Reduced E-Cadherin Expression as a Cause of Distinctive Signet-Ring Cell Variant in Colorectal Carcinoma

Colorectal signet-ring cell carcinoma (SRCC) is a rare type of adenocarcinoma and presents with distinctive clinicopathological features. This study was performed to assess the biological characteristics of colorectal SRCC regarding the E-cadherin expression. Seventeen patients with primary colorectal SRCC were identified and their clinicopathological characteristics were analyzed. The mean age of the 17 patients was 45.3 yr (14-68). Immunohistochemical staining of E-cadherin and β-catenin were performed in ten colorectal SRCCs and in 30 ordinary colorectal adenocarcinomas as control. Primary colorectal SRCC occurred in 0.7% of 2,388 colorectal adenocarcinomas. Most patients had advanced stage tumor at surgery (stage III and IV, AJCC: 82%). Five-year survival rate was 16%. Peritoneal seeding was the most common recurrence pattern (41%) and liver metastasis was not identified. All SRCCs showed a markedly reduced or absent expression of E-cadherin on immunohistochemical staining, whereas seven (23.3%) of ordinary carcinomas showed reduced expression, thereby indicating a significant difference between the two groups ($p<0.005$). In immunohistochemical staining for β-catenin, eight of ten SRCCs showed reduced membrane expression that did not attain statistical significance compared to ordinary adenocarcinomas. It is suggested that aberrant E-cadherin expression may explain the distinct clinicopathological features in primary colorectal SRCC.

Key Words : *Colorectal Neoplasms; Carcinoma, Signet-ring cell; Cadherins; β-catenin*

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INTRODUCTION

Colorectal signet-ring cell carcinoma (SRCC) is a rare histological type of tumor reported to be 0.7-2.4% of all primary colorectal carcinomas (1-3). It is a mucin-secreting adenocarcinoma with abundant intracytoplasmic mucin and deviated nuclei. Primary colorectal SRCC consists of signet-ring cells comprising more than half of the entire number of tumor cells. It is also necessary that all other primary cancers, especially gastric cancer, must be excluded (1, 2). Colorectal SRCC is assumed to present distinctive features in contrast to ordinary colorectal adenocarcinoma, i.e. aggressive infiltration to surrounding tissues, frequent peritoneal seeding, and poor prognosis (1-3). In one investigation, the two categories of colorectal adenocarcinoma including SRCC were found to differ in such molecular events as microsatellite instability and *K-ras* mutation, without any clear explanation of their divergent biological behavior (4).

E-cadherin, a cadherin family member of the cell surface glycoprotein, is a Ca^{2+}- dependent cell-to-cell adhesion molecule found mainly in epithelial tissue. It is thought to implicate embryogenesis, cellular migration in inflammatory tissue, and cellular differentiation or dedifferentiation (5). The extracellular domain interacts homotypically with the

E-cadherin molecules of neighboring cells while maintaining intercellular adhesion. Its cytoplasmic tail constitutes complex with a group of intracytoplasmic proteins, such as catenins (6, 7). E-cadherin and catenin unit binds to the cytoskeleton that is essential for the adhesive function of E-cadherin. Furthermore, E-cadherin and catenins appear to act as more of an intercellular glue. β-catenin seems to participate in the signal transduction from the cellular surface to the cytoplasm independently from the cell-to-cell adhesion. Catenins also correlate with molecules of growth regulatory and signaling functions, i.e. adenomatous polyposis coli (APC) protein, axin, and TCF/Lef transcriptional factors (8). APC protein plays a key role in approximately 80% of colorectal carcinogenesis, competing directly with E-cadherin in binding to β-catenin; it also appears to be an indirect regulator of E-cadherin-mediated adhesion (9).

Many investigators have suggested the suppressor role of E-cadherin in tumor invasion (10). Loss or diminished expression by mutation or epigenetic change has been demonstrated in many epithelial cancers (11-13). The down-regulation of E-cadherin is seen most prominently in carcinomas showing infiltrative growth associated with little intercellular cohesion, such as invasive lobular carcinoma of the breast and diffuse gastric adenocarcinoma including gastric SRCC

(14, 15). Therefore, the loss of the E-cadherin function could be associated with invasiveness, lymph node metastasis, and distant metastasis resulting in poor prognosis (10, 16).

Few investigations have explained the mechanism of the distinctive phenotype and aggressive clinical behavior in colorectal SRCC. This study assessed the correlation between the clinicopathological features of colorectal SRCC and the expression of E-cadherin in order to identify the biological characteristics of E-cadherin in colorectal SRCC.

PATIENTS AND METHODS

Patients

Seventeen patients (0.7%) with primary colorectal SRCC were included among the 2,388 patients who underwent primary colorectal carcinoma surgery between 1989 and 1999 at the Asan Medical Center (Seoul, Korea). The clinicopathological findings were evaluated retrospectively. All tumors were examined in duplicate and identified as SRCC when more than 50% of the tumor cells were signet-ring cells. Metastatic SRCCs were excluded by operative findings as well as by preoperative imaging studies and endoscopy. There were nine male and eight female patients. Their mean age was 45.3 yr (range 14-68), and seven patients (41%) were younger than 40 yr.

Thirty colorectal carcinomas with well or moderately differentiated cell types (six well and 24 moderate differentiated cell type) were used as control group. They consecutively underwent surgery during the year 2000. The mean age of the control group was 56.5 yr (35-79), and there were 17 male and 13 female patients. The mean age of the control group was significantly greater than that of the SRCC group ($p<0.05$), whereas the sex ratio, tumor location, and stage were not different between the two groups.

Immunohistochemical Staining and Interpretation

Immunohistochemical staining was available in ten SRCCs using archival paraffin blocks. Early seven tissue blocks were lost in flood accident of the Asan Medical Center in 1988. Briefly, endogenous peroxidase activity was blocked by incubation in 0.5% hydrogen peroxide in methanol for 15 min. Antigen was retrieved by heating in a sodium citrate solution (0.1 M, pH 6.0) in a pressure cooker maintaining 15 psi for 30 min. Sections were incubated for 30 min with normal rabbit serum (Dako, Capinteria, CA) and then incubated with a mouse monoclonal antibody for E-cadherin (Takara, Shiga, Japan) (17) and β-catenin (Tranduction Labs., Lexington, KY) (18) overnight at room temperature. After washing in phosphate-buffered saline (PBS), sections were incubated with mouse and horseradish-peroxidase-labeled streptavidin, respectively (1:200 dilution each). Diaminobenzidine hydro-

chloride (DAB) was used as the chromogen.

The percentage of cells showing positive membrane staining for E-cadherin or β-catenin was graded as negative for no staining, one for $\leq 25\%$, two for $>25 - \leq 50\%$, three for $>50 - \leq 75\%$, and four for $>75\%$. The staining intensity was graded according to the color guide (4th ed., Dainippon Ink and Chemicals Inc., Tokyo, Japan) as negative for no staining, weak for light brown (No. 335), moderate for brown (No. 338), and intense for brown (No. 341) (19). A weighted score was obtained by multiplying the percentage by the intensity, and ranged from 0 to 12. Score 9-12 was defined as strong staining, 5-8 as reduced staining, 1-4 as greatly reduced staining, and 0 as negative (20, 21). Nuclear staining for β-catenin was considered as positive when $\geq 5\%$ of the nuclei were stained and as negative when $<5\%$ were stained (21).

Statistical Analysis

Comparison between the clinical factors and immunohistochemical staining was performed using the chi-square test, Fisher's exact test, and the Spearman correlation analysis. The survival time and the cumulative survival rate were estimated using the Kaplan-Meier method. The significance level was set at 5% for each analysis, and the SPSS (version 9.0, SPSS Inc) was used for statistical analysis on an IBM PC.

RESULTS

Clinical Characteristics of Patients with Colorectal SRCC

No patient with colorectal SRCC had a familial history of malignant disease, including colorectal cancer and hereditary non-polyposis colorectal cancer with its associated malignancy. Synchronous colon cancer was also present in one patient. Colorectal SRCC was evenly distributed along the entire colon, i.e. five cases on the right colon, four on the left colon, and eight on the rectum. The gross appearance of linitis plastica was found in four patients (24%). Stage III occurred most frequently (11 cases), followed by Stage IV (three cases) (Table 1).

During a median follow-up of 18 months, nine patients died due to recurrence in 14 patients who had not had distant metastasis on primary surgery. Peritoneal seeding was the most frequent mode of recurrence (seven patients, 41%). Four patients (24%) showed brain metastases and three patients (18%) showed bone metastases; hepatic metastasis was not identified. Two year and five-year overall survival rates were 32% and 16%, respectively. There was no difference in any of the clinicopathologic features in all 17 SRCC cases as well as ten cases of immunohistochemical staining.

Table 1. Clinicopathologic characteristics in 17 patients with colorectal signet-ring cell carcinoma

No	Sex	Age (yr)	Location*	Gross type[†]	Stage[‡]	Recurrence	Survival (months)[§]
1	F	14	Right colon	III	II	Peritoneum, Brain	21
2	F	20	Left colon	II	IV	Lymph node	53
3	M	25	Left colon	IV	III	Peritoneum	16
4	M	29	Rectum	IV	III	Lymph node, Bone	13
5	F	30	Rectum	IV	III	Peritoneum, Bone	23
6	F	35	Left colon	III	III	-	70+
7	M	38	Rectum	II	III	Bone, Brain	8
8	M	45	Right colon	III	IV	Peritoneum	3
9	M	53	Right colon	III	III	Bone, Brain	15
10	M	53	Rectum	III	III	-	28+
11	F	57	Right colon	III	IV	Peritoneum	18
12	F	58	Right colon	III	II	-	49+
13	M	60	Rectum	I	I	-	13+
14	F	60	Left colon	III	III	-	50+
15	F	61	Rectum	IV	III	Peritoneum, Brain	14
16	M	65	Rectum	III	III	Local	18
17	M	68	Rectum	II	III	Peritoneum	14

*Right colon includes the cecum, ascending colon, and transverse colon; Left colon includes descending colon and sigmoid colon. [†]Gross type I, protruding; II, ulcerofungating; III, ulceroinfiltrative; IV, diffuse (Linitis plastica). [‡]Stage according to the American Joint Committee on Cancer (AJCC, 1997). [§] '+' means that patient was alive at the last follow-up.

Table 2. The membrane expression of E-cadherin in colorectal signet-ring cell carcinomas and in ordinary adenocarcinomas

	Weighted score*			
	Preserved	Reduced		
	9-12 (%)	5-8	1-4	0	Reduced (%)
Ordinary adenocarcinoma	23 (76.7)	6	1	0	7 (23.3)
SRCC[†]	0 (0)	1	5	4	10 (100)

*A weighted score was obtained by multiplying percentage by the intensity. [†]Colorectal signet-ring cell carcinoma. Chi-square test (9-12 vs. 5-8, 1-4, and 0), $p<0.005$.

Immunohistochemical Staining of E-cadherin and β-catenin in Colorectal SRCC

Normal colonic epithelium showed punctuate basolateral membrane staining for E-cadherin along the entire length of the crypt; this served as an internal positive control. All colorectal SRCC showed variable degrees of decreased membrane expression (Table 2). It was noted that no expression was found in four of ten cases. In 30 control cases, normal E-cadherin expression was found in 23 cases and reduced expression was identified in seven cases. The E-cadherin expression of colorectal SRCC significantly differed from that of ordinary colorectal cancers ($p<0.001$)

The membrane expression of β-catenin was preserved in two cases of colorectal SRCC and 10 cases in the control group without a significant difference between the two groups. Nuclear expression of β-catenin was identified in nine cases of colorectal SRCC and in 22 cases in the control group (Table 3). All cases showing decreased membrane

Table 3. Membrane and nuclear expression of β-catenin in colorectal signet-ring cell carcinoma and in ordinary adenocarcinomas

	Ordinary adenocarcinoma (%)	SRCC* (%)
Membrane expression (Weighted score[†])		
Preserved (9-12)	10 (33.3)	2 (20)
Reduced	20 (66.7)	8 (80)
5-8	12	4
1-4	8	3
0	0	1
Nuclear expression[‡]		
Positive	22 (73.3)	9 (90)
Negative	8 (26.7)	1 (10)

*Colorectal signet-ring cell carcinoma. [†]A weighted score was obtained by multiplying percentage by the intensity: chi-square test (9-12 vs. 5-8, 1-4, and 0), $p=0.69$. [‡]Chi-square test, $p=0.40$.

expression showed nuclear expression. There was no correlation between the membrane expression of E-cadherin and that of β-catenin (Spearman correlation coefficient=-0.092).

DISCUSSION

Laufman and Saphir (1951) described colorectal SRCC as a distinct type of primary neoplasm of the large bowel. A substantial number of studies have reported that colorectal SRCC presents with different biologic behavior and a different clinical course compared to ordinary colorectal adenocarcinomas (1-3). Colorectal SRCC is rarely associated with adenomatous polyps and a family history of colorectal cancer
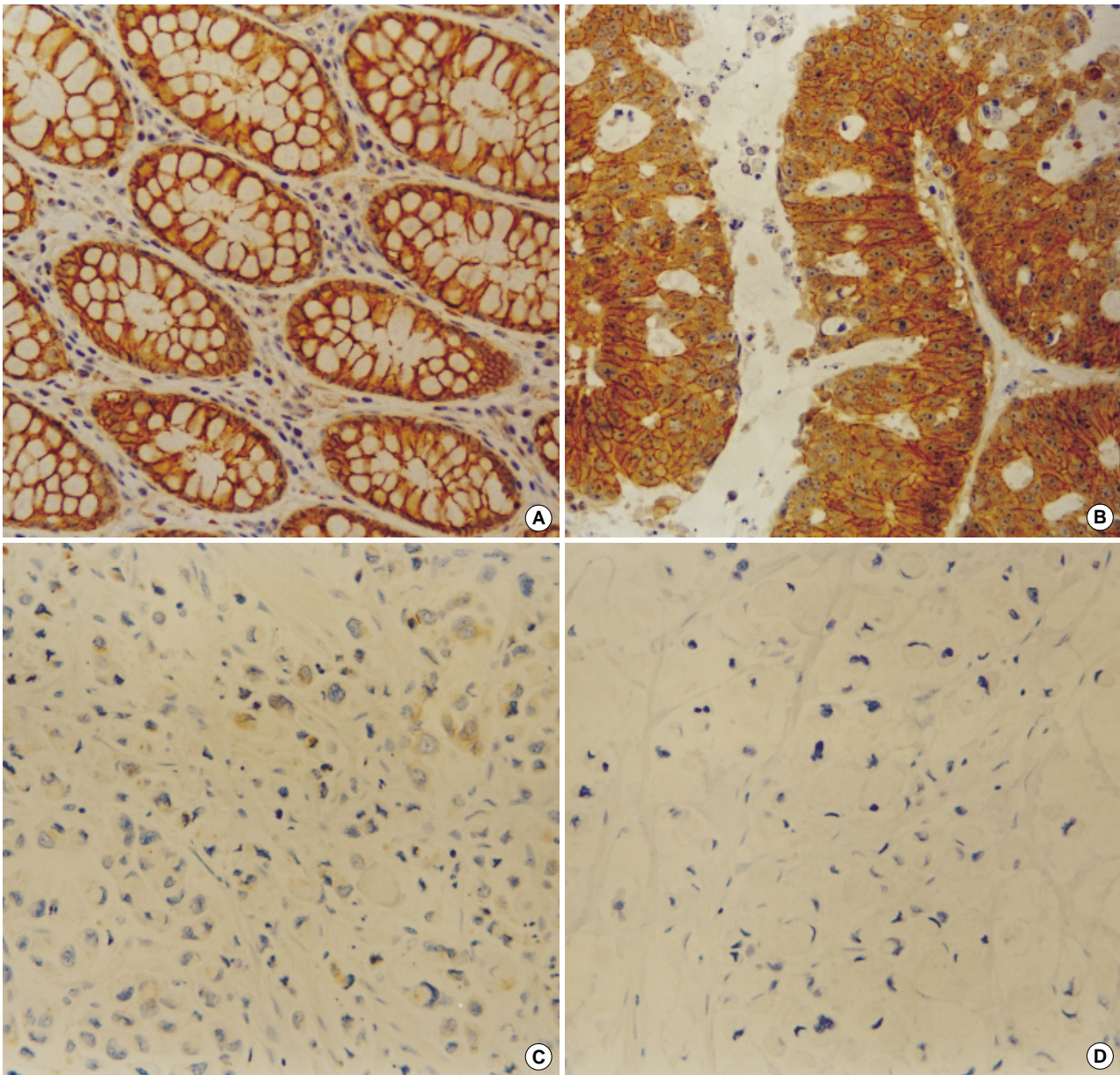
Fig. 1. A: Normal mucosa showing uniformly membranous expression of E-cadherin (×200). B: Preserved expression of E-cadherin (Grade 3 with strong intensity in moderately differentiated adenocarcinoma, ×200). C: Reduced expression of E-cadherin (Grade 1 with weak intensity in colorectal SRCC, ×200). D: Absent expression of E-cadherin in SRCC (×200).

in contrast to ordinary colorectal adenocarcinoma showed a 15-20% rate of familial clustering. According to the three recent controlled studies, the survival outcome of SRCC was uniformly poorer than that of ordinary adenocarcinomas (1-3). Furthermore, the metastatic pattern of frequent peritoneal dissemination, and the relatively rare occurrence of liver metastasis, are the features of colorectal SRCC, which distinguish it from ordinary colorectal adenocarcinoma. In this study, we also identified these clinical characteristics of colorectal SRCC. These observations might suggest a different molecular behavior implying the characteristic carcino-

genesis and progression of colorectal SRCC.

The loss of function in any of the E-cadherin-catenin complex components has been suggested as the cause of loss of epithelial differentiation and architecture or the acquisition of a motile and invasive phenotype (22). Alteration in the expression or function of E-cadherin in carcinomas may allow certain carcinoma cells to be readily detached from the surrounding structure and thereby develop a more infiltrative growth pattern. A variety of human malignancies, including thyroid, esophagus, gastric, and colon adenocarcinomas, have shown that reduced E-cadherin expression is correlated
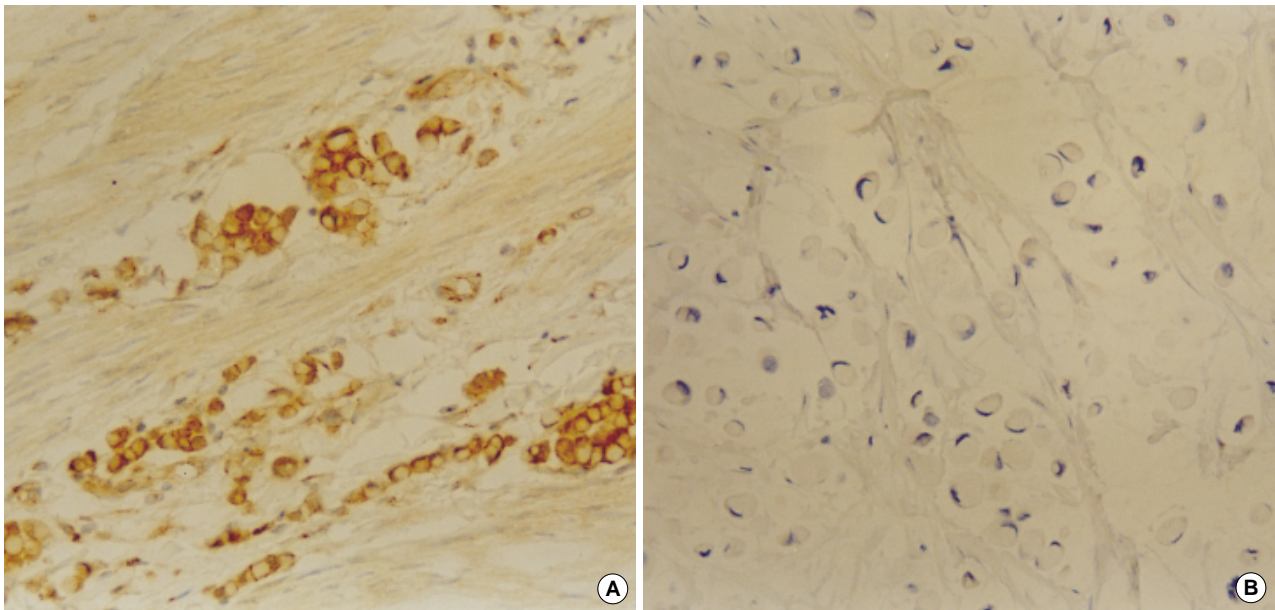
Fig. 2. A: Membranous and nuclear expression of β-catenin in SRCC ($\times$200). B: Absent expression of β-catenin either on the membrane or on the nucleus in SRCC ($\times$200).

with tumor dedifferentiation, infiltrative growth, and lymph node involvement (23, 24). E-cadherin loss has frequently been reported in gastric SRCC and lobular breast carcinoma. Approximately half of gastric SRCCs included mutations in the E-cadherin gene *(CDH1)* resulting in a low or aberrant expression of protein. In our study, the significantly reduced E-cadherin expression in colorectal SRCC was found and suggest that an aggressive biological behavior of colorectal SRCC is partly contributed by aberrant E-cadherin expression.

E-cadherin appears to be the common target for soluble growth factors and cytokines, such as the epidermal growth factor (EGF), the transforming growth factor α (TGF α), the hepatocyte growth factor/scatter factor (HGF/SF), and the trefoil factor (25). The trefoil peptides of secreted proteins found predominantly in mucus-secreting cells, have been thought to promote migration of intestinal epithelial cells and to enhance mucosal healing and epithelial restitution (25, 26). In colon cancer cell lines, trefoil factor has been shown to modulate epithelial cancer cell adhesion, migration, and apoptosis by disturbing the complexes among E-cadherin, β-catenin, and associated proteins (11, 27). Although the mechanism of the trefoil factor is not precisely known, the trefoil factors activate the EGF receptor pathway to effect cell-to-cell adhesion (27, 28). It has been found that trefoil factors and mucins are often coexpressed in mucous cells in a closely related manner. Moreover, a recent study reported that the trefoil factor participated in mucus formation and protected the gastrointestinal mucosa via mucin binding (29). SRCC characteristically includes abundant intracytoplasmic mucin. The down-regulation of E-cadherin in colorectal SRCC may be associated with mucin, trefoil factors, and other growth factors, which produce an aggressive phenotype of colorectal SRCC.

β-catenin is known to participate in the Wnt signaling pathway (30). In the presence of the *APC* mutation, degradation of β-catenin diminished with accumulation of nuclear β-catenin bound to transcriptional factors. As E-cadherin competes with APC protein in binding to β-catenin, cytoplasmic concentration and nuclear shifting of β-catenin may be influenced by both proteins (9). In our study, the membrane expression of β-catenin was predominantly reduced, whereas nuclear expression was prominent, in both SRCC and in the control groups. Down-regulation of E-cadherin may affect the decreased membrane expression and nuclear shifting of β-catenin, even though we could not find any correlation between the membrane expression of E-cadherin and β-catenin. Interestingly, colorectal SRCC showing nuclear expression of β-catenin may also share common characteristics with the ordinary colorectal tumorigenesis at an early stage.

In conclusion, the aggressive behavior of colorectal SRCC appears to be closely related to the down-regulation of E-cadherin. However, the precise genetic and epigenetic changes involved in SRCC remains to be verified in order to understand the various phenotypic expression of E-cadherin.

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