ABSTRACT

**Purpose:** Promoter DNA methylation of various genes has been associated with metachronous gastric cancer (MGC). The cancer-specific methylation gene, **cysteine dioxygenase type 1 (CDO1)**, has been implicated in the occurrence of residual gastric cancer. We evaluated whether DNA methylation of **CDO1** could be a predictive biomarker of MGC using specimens of MGC developing on scars after endoscopic submucosal dissection (ESD).

**Materials and Methods:** **CDO1** methylation values (TaqMeth values) were compared between 33 patients with early gastric cancer (EGC) with no confirmed metachronous lesions at >3 years after ESD (non-MGC: nMGC group) and 11 patients with MGC developing on scars after ESD (MGCSE groups: EGC at the first ESD [MGCSE-1 group], EGC at the second ESD for treating MGC developing on scars after ESD [MGCSE-2 group]). Each EGC specimen was measured at five locations (at tumor [T] and the 4-point tumor-adjacent noncancerous mucosa [TAM]).

**Results:** In the nMGC group, the TaqMeth values for T were significantly higher than that for TAM (P=0.0006). In the MGCSE groups, TAM (MGCSE-1) exhibited significantly higher TaqMeth values than TAM (nMGC) (P<0.0001) and TAM (MGCSE-2) (P=0.0041), suggesting that TAM (MGCSE-1) exhibited **CDO1** hypermethylation similar to T (P=0.3638). The area under the curve for discriminating the highest TaqMeth value of TAM (MGCSE-1) from that of TAM (nMGC) was 0.81, and using the cut-off value of 43.4, **CDO1** hypermethylation effectively enriched the MGCSE groups (P<0.0001).

**Conclusions:** **CDO1** hypermethylation has been implicated in the occurrence of MGC, suggesting its potential as a promising MGC predictor.

**Keywords:** Cysteine dioxygenase; Early gastric cancer; Endoscopic submucosal dissection; Methylation
INTRODUCTION

Presently, gastric cancer ranks as the sixth most common type of malignant tumor and is the third leading cause of mortality in men worldwide [1]. Due to the eradication efforts for *Helicobacter pylori*, the incidence of gastric cancer has decreased in recent years in Japan; however, it remains the third leading cause of mortality among malignant tumors [2]. With the development and increasingly common use of endoscopic submucosal dissection (ESD), ESD has become the established standard treatment for early gastric cancer (EGC) [3]. With the widespread treatment of EGC by ESD, it has become well known that metachronous gastric cancer (MGC) can develop after ESD, and surveillance endoscopy for MGC is recommended after ESD in these patients [4]. There are certain cases in which MGC develops on scars after curative ESD. The incidence of MGC after ESD has been reported to range from 4.0% to 13.0% [5-7].

Various genetic abnormalities such as genomic gain, genomic loss, and genomic mutations are involved in gastric cancer development [8]. Furthermore, epigenetic abnormalities, including DNA methylation, are more dominant during gastric carcinogenesis [9] and are affected by epigenetic field cancerization in the tumor-adjacent noncancerous mucosa (TAM), where DNA methylation abnormalities in various genes increase because of chronic inflammation due to *H. pylori* infection in the gastric mucosa [10-12].

Promoter DNA methylation, which is associated with gastric carcinogenesis, has recently attracted attention as a cancer-specific biomarker [13]. Among them, *cysteine dioxygenase type 1 (CDO1)* has been recognized as a novel tumor suppressor gene candidate in human cancers [14,15]. Previous reports have described the excellent diagnostic performance and prognostic relevance of *CDO1* promoter hypermethylation in various gastrointestinal cancers, including gastric cancer [16-21]. However, there have been no reports on *CDO1* promoter hypermethylation as a predictor of MGC after ESD. Therefore, we investigated the association between the subsequent development of MGC after ESD and epigenetic abnormalities using specimens of MGC developing on scars after curative ESD.

MATERIALS AND METHODS

Patients and materials

A total of 2,055 patients underwent ESD for EGC at Kitasato University Hospital and Kitasato University East Hospital between September 2002 and December 2016 (Fig. 1). A total of 33 patients with the latest consecutive EGC with no confirmed evidence of metachronous lesions at ≥3 years after curative ESD were selected as controls from 1,896 patients with no metachronous lesions (non-MGC: nMGC group). All 33 patients tested positive for *H. pylori* infection (current infection or after eradication). Among the 2,055 patients, 11 (0.5%) exhibited the development of MGC on scars after curative ESD (MGCSE groups) (Fig. 2), all 11 tested positive for *H. pylori* (current infection or after eradication). All patients were investigated for *H. pylori* status at the time of the initial medical examination. Among the MGCSE groups, we also examined patients who underwent the first ESD for EGC (MGCSE-1 group) and those who underwent a second ESD to treat MGC developing on scars after curative ESD (MGCSE-2 group). Both groups (MGCSE-1 and MGCSE-2) were investigated in the same patients. Furthermore, in the MGCSE-2 group, no cases of new MGCSE occurred during the observation period after the second ESD.
Predictive Significance of *CDO1* in MGC

Patients with EGC treated by ESD between 2002 and 2016 (n=2,055)

Patients with no MGC after ESD (n=1,896)

*H. pylori* infection (+)

Patients with the latest consecutive EGC with no confirmed metachronous lesions for over 3 years (nMGC group; n=33)

Patients with MGC after ESD (n=159)

Patients with MGC not developing on scars after ESD (n=148)

Patients with MGC developing on scars after ESD (MGCSE groups; n=11)

**Fig. 1.** Flowchart of study participants treated for early gastric cancer by endoscopic submucosal dissection. EGC = early gastric cancer; ESD = endoscopic submucosal dissection; MGC = metachronous gastric cancer.

**Fig. 2.** Representative cases in the MGCSE groups. Representative case from MGCSE group. Three years after the first ESD (the upper left panel shows the lesion (yellow arrowhead) before ESD (MGCSE-1) and the upper right panel shows after ESD), we diagnosed a new lesion (lower left panel) categorized as MGCSE-2 (yellow arrowhead). The lower-right panel shows the lesion after the second ESD. While performing the first ESD, we could recognize the submucosal layer with a blue area (indigo carmine) (upper middle panel). On the second ESD, it was difficult to recognize the border between the submucosal and muscular layers due to fibrosis (white area), and it was difficult to treat (lower middle panel).

MGCSE = metachronous gastric cancer developing on scars after curative ESD; ESD = endoscopic submucosal dissection; MGCSE-1 = early gastric cancer at the first ESD; MGCSE-2 = EGC at the second ESD performed for treating MGC developing on scars after curative ESD.
This study was conducted in accordance with the ethical guidelines outlined in the Declaration of Helsinki and was approved by the Kitasato University Hospital Ethics Committee (no. B18-036). All patients provided informed consent prior to enrollment.

**Cell lines**

The hepatocellular carcinoma cell line HepG2 and colorectal cancer cell line DLD1 were used as positive and negative controls for CDO1 methylation, respectively, as previously described [22]. The DLD1 cells were provided by the Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University (Sendai, Japan), whereas HepG2 cells were purchased from the RIKEN BioResource Research Center (Ibaraki, Japan). DLD1 cells were maintained in Roswell Park Memorial Institute-1640 medium (GIBCO, Carlsbad, CA, USA). HepG2 cells were maintained in Dulbecco’s modified Eagle’s medium (GIBCO) containing 10% fetal bovine serum and penicillin-streptomycin (GIBCO).

**Genomic DNA extraction and bisulfite treatment**

The locations of the tumor (T) and TAM were identified in EGC specimens pathologically diagnosed using hematoxylin-eosin staining after ESD. Formalin-fixed paraffin-embedded tissues of excised specimens of the T and TAM were cut into 20 10-μm-thick slices. After deparaffinization, genomic DNA was extracted from the T and the separated TAMs (four points: oral TAM, anal TAM, right TAM, and left TAM) tissues using a QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany) (Fig. 3). TAMs were extracted from noncancerous mucosa at a distance of more than 2 mm from the T site to prevent contamination of the tumor components. The extracted genomic DNA (2 μg) was chemically converted by bisulfite treatment using the EZ DNA Methylation-Gold™ Kit (Zymo Research, Irvine, CA, USA). The bisulfite-treated DNA was subsequently amplified as a template via quantitative methylation-specific polymerase chain reaction (Q-MSP).

![Fig. 3. Definition of sample locations for DNA extraction. Sample locations for DNA (left panel) and genomic DNA extraction from the T and separate TAMs (four points: oral TAM, anal TAM, right TAM, and left TAM) tissues. Corresponding pathological findings are shown in the right panels (hematoxylin and eosin staining, ×20, ×40). TAMs were extracted from noncancerous mucosa at a distance of more than 2 mm from the T site to prevent contamination of the tumor components. T = tumor; TAM = tumor-adjacent noncancerous mucosa.](https://jgc-online.org)
Q-MSP
Q-MSP for CDO1 was performed using a C1000 Touch™ thermal cycler with a CFX96 Real-Time System (Bio-Rad, Hercules, CA, USA). Q-MSP was conducted at 95°C for 3 minutes, followed by 40 cycles at 95°C for 20 s, annealing temperature for 30 seconds, and 72°C for 30 seconds, in a 25-μL reaction volume containing 1 μL of bisulfite-treated genomic DNA, 300 nmol/L of each primer, 200 nmol/L of a fluorescent probe, and 12.5 μL of iQ Supermix (Bio-Rad). PCR conditions and primer and probe sequences were designed as previously described [22]. All reactions were performed in triplicate. The CDO1 methylation value was defined as the ratio of the amplified signal value of methylated CDO1 to the value of β-actin, which was subsequently multiplied by 100. The CDO1 methylation value was designated as the TaqMeth value throughout the text.

Statistical analysis
Continuous and categorical variables were analyzed using the Student’s t-test and χ² test, respectively. Univariate analyses of factors for the clinicopathological characteristics of EGC were performed using the log-rank method. The observation period was defined as the time from the first ESD to the day of the final upper gastrointestinal endoscopy (median, 59 months; range, 37–179 months). The median observation period was 59 months (37–66 months) in the nMGC group and 70 months (44–179 months) in the MGCSE group. The occurrence period for MGC was defined as the time from the day of the first ESD to the day of the second ESD (median, 22 months; range, 14–49 months). Statistical analyses were performed using the JMP 11 software (SAS Institute Inc., Cary, NC, USA). Statistical significance was set at P<0.05.

RESULTS
Clinicopathological characteristics
Table 1 summarizes the clinicopathological characteristics of the 44 patients with EGC (nMGC group, n=33; MGCSE-1 group, n=11). With respect to H. pylori status at the time of ESD treatment, all 44 patients had been infected (current infection, n=16; after eradication, n=28; P=1.0000). Atrophic gastric mucosa was defined according to the Kimura-Takemoto classification [23-25]; all 44 patients (nMGC and MGCSE-1 group) had atrophic gastric mucosa (closed type: n=4, open type: n=40) (P=1.0000). With regard to histological type,
39 lesions were well-differentiated adenocarcinomas, whereas five lesions were moderately differentiated adenocarcinomas; however, the histological types were not significantly different among all patients (P=1.0000). Furthermore, among the MGCSE groups, the histological type of the lesion in the MGCSE-1 and MGCSE-2 groups was the same in 9 of 11 patients (Supplementary Table 1).

**Quantification of CDO1 promoter DNA methylation in the nMGC group**

In the nMGC group, the CDO1 TaqMeth values significantly differed between the T (n=33; median, 26.0; range, 3.1–81.2) and the TAM (n=33×4=132; median, 18.3; range, 0.0–65.8) tissues (P=0.0006; Fig. 4A). The CDO1 TaqMeth values for TAM were significantly lower than those for T. In addition, our recent study on CDO1 methylation status demonstrated that the methylation value was nearly zero (median, 0; range, 0.0–3.4) in 160 samples of the corresponding non-cancerous pancreas (non-CP) among patients with pancreatic ductal adenocarcinoma (PDAC) [26]. Therefore, considering the results of PDAC, the TaqMeth values for TAM in this study were surprisingly higher than expected. Thereafter, we quantified values in each of the TAMs separately (four points: oral TAM, anal TAM, right TAM, and left TAM) compared to the corresponding T value and found significant differences (P=0.0111, P=0.0451, P=0.0201, and P=0.0220, respectively; Fig. 4B).

**Quantification of CDO1 promoter DNA methylation in the MGCSE-1 group**

In the MGCSE-1 group, no significant difference in CDO1 TaqMeth values was identified between the tissue from the T (n=11; median, 40.1; range, 16.2–85.3) and TAM (n=11×4=44; median, 33.2; range, 7.1-100.6) (P=0.3914; Fig. 4C). Thereafter, we quantified the TAM values separately (four points: oral TAM, anal TAM, right TAM, and left TAM) in comparison with the corresponding T value, and found no significant differences (P=0.3606, P=0.9999, P=0.2535, and P=0.5242, respectively; Fig. 4D).

**Quantification of CDO1 promoter DNA methylation in the MGCSE-2 group**

In the MGCSE-2 group, no significant difference in CDO1 TaqMeth values was identified between the tissue from the T (n=11; median, 24.0; range, 8.6–47.2) and TAM (n=11×4=44; median, 24.1; range, 0.0–56.2) (P=0.5677; Fig. 4E). We subsequently quantified the TAM values separately (four points: oral TAM, anal TAM, right TAM, and left TAM) in comparison with the corresponding T value and found no significant differences (P=0.8779, P=0.3751, P=0.1948, and P=0.8386, respectively; Fig. 4F).

**Unique characteristics of the TAM in the MGCSE-1 group**

In all the groups (nMGC, MGCSE-1, and MGCSE-2 groups), we separately analyzed the values of T (Fig. 5A) and TAM samples (Fig. 5B). The median CDO1 TaqMeth value for the T was 26.0 (range, 3.1–81.2) in the nMGC group (n=33), 40.1 (range, 16.2–85.3) in the MGCSE-1 group (n=11), and 24.0 (range, 8.6–47.2) in the MGCSE-2 group (n=11), albeit without significant differences (P=0.1096, P=0.6180, P=0.6314, respectively; Fig. 5A). The median CDO1 TaqMeth value for the TAM was 18.3 (range, 0.0–65.8) in the nMGC group (n=132), 33.2 (range, 7.1-100.6) in the MGCSE-1 group (n=44), and 24.1 (range, 0.0–56.2) in the MGCSE-2 group (n=44), with significant differences between the MGCSE-1 group and the nMGC and MGCSE-2 groups (P=0.0001 and P=0.0041, respectively; Fig. 5B), and there was no significant difference between the nMGC and MGCSE-2 groups (P=0.0560). Intriguingly, no significant difference was detected between the TAM values in the MGCSE-1 group and the T value for the 55 cases pooled from all groups (P=0.3638; Fig. 5C).
Prediction according to the CDO1 TaqMeth values in MGC

The optimal cut-off value for the prediction of MGC was analyzed from the TAM data in the nMGC and MGCSE-1 groups using receiver operating characteristic curves. The optimal TaqMeth value for all TAMs (n=176) in both groups was 29.1 (area under the curve [AUC], 0.74; P<0.0001; sensitivity, 56.8%; specificity, 79.6%; Fig. 6A). Furthermore, when the highest value for the separate four-point TAMs in both groups was extracted, the optimal TaqMeth value was 43.4 (AUC, 0.81; P<0.0001; sensitivity, 81.8%; specificity, 78.8%; Fig. 6B).
The \( CDO1 \) \( \text{TaqMeth} \) values for all cases are presented in Supplementary Tables 2-4. In seven cases (21.2%), the highest value for the TAM was equal to or greater than the cut-off value (43.4%) among the 33 patients in the nMGc group. In contrast, in nine cases (81.8%), the

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Predictive Significance of \( CDO1 \) in MGC

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Fig. 5. Quantitative methylation-specific polymerase chain reaction for \( CDO1 \) among the nMGc, MGcSE-1, and MGcSE-2 groups. (A) In the T tissue, there was no significant difference in the \( \text{TaqMeth} \) value of \( CDO1 \) among the nMGc, MGcSE-1, and MGcSE-2 groups (\( P=0.1096, P=0.6180, P=0.6314 \), respectively). (B) In the TAM tissue, the \( \text{TaqMeth} \) value of \( CDO1 \) in the MGcSE-1 group was significantly higher than that in the nMGc and MGcSE-2 groups (\( P<0.0001 \) and \( P=0.0041 \), respectively), although there was no significant difference between the nMGc and MGcSE-2 groups (\( P=0.0560 \)). (C) In the T and TAM tissues, there was no significant difference in the \( \text{TaqMeth} \) values of \( CDO1 \) between the pooled T tissue from all groups and the TAM in the MGcSE-1 group (\( P=0.3638 \)).

T = tumor; TAM = tumor-adjacent noncancerous mucosa; \( \text{TaqMeth} \) values = methylation values; nMGc = EGc with no confirmed evidence of metachronous lesions >3 years after curative ESD; EGc = early gastric cancer; ESD = endoscopic submucosal dissection; MGcSE = MGc developing on scars after curative ESD; MGcSE-1 = EGc at the first ESD; MGcSE-2 = EGc at the second ESD for treating MGc developing on scars after curative ESD; MGc = metachronous gastric cancer; \( CDO1 \) = cysteine dioxygenase type 1.

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Fig. 6. Quantitative methylation-specific polymerase chain reaction for cysteine dioxygenase type 1 at all points and the optimal cut-off value for MGc occurrence. (A) ROC curve for all TAMs (nMGc and MGcSE-1 groups). The AUC was 0.74, and there was a significant difference (\( P=0.0001 \)). (B) ROC curve with the highest value for the separate four-point TAMs (nMGc and MGcSE-1 groups). The AUC was 0.81, and there was a significant difference (\( P=0.0001 \)).

AUC = area under the curve; MGc = metachronous gastric cancer; ROC = receiver operating characteristic; TAM = tumor-adjacent noncancerous mucosa; nMGc = EGc with no confirmed evidence of metachronous lesions >3 years after curative ESD; MGcSE = MGc developing on scars after curative ESD; MGcSE-1 = EGc at the first ESD; EGc = early gastric cancer; ESD = endoscopic submucosal dissection.
highest value for the TAM was equal to or greater than the cut-off value (43.4) among the 11 patients in the MGCSE-1 group. The values significantly differed between the MGCSE-1 and nMGC groups (P<0.0001) (Supplementary Table 5). In three cases (27.2%), the highest value for the TAM was equal to or greater than the cut-off value (43.4) among the 11 patients in the MGCSE-2 group.

**DISCUSSION**

This is the first study to report on the molecular alterations associated with MGC using specimens of MGC developing on scars after curative resection by ESD. MGCSE was detected in only 0.5% of all ESD cases. The median duration for the development of new atypical EGC on scars after ESD was 22 months (range, 14–49 months). The MGCSE-1 group was compared to the nMGC group, and no clear clinicopathological differences were identified them (Table 1). This result suggests that MGCSE might have formed because of molecular changes in the background atrophic mucosa. These changes may play a critical role in carcinogenesis among epigenetic factors. This is also the first report of the clinicopathological features of MGCSE.

The carcinogenic process in the gastric mucosa with atrophy is mainly attributable to epigenetic field abnormalities [10]. Atrophy related to old age and *H. pylori* infection is caused by irritation due to chronic inflammation [10,27]. Chronic inflammation leads to DNA and epigenetic abnormalities in the gastric mucosa [11,28]. Particularly, epigenetic alterations involved in gastric carcinogenesis are considered to contribute to a two-fold higher risk than that associated with esophageal squamous cell carcinoma [9], which may be due to so-called “field cancerization,” in which epigenetic changes responsible for altering gene expression have already occurred in the background gastric mucosa [12]. Although the histological types of the MGCSE-1 and MGCSE-2 groups were almost equivalent in our study, the ESD results of all MGCSE groups showed that the lateral and horizontal margins were negative, and all cases achieved curative resection (R0 resection). Therefore, we suggest that MGCSE-1 was pathologically confirmed after curative resection by the first ESD and that MGCSE-2 was not caused by residual cancer. However, considering the presence of newly developed cancers in the same area, it is strongly suggested that the TAM in the MGCSE-1 group already harbored cancer-like changes. Furthermore, by measuring TAMs at four points rather than one, it is possible to confirm whether epigenetic changes have occurred in the entire TAM.

We focused on *CDO1*, a hypermethylated gene with particularly high specificity in human cancers because *CDO1* is the most promising candidate gene for evaluating cancer-specific epigenetic changes. *CDO1* is an enzyme that converts cysteine to cysteine sulfenic acid in cells, leading to an increase in SO\(^2\) mediated by aspartate aminotransferase (GOT1) and replenishing cystine (CYS2) in the extracellular compartment [29]. xCT mediates the transport of cystine from the extracellular space to intracellular compartments, facilitating cancer stemness [29]. Its overexpression is accompanied by the production of nuclear factor erythroid 2-related factor 2 (NRF2), a central redox sensor, and results in the generation of reactive oxygen species, thereby promoting apoptosis [30].

Among the cancer-specific methylation genes in gastric cancer, *CDO1* has the highest AUC (0.95) for distinguishing cancerous from noncancerous tissues [14]. This finding suggests that *CDO1* methylation in cancer cells may be strongly associated with carcinogenesis. This study revealed that the TAM tissues in the nMGC group were hypermethylated, although...
not as much as the T tissue (Fig. 4A). Our previous study on CDO1 methylation in PDAC showed that 90% of cancerous tissues were methylated, whereas non-CP tissues exhibited no methylation [26]. In addition, in remnant gastric cancer, CDO1 was shown to be more highly methylated in cancerous tissue than in the noncancerous mucosa far from the tumor; however, its methylation level was detectable in the noncancerous mucosa [31]. Furthermore, CDO1 methylation has been reported to be significantly higher in precancerous lesions of other cancers, such as small bowel cancer, colorectal cancer, and intraductal papillary mucinous neoplasm, than in noncancerous tissues [20,21,32]. This study demonstrated for the first time that CDO1 hypermethylation in the TAM was more frequently detected in the MGCSE-1 group than in the nMGC group. In addition, the CDO1 methylation values for the TAM in the MGCSE-1 group were significantly higher than that in the MGCSE-2 group (Fig. 5B). Moreover, there was no significant difference in the CDO1 methylation values for the TAM between the MGCSE-2 and nMGC groups (Fig. 5B). Since there were no confirmed cases of a new MGCSE after the second ESD in the MGCSE-2 group, we propose that CDO1 hypermethylation is involved in carcinogenesis. Moreover, using the optimal CDO1 methylation value (43.4), we identified 9 out of 11 cases in the MGCSE-1 group. Maeda et al. reported an association between the development of MGC and hypermethylation of three genes (miR-124a-3, EMX1, and NKX6-1) in the gastric mucosa [33]. In this study, although the target genes were different, the TAM of the MGCSE group exhibited CDO1 hypermethylation, indicating a high risk of carcinogenesis even after curative ESD.

Furthermore, MGCSE groups are considered useful as models for investigating the carcinogenesis of gastric cancer. The results of this study are essential, as the results in the MGCSE groups reflect epigenetic abnormalities that had already occurred. In predicting MGC, Asada et al. [34] examined gene methylation at a fixed point in the antrum (the lesser curvature, 2 cm from the pyloric ring) and reported a 2.3-fold increased risk of MGC. However, identifying the site of development of gastric cancer is difficult and cannot be accurately determined by prior biopsy. In contrast, in the MGCSE groups, MGC developed in the same location (MGCSE-2) as that of the first ESD, and the resection was curative (MGCSE-1). Furthermore, in the MGCSE-2 group, there was no development of MGCSE after the second ESD. Therefore, measuring CDO1 methylation of the T and TAM in nMGC, MGCSE-1, and MGCSE-2 specimens may predict the occurrence of new cancers in the same location in which the first ESD was performed for EGC. Therefore, MGCSE may be an important model for predicting the development of EGC.

Our study has several important limitations. First, DNA methylation does not reflect a change that occurs in only a single gene; there is a strong association between methylated genes [35]. We have recently reported that when combined with HOPX/Reprimo/CDH1, CDO1 methylation can predict future occurrences of remnant gastric cancer [31]. It has been shown that the analysis of methylation of only a single gene, CDO1, may predict MGC; however, other methylated genes should be considered in combination with CDO1 for the prediction of MGC and in clinical applications. Second, we did not examine the case of MGC that did not develop scars after ESD in this study. We suggest that CDO1 hypermethylation may be useful in predicting MGC compared to nMGC and MGCSE groups. However, since MGCSE is relatively rare, a comparison with MGC not developing on scars after ESD should be performed in the future to improve the accuracy of predicting MGC in CDO1 hypermethylation. Furthermore, in this study, using the nMGC group as a control, the optimal methylation value of CDO1 (43.4) was used to identify nine of the 11 cases in the MGCSE-1 group. However, among the nMGC group, we found seven cases in which the highest value for the TAM exceeded the
cut-off value (43.4). These cases need to be closely monitored because of the possibility of developing metachronous lesions.

In conclusion, MGCSE specimens can be used to evaluate CDO1 DNA methylation. The results showed that CDO1 hypermethylation of the TAM in the MGCSE-1 group was comparable to that of T. Thus, CDO1 promoter DNA methylation was implicated in the occurrence of MGC and may be an important biomarker for predicting MGC.

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SUPPLEMENTARY MATERIALS

Supplementary Table 1
Histological type in MGCSE groups (MGCSE-1 and MGCSE-2 groups)

Click here to view

Supplementary Table 2
CDO1 TaqMeth values in the nMGC group

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Supplementary Table 3
CDO1 TaqMeth values in the MGCSE-1 group

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Supplementary Table 4
CDO1 TaqMeth values in the MGCSE-2 group

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Supplementary Table 5
Comparison of the highest value TAM and the cut-off value (43.4) in the nMGC and MGCSE-1 groups

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