pancreas, prostate and

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kidney [8]. Recent studies showed that CEACAM1 is a negative regulator of tumor cell growth, suggesting that it may function as a tumor suppressor [9–11]. It has also been reported that the expression of CEACAM1 is downregulated in more than 85% of early colorectal adenomas and carcinomas [12, 13]. Furthermore, Nittka et al. has reported that aberrant crypt foci (ACF) and hyperplastic polyp lesions, representing the earliest stage of colorectal cancer, have reduced levels of expression of CEACAM1. Expression levels of adenomatous polyposis coli (APC) protein were normal in these lesions, as was the expression pattern for β-catenin [14], indicating that silencing of CEACAM1 expression precedes changes in the gatekeeper gene APC. In functional analysis, CEACAM1 is implicated in generating apoptotic signals in epithelial cells [8, 12] and associated with early stage of angiogenesis and invasion [15–17]. In murine model, when mice are treated with azoxymethane to induce colonic tumors, CEACAM1 knockout mice develop a significantly greater number of tumors than their controls [18].

Here, in order to investigate whether the CEACAM1 contribute to the development or progression of colorectal cancer, we evaluated the genetic alterations, including mutations and allelic loss of the CEACAM1 gene in 17 colonic adenomas and 123 sporadic colorectal cancers by polymerase chain reaction (PCR)-single strand conformation polymorphism (SSCP) and loss of heterozygosity (LOH). In addition, the expression pattern of CEACAM1 was examined in 60 colorectal adenomas and 123 sporadic colorectal cancers by immunohistochemistry.

Materials and Methods

Tissue Samples

A total of 60 formalin-fixed and paraffin-embedded colorectal adenomas and 123 colorectal cancers between 2001 and 2002 were enrolled in this study. Colorectal adenomas with low- and high-grade dysplasia were 19 and 41 cases, respectively. No patient had a family history of colorectal cancer. Cancer size ranged from 13 to 80 mm in longest diameter, with a median of 42.3 mm. Of them, 66 cancers were ≥5 cm in tumor size. Twenty-five cases were located in right-side colon and 98 in left-side. Histologically, cancers consisted of 6 well-, 112 moderate- and 5 poorly-differentiated adenocarcinomas. The tumors were staged according to Dukes' criteria [19]. There were 12, 47, 56, and 8 cases with stage A, B, C, and D, respectively. For cancer samples, two pathologists screened histological sections and selected areas of the representative tumor cells. Three tissue cores from each cancer sample (0.6 mm in diameter) were taken and placed in a new recipient paraffin block using a commercially available microarray instrument (Beecher Instruments, Micro-Array Technologies, Silver Spring, MD, USA), according to established methods [20]. One cylinder of normal colonic mucosa adjacent to each tumor was also transferred to the recipient block.

Microdissection and DNA Extraction

Tumor cells were selectively procured from hematoxylin and eosin-stained slides using a laser microdissection device (ION LMD; JungWoo International, Seoul, Korea). We also obtained inflammatory or surrounding normal mucosal cells for corresponding normal DNA samples from the same slides in all cases. DNA extraction was performed by a modified single step DNA extraction method, as described [21]. For mutation and allele loss analysis of the CEACAM1 gene, 17 colorectal adenomas and 123 tumor matched normal DNA sample pairs were used.

SSCP Analysis

Genomic DNAs from tumor cells and corresponding normal cells were amplified with 13 set of primers covering the entire coding region (exon 1-9) of the CEACAM1 gene (Table 1). Each PCR was performed under standard conditions in a 10 μl reaction mixture containing 1 μl of template DNA, 0.5 μM of each primer, 0.2 μM of each deoxynucleotide triphosphate, 1.5 mM $MgCl_2$, 0.4 unit of Ampli Taq gold polymerase (Perkin-Elmer, Foster City, CA, USA), 0.5 μCi of [^{32}P]dCTP (Amersham, Buckinghamshire, UK), and 1 μl of 10X buffer. The reaction mixture was denatured for 12 min at 94°C and incubated for 35 cycles (denaturing for 40 s at 94°C, annealing for 40 s at 56°C, and extending for 40 s at 72°C). Final extension was continued for 5 min at 72°C. After amplification, PCR products were denatured for 5 min at 95°C at a 1:1 dilution of sample buffer containing 98% formamide/5 mmol/L NaOH and were loaded onto a SSCP gel (FMC Mutation Detection Enhancement system; Intermountain Scientific, Kaysville, UT, USA) with 10% glycerol. After electrophoresis, the gels were transferred to 3 MM Whatman paper and dried, and autoradiography was performed with Kodak X-OMAT film (Eastman Kodak, Rochester, NY, USA). We repeated the experiment three times, including tissue microdissection, PCR, SSCP, and sequencing and found that the data were consistent.

Loss of Heterozygosity Analysis

Tumor and corresponding normal colonic mucosa DNAs were amplified by thermal cycler (MJ Research Institute, Watertown, MA, USA) with D19S211 and D19S872

Table 1 Primer sequence for amplifying the coding region of the CEACAM1 gene

Name of primer	Nucleotide sequence	Product size (bp)
E1-F	5′-CCCAAGCTCTCCTCCACAGGTGAA-3′	179
E1-R	5′-TCCCCAGAGGACCCTAGCCATTCT-3′	
E2A-F	5′-GGACCCAAGGCCCCATTTTTCTA-3′	242
E2A-R	5′-TGACGGTTGCCATCCACTCTTTC-3′	
E2B-F	5′-TACAGCTGGTACAAAGGGGAAAG-3′	251
E2B-R	5′-GTCATGGGGAAATACTCACGGTA-3′	
E3A-F	5′-TGGAGGAATCAAAGGTGCCACAC-3′	210
E3A-R	5′-GACAGCTGCAGCCTGGGACTGAC-3′	
E3B-F	5′-TGTGGTGGATAAACAATCAGAGC-3′	202
E3B-R	5′-GGGCCACAGAGGAACAGAAGATA-3′	
E4A-F	5′-AGCTTCCCCTTCCCTCTGATGACA-3′	199
E4A-R	5′-TCTTGTGTGCTTTGCTGGAATGTT-3′	
E4B-F	5′-CTGCACAGTACTCCTGGCTTATCA-3′	236
E4B-R	5′-CTTCTCTGCTCCTATTTGAAAACC-3′	
E5A-F	5′-GCCCCAGGCCCCAACCCTATTCTC-3′	238
E5A-R	5′-TGGTGTTGCCCTGGGACAGCTTCA-3′	
E5B-F	5′-CAAAAACCAGAGTCTCCCGTCCTC-3′	232
E5B-R	5′-TCCATTTTGCACACCATTGACAGA-3′	
E6-F	5′-TCCCAGCCCCCATTGTAAAATAAT-3′	236
E6-R	5′-GTCAGCCTTGGAGGAAACAGAACA-3′	
E7-F	5′-TTGGGAATAGCAGTCACAGTTTC-3′	185
E7-R	5′-AGGGAGGGAGTGGTTTGGGAATC-3′	
E8-F	5′-TCCAACTGCCACCTCCTTCAGCTA-3′	153
E8-R	5′-TGAGAAAATAAGCCCATGAAAACC-3′	
E9-F	5′-AACTGATCATTATTTGTGCTTTTC-3′	206
E9-R	5′-TAGTGATGAGGGTGAGAGACTTGA-3′	

markers. Each PCR reaction was performed under standard conditions in a 10 μl reaction mixture containing 20 ng of template DNA, 0.5 μM of each primer, 0.2 μM of each deoxynucleotide triphosphate, 1.5 mM $MgCl_2$, 0.4 unit of Taq polymerase, 0.5 μCi of [^{32}P]dCTP (Amersham), and 1 μl of 10X buffer. PCR products were then denatured and electrophoresed in 6% polyacrylamide gel containing 7 M urea. After electrophoresis, the gel was transferred to 3 MM Whatman paper, dried, and subjected to autoradiography using Kodak-OMAT film (Eastman Kodak). Complete absence of one allele in the tumor DNA of informative cases, which was determined visually, was considered as LOH.

Immunohistochemistry for CEACAM1 Protein

For immunohistochemical analysis, 2 μm sections were cut the day before use and stained as previously described [22]. To maximize the signal on immunohistochemistry, two strategies were used in the present study, i.e. antigen retrieval in citrate buffer, and signal amplification with biotinylated tyramide. For the former, heat-induced epitope retrieval was conducted by immersing the slides in Coplin jars filled with 10 mmol/L citrate buffer (pH 6.0) and boiling the buffer for 30 min in a pressure cooker (Nordic Ware, Minneapolis, MN, USA) inside a microwave oven at 700 W; the jars were then cooled for 20 min. For the latter, the Renaissance TSA indirect kit (NEN Life Science, Boston, MA, USA), which included streptavidin-peroxidase and biotinylated tyramide, was used. After rinsing with PBS, the slides were treated with 1% H_2O_2 in PBS for 15 min at room temperature to abolish endogenous peroxidase activity. After washing with TNT buffer (0.1 mol/L Tris-HCl, pH 7.4, 0.15 mol/L NaCl and 0.05% Tween 20) for 20 min, the slides were treated with TNB buffer (0.1 mol/L Tris-HCl, pH 7.4, 0.15 mol/L NaCl and 0.5% blocking reagent). Sections were incubated overnight at 4°C with anti-CEACAM1 antibody (1:100 dilution; R&D Systems, Minneapolis, MN, USA). Detection was carried out using biotinylated goat anti-mouse antibody (Sigma, St. Louis, MO, USA), followed by incubation with peroxidase-linked avidin-biotin complex. Diaminobenzidine was used as chromogen, and the slide was counterstained with Mayer's hematoxylin. The results were reviewed independently by two pathologists. As negative controls, the slide was treated by replacement of primary antibody with non-immune serum.

Statistical Analysis

The correlation between CEACAM1 expression and various clinicopathologic parameters was assessed using the Chi-square and Bartholomew tests. Survival curves were constructed using the Kaplan–Meier method and compared using the log rank test. A P value less than 0.05 was considered the limit of statistical significance.

Results

Mutational Analysis of CEACAM1 Gene

Enrichment and sequencing analysis of aberrantly migrating bands on SSCP gel led to the identification of CEACAM1 mutations in four (3.3%) of the cancer samples (Fig. 1a–d) and no mutation in colonic adenomas. As shown in Fig. 1 and Table 2, three of the mutations were missense mutations caused by single-nucleotide substitutions within immunoglobulin domain of the CEACAM1 and one in the transmembrane domain, a C to T transition at codon 36 (CTC→TTC), 312 (ACA→ATA) and 445

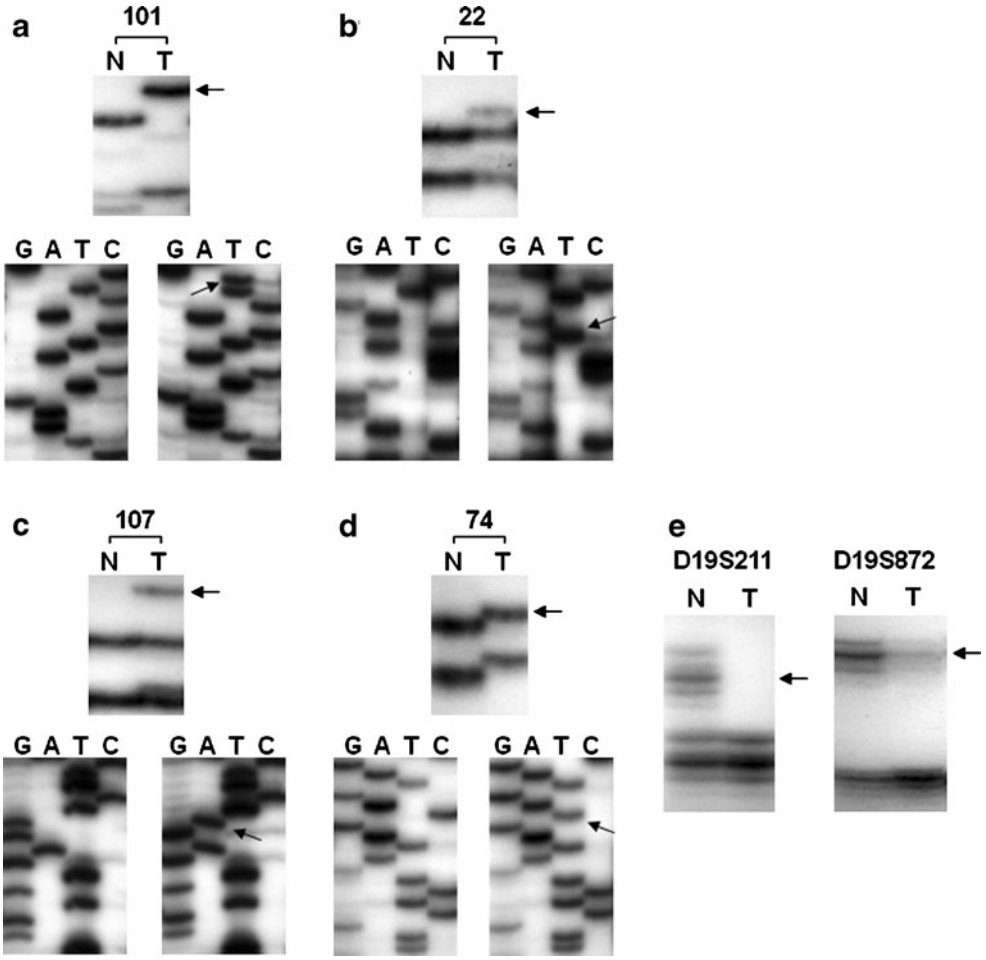

Fig. 1 Representative results showing SSCP, sequencing and LOH analyses of the CEACAM1 gene in colorectal cancer. SSCP of case No. 74 and 101 showed only two aberrant bands without any wild-type bands as compared to SSCP from corresponding normal tissue and case No. 22 and 107 demonstrated one aberrant bands with two wild-type bands. All of the mutations were missense mutation: a C to T at codon 36 (**a**), a C to T at codon 312 (**b**), a G to A at codon 398 (**c**), a C to T at codon 445 (**d**) in exon 2, 4, 5 and 6 of the gene, respectively. Representative results of loss of heterozygosity at microsatellite markers, D19S221 and D19S872 (**e**). (*N* normal; *T* tumor). *Arrows* indicate allele exhibiting LOH

(GCA→GTA), and a G to A transition at codon 398 (GTC→ATC). The mutations were present in tumor tissue, but absent in corresponding normal DNAs extracted from surrounding non-cancer cells, suggesting that the mutations had arisen somatically. Clinically, all of the mutations were found in left-side colon cancers of the patients with clinical stage III. Three mutations were found in the cases with surrounding lymph node metastasis and three patients died of colorectal cancer within 3 years after operation (Table 2). Interestingly, two cases with mutation (case No. 74 & 101) showed only aberrant migrating mutant bands with absence of wild-type bands, indicating mutation of one allele and loss of the remaining allele (Fig. 1a–d).

LOH Analysis

Nine (52.9%) of 17 colonic adenoma cases without microsatellite instability (MSI) were heterozygous for the D19S221 marker and four (44.4%) of them showed allelic loss. For D19S872, four (16.7%) of 16 colonic adenomas cases without MSI were informative and one (25%) of them

Table 2 Summary of mutations of the CEACAM1 gene in colorectal cancers

Case no.	Stage	Site	Size (cm)	LN metastasis	Allele loss	CEACAM1 expression	Mutation		Survival (months)
							Nucleotide	Amino acid	
22	III	Left	5	+	−	+	ACA→ATA	T312I	55
74	III	Left	4	+	+	−	GCA→GTA	A445V	21
101	III	Left	2.5	+	+	+	CTC→TTC	L36F	36
107	III	Left	5	−	−	+	GTC→ATC	V398I	29

LN lymph node

demonstrated allelic deletion. Of 17 colonic adenomas, nine cases were informative for at least one of the markers studied and five (55.6%) showed allelic loss at these markers. In cancer samples, 33 (39.6%) of 83 cases without MSI were heterozygous for D19S221 marker and 14 (43.8%) of them showed allelic loss. For D19S872, 16 (16.7%) of 96 colorectal cancer cases without MSI were informative and nine (56.3%) of them demonstrated allelic deletion (Fig. 1e). Of 123 colorectal cancers, 38 cases were informative for at least one of the markers studied and 21 (55.3%) showed allelic loss at these markers. Interestingly, LOH was found in five (71.4%) of seven right-side and 16 (51.6%) of 31 left-side informative colorectal cancers, respectively. The frequencies of allelic loss were 0%, 63.3%, and 0% in well-, moderately- and poorly-differentiated cancer, respectively. Statistically, there was no significant difference in LOH frequency according to tumor location (P=0.2707), tumor size (P=0.5349), histologic grade (P=0.2699), lymph node metastasis (P=1.000) and clinical stage (Bartholomew test, P>0.05). The autoradiograms showing LOH are displayed in Fig. 1e. Interestingly, two of the cases with CEACAM1 mutation showed allelic loss at these markers, consistent with the SSCP data and the one case did MSI.

Immunohistochemistry of CEACAM1 Protein

In immunohistochemistry, luminal surface of glandular epithelial cells of colonic mucosa demonstrated moderate positive staining [23]. Expression of CEACAM1 in tumor cells compared to their corresponding normal colonic mucosa. Loss of expression of CEACAM1 protein was clearly marked on the surface of colorectal tumor cells (Fig. 2). The tumor tissues, even the different portions in the same tumor, showed similar expression levels, indicating no evidence of intratumoral heterogeneity. Interestingly, loss of expression of CEACAM1 protein was detected in nine (15.0%) and 30 (24.4%) of 60 colorectal adenomas and 123 colorectal adenocarcinomas, respectively. For colorectal adenomas, CEACAM1 expression was observed in 18 (94.7%) and 33 (80.5%) of 19 low- and 41 high-grade adenomas, respectively. There was no statistically significant association between CEACAM1 expression and histological grade of the tumor cells (P=0.1504). Based on the differentiation grade of cancer cells, loss of expression was detected in 0% (0/6), 25% (28/112), and 40% (2/5) of well, moderately, and poorly differentiated colorectal cancers. In this classification, expression of the CEACAM1 protein was not associated with the differenti-

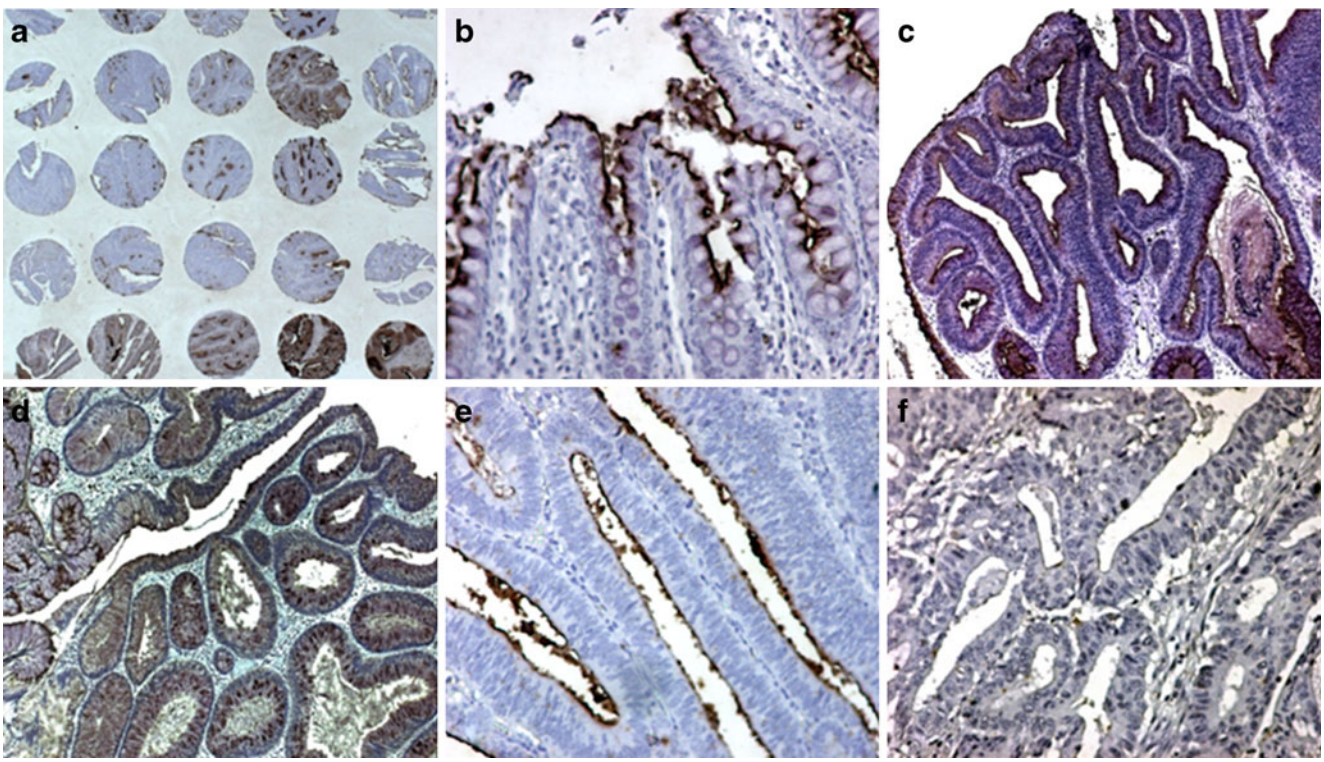

Fig. 2 Altered expression of CEACAM1 protein by immunohistochemistry. Low power view of tissue cores stained against CEACAM1 (**a**). Expression of the CEACAM1 protein was observed in the luminal surface of normal colonic mucosa (**b**). Colon adenoma and cancer demonstrating moderate immunostaining on the surface of tumor cells (**c** & **e**). Colon adenoma and cancer cells showing negative staining for CEACAM1 protein (**d** & **f**). Original magnification: **a**, ×25; **b**, **e**, **f**, ×200; **c**, **d**, ×100

ation of tumor cells (P=0.2699). And CEACAM1 expression loss was seen in two (16.7%) of 12 cases corresponding to stage A, nine (19.1%) of 47 to stage B, 18 (32.1%) of 56 to stage C, and one (12.5%) of eight to stage D, respectively. In addition, CEACAM1 was not expressed in 18 (30.5%) of 59 cases with lymph node metastasis. Statistically, there was no significant relationship between loss of CEACAM1 expression and clinicopathologic parameters, including clinical stage (Bartholomew test, P>0.05), tumor location, tumor size and lymph node metastasis (P>0.05) (Table 3). In addition, no significant association between CEACAM1 expression and 5-year survival was found (P=0.6841).

Discussion

CEACAM1 is an important member of the CEA family and plays a role in the development of many types of cancers [12–14, 17]. Expression of the CEACAM1 protein in the CT51 colonic carcinoma cell line reduced the number of colonies in a clonogenic assay and the number and size of tumors formed in syngeneic BALB/c mice [24]. In addition, Ceacam1$^{-/-}$ mice develop a significantly greater number and size of tumors than their littermate controls [18, 25]. CEACAM1 expression is dramatically down-regulated in colorectal adenomas and cancers [14], although its molecular mechanism is unclear. Furthermore, epithelial down-regulation of CEACAM1 induces angiogenesis via increased expression of vascular endothelial growth factor [15] and CEACAM1 acts as a specific trigger for apoptosis and actively participates in Wnt signaling pathway in intestinal cells [14, 25]. Recently, Bastide et al. has reported that the HMG box transcription factor SOX9 inactivation altered the colon epithelium with the appearance of hyperplasia and crypt dysplasia [26]. Interestingly, CEACAM1 was identified as the direct transcriptional target of SOX9 in the colonic epithelium [23]. Thus, CEACAM1 has been considered as a tumor suppressor gene that contributes to the development of colorectal cancer, as an early event.

Finally, we found CEACAM1 mutations in four (3.3%) cancer samples (Fig. 1a–d), but not in adenoma cases. All the mutations were somatic missense mutations in the colorectal cancer with clinical stage III. Clinicopathologically, the mutations were found in left-side colon cancers with moderately-differentiated cancers and three of them were detected in the cases with lymph node metastasis (Table 2). Interestingly, two cases with mutation showed only aberrant migrating mutant bands with the absence of wild-type bands, which indicated inactivation of both alleles. It has been reported that the CEACAM1 with a long cytoplasmic domain inhibited the development of tumors whereas a splice variant lacking the cytoplasmic domain did not [24, 27]. In addition, CEACAM1 functions as an intercellular adhesion molecule through its first immunoglobulin domain [7, 28] and the transmembrane domain plays a crucial role in CEACAM1 targeting to cell–cell contact [29].

In the present study, the frequency of allelic loss at the CEACAM1 locus in colonic adenoma cases (55.6%) was similar to that seen in colorectal cancers (55.3%), which suggested that CEACAM1 loss may be an early event in the pathogenesis of colorectal cancer. Comparing the frequency of allelic loss at the CEACAM1 locus with the patient characteristics, no statistical association was found between the frequency of CEACAM1 allelic loss and clinical parameters, including tumor location, tumor size, lymph node metastasis and clinical stage. Unexpectedly, loss of CEACAM1 expression was detected in only one case with somatic mutation and allelic loss (Table 2). Therefore, molecular mechanisms other than a structural alteration might be responsible for the CEACAM1 gene silencing. Recently, Phan et al. have reported that there is no evidence for promoter methylation of the CEACAM1 gene in prostate cancer and that the gene may be silenced by high expression levels of the transcription factor Sp2 in prostate cancer and recruitment of histone deacetylase

Table 3 Correlation of CEACAM1 protein expression with clinicopathologic parameters

Parameters	CEACAM1 expression		P value
	+	–	
Differentiation			0.2699[a]
Well	6	0	
Moderate	84	28	
Poorly	3	2	
LN metastasis			0.1292[a]
+	41	18	
–	52	12	
Stage			0.1504[b]
A	10	2	
B	38	9	
C	38	18	
D	7	1	
Side			0.6375[a]
Right	18	7	
Left	75	23	
Size			0.7043[a]
< 5 cm	44	13	
≥ 5 cm	49	17	

[a] Chi-square test

[b] Bartholomew test

(HDAC) to the promoter [30]. Thus, more studies on the molecular mechanism of CEACAM1 silencing in colon cancer will be needed to verify this initial observation.

Recently, Nittka et al. have reported that the loss of CEACAM1 expression in ACF and hyperplastic polyp was similar to that seen in adjacent tumor [14]. Since reduced expression of CEACAM1 has been correlated with the progression from normal to adenoma to overt adenocarcinoma of colon, we were especially interested in CEACAM1 expression pattern in colonic adenomas and cancers. In immunohistochemistry, normal corresponding colonic epithelial cells has strong membrane staining of CEACAM1 in luminal surfaces of colonic mucosa, while colonic adenoma and cancer cells demonstrated little staining or immunonegativity for CEACAM1 (Fig. 2). Finally, we found CEACAM1 expression loss in nine (15.0%) colorectal adenoma and 30 (24.4%) cancer samples. Statistically, there was no significant relationship between loss of CEACAM1 expression and clinicopathologic parameters, including clinical stage (Bartholomew test, $P>0.05$), tumor location, tumor size and lymph node metastasis ($P>0.05$) (Table 1). In addition, no significant association between CEACAM1 expression and 5-year survival was found ($P=0.6841$). Loss of CEACAM1 led to reduced levels of apoptosis, thus leading to a net increase in proliferation in these cells and acquiring an oncogenic mutation [14]. Thus, our findings suggest that the loss of expression of CEACAM1 may contribute to the colorectal carcinogenesis and further support that CEACAM1 gene may be one of the candidate tumor suppressor gene in colon.

Although the Ceacam1$^{-/-}$ colon showed increased proliferation and decreased apoptosis with a higher tumor formation [18, 25] and we found loss of expression of CEACAM1 protein in colorectal cancer, there are still controversial in the role of CEACAM1 as a tumor suppressor. In addition to colon cancer, CEACAM1 expression is downregulated in hepatocellular carcinoma, prostatic and bladder cancer, and breast cancer [15, 31–33]. However, CEACAM1 activity was associated with metastatic spread and CEACAM1 promoted cancer cell migration and invasion in thyroid cancer and melanoma cells [34, 35]. In the lung cancer, high CEACAM1 expression was associated with an increased angiogenic activity in non-small-cell lung cancer [36]. Furthermore, although no CEACAM1 expression was found in normal gastric mucosa, all of the gastric carcinomas expressed CEACAM1, membranous staining in the intestinal-type carcinoma and cytoplasmic staining in the diffuse-type carcinoma. High microvessel density was observed more frequently in the gastric cancers with membranous expression [37]. Therefore, different CEACAM1 expression pattern has been observed in the different tumors, suggesting that CEACAM1 does not generally act as a tumor suppressor. Further analysis is strongly necessary to identify the role of CEACAM1 in specific cell type and the molecular mechanism of CEACAM1 silencing in the colon cancer.

In conclusion, We found four somatic mutations and more than 50% allelic loss in colorectal adenomas and carcinomas as well as CEACAM1 expression loss in nine (15.0%) and 30 (24.4%) colorectal adenoma and cancer samples, respectively, and altered expression of CEACAM1 was not associated with clinical and pathologic parameters. Thus, we conclude that loss of expression of the CEACAM1 gene may play a role in the development of colorectal cancers, as an early event.

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