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Localization of specialized intestinal metaplasia and the molecular alterations in Barrett's esophagus in a Japanese population: an analysis of biopsy samples based on the “Seattle” biopsy protocol

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Summary

It remains unclear why Barrett's esophagus (BE)-associated adenocarcinoma (EAC) frequently occurs in the 0-3 o'clock area of the BE. The aims of this study were to clarify the localization of specialized intestinal metaplasia (SIM) as a precancerous lesion and of molecular alterations among different locations using four-quadrant biopsies based on the “Seattle” protocol. We prospectively evaluated microsatellite instability, methylation status at the *APC*, *CDKN2A*, *hMLH1*, *RUNX3* and *MGMT* genes, the immunoreactivity of the monoclonal antibody Das-1 for the colonic phenotype, and Ki-67 staining in 10 early EACs and 128 biopsy samples from 32 BE patients. Among the molecular changes, only *APC* gene hypermethylation was an independent predictive marker of EAC (OR=24.4, $p=0.01$). SIM was more frequently identified in the 0-3 o'clock quadrant than in the 6-9 o'clock quadrant ($p=0.08$). The Ki-67 index was higher in SIM than in the columnar-lined epithelium (CLE) without goblet cells ($p<0.0001$), and in both SIM and CLE with Das-1 reactivity than in those without ($p=0.04$ and $p=0.06$, respectively). Furthermore, the index was relatively higher in the 0-3 o'clock quadrant than in the 6-9 o'clock quadrant in cases with Das-1 reactivity. *RUNX3* methylation was more frequently found in SIM than in CLE ($p=0.04$), while the incidence of the other biomarkers did not show a significant difference between the 0-3 o'clock and 6-9 o'clock areas, nor between SIM and CLE. SIM with Das-1

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reactivity, but not molecular alterations, in the 0-3 o'clock quadrant may have higher proliferative activity compared to the other areas of the BE.

Keywords

Barrett's esophagus; specialized intestinal metaplasia; Das-1; columnar-lined epithelium; methylation; microsatellite instability

1. Introduction

The main risk factor for Barrett's esophagus (BE) is considered to be gastroesophageal reflux disease (GERD) [1]. Since 2000, the incidence of GERD has been increasing in Japan as well as in the West [2]. This finding suggests that the prevalence of GERD-associated BE will increase in Japan over the next few decades. The incidence of high-grade dysplasia (HGD) and esophageal adenocarcinoma (EAC) arising in individuals with BE is also increasing in the West [3]. Current surveillance guidelines for BE from the West recommend that quadrantic biopsies should be performed every 1 to 2 cm in the columnar segment together with biopsies of any visible lesions (the "Seattle protocol") to detect dysplasia and EAC at an early stage and subsequently improve survival [4,5].

A Japanese group first showed that HGD or EAC in patients with short-segment BE (SSBE) was most frequently found in the right anterior wall of the distal esophagus (the 0 to 3 o'clock area) [6]. Since then, similar studies from the West on the localization of dysplasia or EAC in SSBE were reported [7-9]. It remains elusive, however, why neoplasia in SSBE has a predilection for this location of the esophagus.

BE diagnosis requires histological confirmation of specialized intestinal metaplasia (SIM) in the USA [4,10] but not in the UK [5] or Japan [11]. SIM is generally considered to be a precancerous condition [4,10], whereas some researchers have shown that molecular abnormalities in the columnar-lined epithelium (CLE) without SIM were statistically similar to those in BE epithelium with SIM [12,13]. Additionally, it has been reported that non-SIM BE mucosa (CLE) has the same cancer risk as intestinal-type mucosa [13,14]. Therefore, whether the presence of SIM is actually associated with a risk of developing dysplasia or EAC is unclear.

To date, many reviews regarding molecular biomarkers, including DNA methylation, that are associated with an increased risk of neoplastic progression (HGD or EAC) have been reported [15-22]. A genome-wide profiling of CpG methylation in esophageal tissue samples found that there was a substantial difference in the methylation pattern between normal esophagus samples and those with BE or EAC, but that the difference between BE and EAC was still unclear [21]. The previous studies also had some limitations; all of the BE samples were obtained from patients who developed HGD or EAC, as opposed to the vast majority of cases of BE that do not progress [19-22].

We herein prospectively: 1) investigated molecular events including microsatellite instability (MSI), the methylation of CpG islands at various genes, and the reactivity of monoclonal

antibody (mAb) Das-1, a unique peptide expressed by the colonic phenotype [23,24] in EAC and BE without EAC; and 2) compared the incidence of SIM at different circumferential locations of BE and the differences in molecular alterations between SIM and CLE using four-quadrant biopsies based on the “Seattle” protocol.

2. Patients, Materials and Methods

2.1. Patients

Ten consecutive HGDs/EACs resected by endoscopic submucosal dissection (ESD) (EAC group) and 128 BE samples from 32 patients (BE group) who underwent endoscopy in our department between August 2014 and April 2015 were evaluated. All BE patients were outpatients who understood the purpose of this study. The exclusion criteria were as follows: (1) patients with a history of esophagectomy or gastrectomy, (2) patients to whom the guidelines of the Japan Gastroenterological Endoscopy Society for gastroenterological endoscopy for patients undergoing antithrombotic treatment did not apply [25], and (3) patients who were determined by their physicians to be unqualified for any other reason. A standardized questionnaire was used to obtain a history from each subject regarding GERD symptoms and smoking and alcohol habits prior to endoscopy. Whether or not subjects were taking proton pump inhibitors (PPIs), non-steroidal anti-inflammatory drugs (NSAIDs), low-dose aspirin, or statins was determined by self-report and a review of the medical prescriptions in our database. The Ethics Committee of Hyogo College of Medicine approved this study. Written, informed consent was obtained from all patients prior to this study. This study is registered with the UMIN Clinical Trials Registry, number UMIN000017994.

2.2. Endoscopy protocol

BE was diagnosed endoscopically as Barrett's mucosa between the lower end of the palisade vessels of the lower esophagus (Japanese criteria) [11] or the upper end of the gastric folds (Prague C & M criteria) [26] and the squamocolumnar junction. When the BE length was considered to be < 1.0 cm, the length was judged in comparison to the biopsy forceps (the open forceps were about 7 mm in diameter; Radial Jaw™ 4: Boston Scientific Corporation, Natick, MA). Because endoscopic diagnosis of ultra-short BE (USB), i.e., < 1 cm in length, is difficult and highly unreliable, patients with USBE were excluded from this study.

The circumferential location in the distal esophagus was noted as on a clock-face with the endoscope in the neutral position, where the 8 o'clock area was aligned with the retained fluid in the left lateral decubitus position. Four-quadrant biopsies, one from each site, were taken from the 0, 3, 6, and 9 o'clock regions of the BE according to the “Seattle” protocol [4]. One esophageal biopsy sample (squamous epithelium) taken from the normal esophagus without any findings of esophagitis was examined in each case as a control in the MSI and CpG islands methylation analysis. Although biopsy samples from the BE and ESD specimens were assessed for the grade of dysplasia using the revised Vienna classification [27], HGD (Category 4.1) and EAC including non-invasive carcinoma (Category 4.2) or intramucosal adenocarcinoma (Category 4.4) were grouped together as the EAC group because they represent a common endpoint for endoscopic therapeutic intervention [4,10].

2.3. DNA extraction

From paraffin-embedded blocks of the biopsy specimens, 7- μ m tissue sections were cut for DNA extraction. DNA was extracted only from cancer cells, SIM, or CLE glands in the biopsy samples via laser capture microdissection (LCM) using the PALM MicroBeam (Microlaser Technologies, Munich, Germany) to avoid DNA contamination of inflammatory or stromal cell nuclei based on the methodology previously described [28,29] (Supplementary Fig. S1).

2.4. Analysis of MSI by high-resolution fluorescent microsatellite analysis

As reported [29], we examined five microsatellite loci on chromosomes for MSI based on the revised Bethesda panel [30] as follows: BAT26, BAT25, D2S123, D5S346, and D17S250. The PCR products were evaluated for MSI by capillary electrophoresis using an ABI PRISM® 310 Genetic Analyzer (Applied Biosystems, Foster City, CA) and automatic sizing of the alleles using a GeneMapper® (Applied Biosystems). The MSI status was judged according to previous reports (Supplementary Fig. S2) [29].

2.5. Sodium bisulfite modification of DNA

Purified DNA samples were chemically modified by sodium bisulfite with the EpiTect® Plus DNA Bisulfite Kit (Qiagen). The bisulfite-modified DNA was amplified using primer pairs that specifically amplify either the methylated or unmethylated sequences of several genes related to BE carcinogenesis, such as *Adenomatous polyposis coli* (*APC*), *CDKN2A*, *hMLH1*, Runt-related transcription factor 3 (*RUNX3*), and *O6-methylguanine-DNA methyltransferase* (*MGMT*).

2.6. Methylation-sensitive high-resolution melting (MS-HRM) analyses

We performed a methylation-sensitive high-resolution melting (MS-HRM) analysis as previously described [31]. Briefly, the PCR amplification and MS-HRM analysis were performed using a LightCycler 480 Real-Time PCR System (Roche, Mannheim, Germany). The primer sequences of all genes for the methylated and unmethylated forms and PCR and MS-HRM conditions are summarized in Supplementary Tables S1 and S2. Percentages of methylation of 0%, 10%, 50%, and 100% were used to draw the standard curve (Supplementary Fig. S3). In this study, we regarded only samples with > 10% methylation as methylated.

2.7. Immunoperoxidase assays with mAb Das-1

Serial sections were stained with the mAb Das-1 (an IgM mAb highly specific against the colonic phenotype) using sensitive immunoperoxidase assays as described previously [23,24]. A specimen was considered positive if both a substantial number of cells and more than one gland were reactive to this mAb (Fig. 1) [23,24].

2.8. Detection of proliferation and apoptosis

Dewaxed paraffin sections were examined by the streptavidin-biotin-peroxidase complex method using MIB-1 as the primary antibody against the Ki-67 antigen of proliferating cells (mouse IgG, Invitrogen, CA). The slides were treated with the antigen-retrieval technique

based on microwave oven heating. Apoptotic cells *in situ* were detected by the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) method. The Takara *in situ* Apoptosis Detection Kit (Takara, Tokyo, Japan) was used according to the manufacturer's instructions. In each case, the fractions (%) of cells that showed positive nuclear staining for Ki-67 antigen and TUNEL were considered to be the proliferative indices (PI) and the apoptotic indices (AI), respectively.

2.9. Statistical analysis

The data were assessed by the Mann-Whitney *U*-test for comparisons between two independent groups and by the chi-square test or Fisher's exact test for comparisons between two proportions. Differences in the molecular alterations rate or the incidence of SIM between the 0-3 o'clock and 6-9 o'clock areas of the BE were analyzed with the McNemar test. Predictive factors for progression to dysplasia or EAC with a *p*-value of < 0.05 in univariate analysis were included in the multiple logistic regression model and analyzed using the backward approach. Odds ratios (OR) and 95% confidence intervals (CI) were calculated for risk factors. The 95% CI of the OR was used to assess the statistical significance at the conventional level of 0.05. $P < 0.05$ was considered statistically significant (StatView version 5.0 (SAS Institute Inc., Cary, NC) or EZR (Saitama Medical Centre, Jichi Medical University; <http://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmedEN.html>; Kanda, 2012), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria, version 2.13.0)).

3. Results

3.1. Patient characteristics

The patient characteristics are shown in Table 1. There were no significant differences in age, gender, smoking, GERD symptoms, medication, or body mass index between the EAC and BE groups, although the percentage of males and the smoking rate were somewhat higher in the EAC group. Out of 10 EACs treated with ESD, 8 EACs were histologically diagnosed as intramucosal cancer. The remaining 2 EACs were diagnosed as submucosal cancer and subsequently underwent surgery. Of 10 EACs, tumors were mainly located in the 0-3 o'clock area in 5 (50%), the 6-9 o'clock area in 4 (40%), and the 3-9 o'clock area in 1 (10%). All cases from the BE group showed circumferential flame-shaped BE, and non-circumferential BE was not seen. The mean BE length in the BE and EAC groups was based on the Prague C & M criteria in Table 1. There was no significant difference in the BE length between these two groups.

3.2. MSI, DNA methylation, and mAb Das-1 reactivity in the EAC and BE groups

MSI and hypermethylation all genes were identified at in the EAC group significantly more often than in the BE group (Table 2), but none of these molecular events were seen in the normal esophageal mucosa (control). In the multivariate logistic regression analysis, MSI and *APC* gene hypermethylation were associated with EAC ($p=0.06$ and $p=0.01$, respectively), and only *APC* gene hypermethylation was an independent predictive marker for the development of EAC (OR=24.4, 95% CI 2.14-277.8, $p=0.01$). MSI is thought to be significantly associated with the loss of the *hMLH1* gene [30]. Of the 33 MSI-positive cases,

including 9 in the EAC group and 24 in the BE group, 14 patients (42.4%) showed positivity for hypermethylation at *hMLH1*.

When statistically evaluating the molecular alterations for EAC, *MGMT* was found to be the highest predictor of EAC among a panel of six molecular events, with 100% sensitivity, 99.2% specificity, 90.9% positive predictive value, and 100% negative predictive value (Supplementary Table S3). No significant associations between the medications and the incidence of molecular alterations were found (data not shown).

3.3. Localization of SIM and the differences in molecular alterations among the different locations of BE and between SIM and CLE

SIM was frequently found in the 0 o'clock area among the four different quadrants of BE, and the incidence of SIM in the 0-3 o'clock quadrant tended to be higher than that in the 6-9 o'clock quadrant ($p=0.08$ by McNemar test) (Fig. 2).

The incidence of MSI and hypermethylation at all genes did not show significant differences between the 0-3 o'clock and 6-9 o'clock quadrants in either SIM or CLE (Supplementary Table S4). Although the *RUNX3* hypermethylation frequency was significantly higher in SIM than in CLE ($p=0.04$), there were no significant differences in other molecular markers between SIM and CLE (Table 3).

3.4. mAb Das-1 reactivity and cell kinetics in EAC and BE

mAb Das-1 reactivity was seen in all EACs (100%) and in 30.5% (39 of 128) of the cases in the BE group. In the BE group, the reactivity was 53.7% (22 of 41) in SIM and 19.5% (17 of 87) in CLE, and thus showed a significant difference ($p < 0.0001$) (Table 3). In contrast, mAb Das-1 reactivity showed no significant difference between the 0-3 o'clock and 6-9 o'clock arcs (Supplementary Table S4).

With regard to the cell kinetics in EAC and BE including SIM and CLE, both PI and AI were highest in EAC, second-highest in SIM, and lowest in CLE, and the differences were significant among all of these groups (Fig. 3A), while very few apoptotic cells were observed in the SIM and CLE glands. Interestingly, PI in both SIM and CLE with mAb Das-1 reactivity was significantly higher than that in SIM and CLE without mAb Das-1 reactivity ($p=0.04$ and $p=0.06$, respectively). Furthermore, PI was relatively higher in the 0-3 o'clock quadrant than in the 6-9 o'clock quadrant in the cases with mAb Das-1 reactivity (Figs. 3B and 3C).

4. Discussion

To date, there have been no studies on the localization of SIM in biopsies taken from four different quadrant locations based on the "Seattle" biopsy protocol nor on the molecular alterations in only EAC, SIM or CLE glands captured selectively by LCM. Thus, this is the first study in which the molecular events including genetic, epigenetic DNA alterations, mAb Das-1 reactivity and cell kinetics were evaluated among the different locations of BE and between SIM and CLE.

Our findings showed that all of the molecular alterations investigated were more frequently detected in the EAC group compared to the BE group, suggesting that CpG hypermethylation at all genes is closely associated with EAC, as previously reported [15-22]. Among these DNA alterations, only *APC* hypermethylation was an independent predictive marker for EAC development. This result is consistent with a study by Wang et al. [16]. In contrast to their results, however, *CKDN2A (p16)* was not a predictor of subsequent progression to EAC in our study. The methylation status at other genes was also different from that found in previous studies [15,17,18,20]. Among the biomarkers evaluated in the current study, statistical calculations of *MGMT* methylation showed the highest sensitivity, specificity, and positive and negative predictive value for EAC.

With regard to MSI, there have been several reports with similar findings, including our previous study, in which there was a positive association between MSI and BE or EAC, and in which MSI in BE was found to take place earlier in the development of SIM [29,32]. Thus, the genetic, as well as epigenetic, alterations present in EAC may already be present in BE [29,32]. Although the incidence of molecular changes in SIM and CLE was slightly different from that found in our previous study [29], this might be accounted for by differences in the sample size and method of molecular analysis.

Taking into account previous studies and our present findings, it appears that epigenetic alterations related to the progression to EAC remain controversial. Some investigators mentioned that the differences in the prevalence of hypermethylation are most likely attributable to a lack of standardization in the techniques used for the assay [33]. In our study, we used MS-HRM analysis, which is applicable for the very sensitive and quantitative assessment of methylation levels in an unmethylated background. Furthermore, many previous studies regarding methylation in BE were conducted using DNA extracted from the whole biopsy tissues, and those results were affected by contamination with inflammatory cells. Therefore, we used LCM to extract DNA selectively from SIM or CLE glands as previously reported by El-Serag et al. and in our previous study [28,29].

Interestingly, SIM was frequently found in the 0-3 o'clock area, which was a similar location to where EAC was most preferentially found [6-9]. The presence of SIM can be limited by sampling error in mucosal biopsies and shows a patchy distribution in the BE mucosa; however, one of the goals of the "Seattle" protocol is to detect dysplasia or SIM [10]. Our results may therefore suggest that the biopsies should be taken from the 0-3 o'clock area to confirm the presence of SIM. In contrast, Sharma et al. reported that the use of narrow band imaging (NBI) targeted biopsies and four-quadrant biopsies based on the Seattle biopsy protocol diagnosed SIM in similar proportions, although NBI was able to achieve this with significantly fewer biopsies [34].

Cell proliferation increased significantly in the following order: from CLE to SIM to EAC. Also, the frequency of *RUNX3* methylation was significantly higher in SIM than in CLE. These results may support the pathogenesis of the metaplasia-dysplasia-carcinoma sequence. In the current study, mAb Das-1, which reacts with the colonic phenotype, was significantly associated with SIM ($p < 0.0001$). The incidence of reactivity (19.5%) was relatively lower compared to those (30–63.6%) reported from the West [13,24]; this discrepancy may be

caused by the ethnic difference between the subjects. The important point is that CLE, in some cases, shows phenotypic evidence of intestinal differentiation (Fig. 1). This finding provides support for the possibility that the non-goblet CLE may be intestinalized before SIM [13,24]. Additionally, the PI was significantly higher in SIM with mAb Das-1 reactivity than in SIM without mAb Das-1 reactivity ($p=0.04$). It was interesting that the PIs of both SIM and CLE in the 0-3 o'clock area were relatively higher than those in the 6-9 o'clock area. These results indicate that BE, especially SIM, in the 0-3 o'clock quadrant with mAb Das-1 reactivity may be in a higher proliferative state compared to the other quadrants. In contrast, it has been recently reported that a high number of SIM cases have an inverse relationship with the presence of DNA content flow cytometric abnormalities and the risk of EAC in patients with BE [35].

The present study has several potential limitations. A possible weakness is that the sample size evaluated was small. Also, we did not analyze the association between the localization of SIM, molecular alterations or mAb Das-1 reactivity, and acid reflux to the esophagus using a circumferential pH sensor array catheter [6]. It has been reported that acid reflux in BE patients predominantly occurs in the right anterior side (0-3 o'clock quadrant) of the distal esophagus [6]. Additionally, four-quadrant biopsies were not obtained from the patients with EAC prior to endoscopic resection. Therefore, the localization of SIM and molecular alterations could not be evaluated in the different areas of BE in the patients with EAC.

In conclusion, SIM was frequently identified in the 0-3 o'clock area of BE, and its proliferative activity was higher than that of CLE, especially in cases with mAb Das-1 reactivity. This result may be an important factor in the pathogenesis with regard to why EAC has a predilection to be located in the 0-3 o'clock area of the BE. *APC* hypermethylation was an independent predictive marker for progression to EAC, while the incidence of most molecular alterations including *APC* did not show significant differences among the locations of BE nor between SIM and CLE. Further studies with a larger sample size are needed to clarify the reason for this lack of association.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Serial representative sections of H&E and immunoperoxidase staining with the monoclonal antibody (mAb) Das-1. A, Serial sections of the Barrett's esophagus (BE) specimen obtained by biopsy revealed the columnar-lined epithelium (CLE) without goblet cells with H&E staining (magnification, 100 $\times$). B, Section shows mAb Das-1 staining of the area enclosed by a box in Fig. 1A. The reactivity was identified in many glands of the CLE (magnification, 200 $\times$). C, Serial sections of the BE showed the specialized intestinal metaplasia with H&E staining (magnification, 200 $\times$). D, When stained with mAb Das-1, there is clear reactivity in many goblet cells of SIM glands (magnification, 200 $\times$).

Fig. 2. Axial location of SIM in Barrett's esophagus. SIM was more frequently found in the 0-3 o'clock quadrant than in the 6-9 o'clock quadrant ($p=0.08$ by McNemar test).

Fig. 3.

Cell kinetics in esophageal adenocarcinoma and Barrett's esophagus including SIM and CLE. A) Comparison of cell kinetics between esophageal adenocarcinoma (EAC) and Barrett's esophagus. Both the Ki67 and apoptotic indexes became significantly higher in the following order: from columnar-lined epithelium (CLE) to specialized intestinal metaplasia (SIM) to EAC (* $p < 0.0001$, † $p = 0.003$). Comparison of cell proliferation in SIM (B) and CLE (C) with and without mAb Das-1 reactivity and between the 0-3 o'clock quadrant and the 6-9 o'clock quadrant. The Ki67 index was higher in both SIM and CLE with mAb Das-1 than in those without (* $p = 0.04$ and † $p = 0.06$). Also, the index was relatively higher in the 0-3 o'clock quadrant than in the 6-9 o'clock quadrant with mAb Das-1 reactivity (§ $p = 0.09$).

Table 1**Characteristics of the patients**

	EAC n=10	BE n=32	p-value
Age (y)	61.8 ± 14.3	66.4 ± 12.2	0.26
Male : Female *	10 : 0	22 : 10	0.08
Alcohol drinking *	5 (50.0)	8 (25.0)	0.24
Current smoking *	3 (30.0)	2 (6.3)	0.08
GERD symptoms *	0 (0)	8 (25.0)	0.17
Medication *			
PPI	5 (50.0)	19 (59.4)	0.72
NSAID / LDA	0 (0)	4 (12.5)	0.56
Statin	1 (10.0)	8 (25.0)	0.42
BMI (kg/m ²)	22.0 ± 3.8	21.8 ± 3.1	0.65
Mean BE length †			
C criteria	10.4 ± 16.3	10.1 ± 16.5	0.64
M criteria	18.9 ± 17.7	19.5 ± 18.1	0.73

Continuous and categorical data are summarized as means ± SDs and frequencies (percentages).

EAC, esophageal adenocarcinoma; BE, Barrett's esophagus; PPI, proton pump inhibitor; NSAID, non-steroidal anti-inflammatory drug; LDA, low-dose aspirin; GERD, gastroesophageal reflux disease; BMI, body mass index.

* Fisher's exact test

† Prague C & E criteria

Table 2
Comparison of molecular alterations between the EAC and BE groups

Markers	EAC n=10	BE n=128	Univariate analysis			Multivariate analysis		
			OR	95% CI	P	OR	95% CI	P
MSI	9 (90.0)	24 (18.8)	39.0	4.7-322.7	0.0007	11.2	0.94-134.9	0.06
APC	9 (90.0)	18 (14.1)	55.0	6.6-460.7	0.0002	24.4	2.14-277.8	0.01
CDKN2A	4 (40.0)	9 (7.0)	8.8	2.1-37.0	0.0003	9.8	0.09-1144.6	0.35
hMLH1	8 (80.0)	29 (22.7)	13.7	2.7-67.9	0.001	4.3	0.47-38.79	0.20
RUNX3	10 (100)	22 (17.2)	NA	-	-	-	-	-
MGMT	10 (100)	1 (0.8)	NA	-	-	-	-	-

Data are summarized as frequencies (percentages). Multivariate analysis was adjusted for gender and smoking.

EAC, esophageal adenocarcinoma; BE, Barrett's esophagus; MSI, microsatellite instability; APC, *adenomatous polyposis coli*; NA, not applicable

Note: All EAC cases were positive for CpG island methylation at the *RUNX3* and *MGMT* genes, thus causing the problem of statistical complete separation when using these parameters (genes) in a logistic regression analysis. Therefore, these genes were excluded from the multivariate analysis.

Table 3
Comparison of molecular alterations between SIM and CLE

Markers	SIM n=41	CLE n=87	<i>p</i> -value
MSI	9 (22.0)	15 (17.2)	0.52
<i>APC</i>	5 (12.2)	13 (14.9)	0.79
<i>CDKN2A</i>	1 (2.4)	8 (9.2)	0.27
<i>hMLH1</i>	9 (22.0)	20 (23.0)	0.90
<i>RUNX3</i>	11 (26.8)	11 (12.6)	0.04
<i>MGMT</i>	0 (0)	1 (1.1)	> 0.99
mAb Das-1 reactivity	22 (53.7)	17 (19.5)	< 0.0001

Data are summarized as frequencies (percentages).

SIM, specialized intestinal metaplasia; CLE, columnar-lined epithelium; MSI, microsatellite instability; *APC*, *adenomatous polyposis coli*; mAb, monoclonal antibody