CD44 variant 9 expression in primary early gastric cancer as a predictive marker for recurrence

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Background: Multiple early gastric cancers (EGCs) may develop in 6–14% of patients even after achieving curative endoscopic submucosal dissection (ESD); however, a useful biomarker for predicting recurrence is not available. The present study investigated whether the expression of CD44 variant 9 (CD44v9), a functional cancer stem cell marker, in the primary gastric cancer tissue represents an indicator of recurrence.

Methods: Eighty-eight patients who underwent ESD for EGC from 2008 to 2010 were enrolled and monitored for recurrence for 3 years. The expression levels of CD44v9 in the tissue of initial EGCs were evaluated by immunohistochemistry, and the recurrence rate was compared between CD44v9-positive and CD44v9-negative groups. The mucin phenotype and expression of miRNA-21 (miR-21) and programmed cell death protein 4 (PDCD4) were also analysed.

Results: The recurrence rate of EGC was significantly higher in the CD44v9-positive group than in the CD44v9-negative group (hazard ratio (HR), 21.8; 95% confidence interval (CI), 5.71–83.1). However, mucin phenotypes and the expression of miR-21 and PDCD4 did not predict recurrence after ESD. Meanwhile, grade of gastric atrophy was also identified as a significant marker of multiple recurrence (HR, 4.95; 95% CI, 1.30–18.8).

Conclusion: CD44 variant 9 expression represents a potential predictive marker for recurrence in EGC.
enhanced protection against reactive oxygen species (ROS; Diehn et al, 2009), suggesting that a link exists between CSCs and tumour recurrence and metastasis. CD44, a major adhesion molecule for the extracellular matrix, is implicated in a wide variety of physiological processes, including tumour cell invasion and metastasis (Nagano et al, 2004). Moreover, CD44 has been identified as one of the cell surface markers associated with cancer-stem-like cells in various solid tumours (Collins et al, 2005; Dalerba et al, 2007). We recently reported that CD44 variant 9 (CD44v9) interacts with and stabilises xCT, a glutamate-cystine transporter, resulting in increased intracellular levels of reduced glutathione (GSH). CD44v9-positive cells demonstrate an enhanced ability to suppress the production of ROS, resulting in subsequent therapeutic resistance, recurrence, and metastasis of tumours (Ishimoto et al, 2011; Tsugawa et al, 2012; Yae et al, 2012). These findings suggest that CD44v9 has a specific function in the regulation of ROS defence and tumour growth. Therefore, if the primary EGC tissue is CD44v9 positive with the potential of maintaining such an oxidative stress defense mechanism, multifocal recurrence may be more probable, even after curative resection is achieved by ESD.

A previous report showed that mucin phenotype subclassification is a predictive recurrence marker after surgical resection for advanced gastric cancer, defined as gastric cancer in which the depth of invasion extends beyond the submucosa. Each tumour was phenotypically classified as an intestinal phenotype, a mixed phenotype, or a gastric phenotype on the basis of the sum of their mucin phenotype immunohistochemistry (IHC) scores for gastric markers (MUC5AC and MUC6) and intestinal markers (MUC2 and CD10; Tsukashita et al, 2001). The number of tumours classified as gastric phenotype was significantly higher in tumours with peritoneal recurrence than in tumours without recurrence (Tajima et al, 2004). On the other hand, microRNAs (miRNAs) are small non-coding RNAs that can function as endogenous silencers of target genes and have critical roles in human malignancies (Saito et al, 2006; Saito et al, 2009). MicroRNA-21 (miR-21) is an oncogenic miRNA that is overexpressed in various human malignancies (Chan et al, 2005; Seike et al, 2009) and predominantly targets tumour-suppressor genes such as programmed cell death protein 4 (PDCD4; Fassan et al, 2011). A previous report showed that miR-21 was significantly overexpressed in human gastric cancer tissues and cell lines (Zhang et al, 2008). In addition, the expression level of miR-21 was associated with progression-related factors, such as depth of tumour invasion (Ueda et al, 2010). In addition, PDCD4 is strongly expressed in the nuclei of normal gastric mucosal cells, whereas it is expressed at lower levels in the gastric adenocarcinoma (Kakimoto et al, 2011). Low PDCD4 expression was significantly correlated with poor clinical prognosis (Motoyama et al, 2010). These findings suggest that miR-21 and PDCD4 expression are biomarkers for gastric cancer recurrence (Wu et al, 2010).

The aim of this prospective study was to identify a new biomarker for predicting multiple recurrence of EGC after ESD.

### MATERIALS AND METHODS

Additional details are included in the Supplementary Materials.

**Patients and specimens.** This prospective study included patients who underwent ESD for EGC at the Keio University Hospital (Tokyo, Japan) between February 2008 and March 2010. Early gastric cancer was defined as an adenocarcinoma confined to the mucosa or submucosa of the stomach. Patients who had a history of ESD and in whom multiple ESDs were performed were excluded. Tissue samples for RNA extraction were obtained by endoscopic forceps biopsy. Following forceps biopsy, ESD was performed and resected specimens were obtained for IHC.

The follow-up period was defined as the period from the time of initial ESD to the time of the last osophasagogastroduodenoscopy (EGD). Follow-up EGDs were performed at ~2, 6, 12, 18, 24, and 36 months after initial ESD (median follow-up period 32 months).

Information on the clinical features of the patients such as gender, age, body mass index (BMI), H. pylori infection status at the time of initial ESD, smoking history, tumour location, tumour differentiation, and grade of gastric atrophy were obtained from medical records. H. pylori infection status was determined using the 13C-urea breath test, serological examination, and/or microaerobic bacterial cultivation. The grade of gastric atrophy was evaluated according to the endoscopic-atrophy-border scale described by Kimura and Takemoto (1969), which correlates with the results of histological evaluation (Ito et al, 1996; Satoh et al, 1996). On the basis of this scale, the grade of atrophy was divided into two types: closed-type/mildly extended atrophy and open-type/severely extended atrophy (Kimura and Takemoto, 1969; Ito et al, 1996; Satoh et al, 1996; Suzuki et al, 2004).

The study protocol was approved by the ethics committees of Keio University School of Medicine (Number 19-68-5), and written informed consent was obtained before subject enrolment. The UMIN Clinical Trials Registry number for this study is UMIN000010577 (http://www.umin.ac.jp/ctr/). The study was performed in accordance with the principles of the declaration of Helsinki.

**Immunohistochemistry.** Following deparaffination and rehydration, antibodies for anti-CD44v9 and anti-phospho-p38MAPK antibodies were retrieved by heating samples in citrate buffer (10 mM, pH 6.0) for 10 min at 105 °C. Antigens for anti-MUC2, anti-MUC5AC, and anti-MUC6 antibodies were retrieved by heating samples in citrate buffer (10 mM, pH 6.0) for 5 min at 121 °C. Antigens for anti-CD10 antibodies were retrieved by heating samples in citrate buffer (10 mM, pH 9.0) for 5 min at 121 °C. Antigens for anti-PDCD4 antibodies were retrieved by heating samples in citrate buffer (10 mM, pH 9.0) for 10 min at 121 °C. After antigen retrieval, endogenous nonspecific peroxidases were quenched with 0.3% hydrogen peroxide for 5 min. Nonspecific binding was blocked using Protein block Serum-free (DAKO Japan, Kyoto, Japan), and each section was incubated overnight at 4 °C with the primary antibodies. CD44 variant 9 was detected with a rat monoclonal antibody (1:200; Ishimoto et al, 2011). Phospho-p38MAPK was detected using a rabbit monoclonal antibody (Number 9211, Cell Signaling Technology, Beverly, MA, USA; 1:100). Monoclonal mouse antibodies were used to detect MUC2 (NCL-MUC-2, Novocastra Laboratories, Newcastle-upon-Tyne, UK; 1:100), MUC5AC (NCL-MUC-5AC, Novocastra Laboratories; 1:50), MUC6 (NCL-MUC-6, Novocastra Laboratories; 1:50), and CD10 (NCL-CD10-270, Novocastra Laboratories; 1:50). Programmed cell death protein 4 was detected using a rabbit monoclonal antibody (600-401-965, Rockland, Gilbertsville, PA, USA; 1:100). Primary antibody binding was detected using a Vectastain Elite Kit (Vector Laboratories, Burlingame, CA, USA) and 3,3′-diaminobenzidine tetrahydrochloride solution (Dako Japan). Counterstaining was performed using Gill’s haematoxylin (Dako Japan). The CD44v9 IHC score was defined as the proportion of the tumour area that showed CD44v9-positive staining. The CD44v9 IHC score was calculated by using ImageJ software (US National Institutes of Health, Bethesda, MD, USA) and setting the threshold (0–120) after the red-green-blue stack. The PDCD4 IHC score was defined as the average of the percentage of positive nuclei in each of three high-power fields of areas containing tumour cells.

**Statistical analysis.** Clinical factors were compared between the recurrence group and the non-recurrence group using the Student’s t-test for continuous variables (i.e., age, BMI, serum CEA, serum CA19-9, and follow-up period) and the χ2-test for categorical variables. Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) software (version 21; IBM, Chicago, IL, USA). Differences with a P-value < 0.05 were considered statistically significant.
discrete variables (i.e., gender, H. pylori infection status, smoking history, tumour location, tumour differentiation, and the type of gastric atrophy). The optimal cut-off value for CD44v9 IHC scores, miR-21 expression levels, and PDCD4 IHC scores were determined using the receiver operating characteristic curve. Clinical factors were compared between the CD44v9-positive and CD44v9-negative groups, miR-21-higher and miR-21-lower groups, and PDCD4-higher and PDCD4-lower groups using the Student’s t-test (continuous variables) or the χ²-test (discrete variables). The differences in clinical factors among mucin phenotype subclassifications were analysed using one-way analysis of variance (ANOVA) for continuous variables and the χ²-test for discrete variables. The Student’s t-test, χ²-test, and one-way ANOVA were applied after normality had been established using the Shapiro–Wilk W-test. The associations of CD44v9 and the type of gastric atrophy with miR-21 and PDCD4 were evaluated using a linear regression model. Recurrence-free curves were calculated using the Kaplan–Meier method and were compared using the log rank test. Univariate and multivariate Cox proportional hazard model analyses were performed to calculate hazard ratios (HRs). Statistical significance was defined as a P-value of <0.05. The data are expressed as mean ± s.d. All statistical analyses were performed using PASW Statistics 18 (SPSS Inc., Chicago, IL, USA).

RESULTS

Patient characteristics. A total of 88 patients were enrolled (Figure 1). Samples from 10 patients who had a history of ESD and from 4 patients in whom multiple ESDs were performed were excluded. Among the 74 initial simple EGC patients, 3 who had never undergone endoscopic examination after initial ESD, 5 who underwent gastrectomy after ESD because of a positive margin, and 1 who was treated with chemotherapy for oesophageal cancer that developed after ESD were also excluded. Thirteen of the 65 remaining patients developed multiple recurrences during the follow-up period, whereas recurrence was not identified in 52 patients. Clinically relevant background factors in the non-recurrence and recurrence group are shown in Table 1. A significant difference in the grade of gastric atrophy was identified between these groups (P = 0.046).

Expression of CD44v9 in EGCs. The CD44v9 IHC scores of all 65 EGCs are illustrated in Figure 2. The average CD44v9 IHC score was 3.58 ± 7.74%. Interestingly, the case that showed an extraordinarily high score (60.3%) was the only case that showed recurrence twice during the follow-up period (22 and 40 months after the initial ESD). Subsequently, patients were allocated into the CD44v9-positive group (n = 13) or the CD44v9-negative group (n = 52).

![Figure 1. Study design](image-url)

Table 1. Patients’ characteristics

| Non-recurrence (n = 52) | Recurrence (n = 13) | P-value |
|------------------------|---------------------|---------|
| Median follow-up period | 32 months          | 36 months |         |
| Gender, number          |                     |         |
| Men (%)                 | 46 (88.4)           | 10 (76.9) | 0.28   |
| Women (%)               | 6 (11.5)            | 3 (23.1)  |         |
| Age, years              |                     |         |
| Mean ± s.d. (range)     | 66.7 ± 9.8 (46–90)  | 71.1 ± 8.3 (53–80) | 0.096 |
| BMI (kg m⁻²), %         |                     |         |
| mean ± SD (range)       | 23.0 ± 2.9 (18.6–30.8) | 24.0 ± 4.2 (18.4–33.5) | 0.45 |
| H. pylori, number       |                     |         |
| Positive (%)            | 31 (59.6)           | 8 (61.5)  | 0.83   |
| Negative                | 5 (9.6)             | 2 (15.4)  |         |
| Non-eradication history | 14 (26.9)           | 3 (23.1)  |         |
| Post-eradication history| 2 (3.8)             | 0 (0.0)   |         |
| Smoking history, number |                     |         |
| Smoker (%)              | 11 (21.2)           | 3 (23.1)  | 0.62   |
| Ex-smoker (%)           | 12 (23.1)           | 3 (23.1)  |         |
| Non-smoker (%)          | 17 (32.7)           | 6 (46.2)  |         |
| Unknown (%)             | 12 (23.1)           | 1 (7.7)   |         |
| Location, number        |                     |         |
| Upper (%)               | 1 (1.9)             | 0 (0.0)   | 0.88   |
| Middle (%)              | 31 (59.6)           | 8 (61.5)  |         |
| Lower (%)               | 20 (38.5)           | 5 (38.5)  |         |
| Differentiation, number |                     |         |
| tub1 (%)                | 46 (88.5)           | 11 (76.9) | 0.71   |
| tub2 (%)                | 6 (11.5)            | 2 (15.4)  |         |
| Atrophy, number         |                     |         |
| Closed-type (%)         | 32 (61.5)           | 4 (30.8)  | 0.046  |
| Open-type (%)           | 20 (38.5)           | 9 (69.2)  |         |

Abbreviation: BMI = body mass index. Bold value indicates a significant difference.
based on a cut-off level of 3.55% (sensitivity, 76.9%; specificity, 93.8%, Supplementary Table 1).

Representative CD44v9 immunohistochemical staining is shown in Figure 3. Although CD44v9 staining was barely detectable in the CD44v9-negative group (Figure 3A), CD44v9 was expressed in the epithelium of tumour glands with a heterogeneous expression pattern in the CD44v9-positive group (Figure 3B). In the high-power field, CD44v9 expression was identified along the cell membrane (Figure 3C). Significant correlations were not identified between CD44v9 positivity and any of the clinical background factors (Supplementary Table 2).

We examined the activation of p38MAPK, a major target of ROS, in cancer tissues to assess the role of CD44v9 in the regulation of intracellular oxidative stress. Immunostains for CD44v9 and phospho-p38MAPK, the active form of p38 MAPK, are shown in Supplementary Figures 1A and B, respectively. Phospho-p38MAPK staining was barely detectable in the CD44v9-positive area but was apparent in the CD44v9-negative area. The immunostaining patterns of CD44v9 and phospho-p38MAPK were inversely correlated in the tumourous glands of EGC, indicating that CD44v9 expression reduces oxidative stress in cancer cells.

Expression of epithelial mucins in EGCs. All 65 EGCs were classified into three mucin phenotypes based on immunohistochemical staining against CD10, MUC2, MUC5AC, and MUC6 (Supplementary Figure 2): the gastric phenotype, 17 (26.2%) patients; the mixed phenotype, 23 (35.4%) patients; and the intestinal phenotype, 25 (38.5%) patients. A significant association between mucin phenotypes and EGC location (P = 0.022) was identified (Supplementary Table 2).

Expression of miR-21 and PDCD4 in EGCs. The average miR-21 expression and PDCD4 IHC scores were 5.70% ± 5.45% and 20.2% ± 11.4%, respectively. Sufficient quantities of RNA could not be obtained for four samples, and these were excluded from the miR-21 evaluation. Patients were allocated into the miR-21-lower group (n = 27) or the miR-21-higher group (n = 34) based on a cut-off level of 3.58 (sensitivity, 53.8%; specificity, 43.8%), and the PDCD4-lower group (n = 27) and the PDCD4-higher group (n = 38) based on a cut-off level of 17.7% (sensitivity, 53.8%; specificity, 43.8%; Supplementary Table 1). Significant correlations were not identified between the expression of miR-21 and clinical factors, but age was significantly associated with PDCD4 expression (Supplementary Table 2). In addition, significant associations were not identified between CD44v9 expression or gastric atrophy grade and miR-21 or PDCD4 (CD44v9-miR-21: R = 0.162, P = 0.236; CD44v9-PDCD4: R = 0.035, P = 0.791; atrophy-miR-21: R = 0.136, P = 0.297; atrophy-PDCD4: R = 0.103, P = 0.413).

CD44v9 expression is a potential marker for the development of multiple cancers. Kaplan–Meier curves for the development of EGC after ESD are shown in Figure 4. During the follow-up period, the development of EGC after ESD was identified in 10 cases (76.9%) in the CD44v9-positive group and in 3 cases (5.8%) in the
CD44v9-negative group. When the recurrence rate was analysed using the log rank test, the recurrence rate was significantly higher in the CD44v9-positive group than in the CD44v9-negative group ($P < 0.001$, Figure 4A). On the other hand, the recurrence rate was not significantly different when patients were divided based on the subclassification of tumours into mucin phenotypes ($P = 0.94$, Figure 4B), miR-21 expression ($P = 0.71$, Figure 4C), or PDCD4 expression ($P = 0.67$, Figure 4D). Moreover, the recurrence rate was significantly higher for open-type gastric atrophy than that for closed-type gastric atrophy ($P = 0.013$, Figure 4E). The HRs are shown in Table 2. CD44 variant 9 positivity significantly correlated with multiple recurrences on using the univariate Cox proportional hazards model ($P < 0.001$; HR, 19.7; 95% confidence interval (CI), 5.37–72.4) and the multivariate Cox proportional hazards model ($P < 0.001$; HR, 21.8; 95% CI, 5.71–83.1). The type of gastric atrophy also significantly correlated with multiple recurrences using the univariate Cox proportional hazards model ($P = 0.022$; HR, 4.17; 95% CI, 1.23–14.1) and the multivariate Cox proportional hazards model ($P = 0.019$; HR, 4.95; 95% CI, 1.30–18.8). However, no significant correlations were identified with regard to mucin phenotypes, miR-21 expression, or PDCD4 expression using the univariate Cox proportional hazard model.

**DISCUSSION**

This study represents the first report that CD44v9 expression, a marker characteristic of cancer stem-like cells, in primary EGCs can clearly predict multiple recurrence of EGC after ESD. Evaluation of CD44v9 expression represents a clinically ideal system, because it can be performed non-invasively on resected specimens when monitoring the clinical course after endoscopic treatment for gastric cancer. The HR for multiple recurrences was 21.8 in patients with CD44v9-positive tumours in our study, suggesting that the follow-up after ESD should be performed more frequently in patients with CD44v9-positive tumours than in patients with CD44v9-negative tumours. The present results support prophylactic CD44v9 monitoring following ESD that would ultimately lead to reduced medical expenditure and mortality associated with EGC recurrence.

Previously, we showed that CD44v9 expression maintains a high level of GSH, which resulted in the suppression of ROS-p38MAPK signaling and subsequent resistance to intracellular ROS in gastric cancer (Ishimoto *et al.*, 2011). In the present study, as well as in our previous study, a reduced level of phospho-p38MAPK was identified...
In CD44v9-positive cells in EGC. Moreover, we have shown that CD44v9 expression was associated with the lung metastasis in the mouse breast cancer cells (Yae et al., 2012). Furthermore, Chen et al. (2013) recently reported the existence of CD44+ cells within the tumours of gastric cancer patients that are endowed with stem cells properties (Chen et al., 2013). These findings suggest that CD44v9-positive cells have CSC-like potential, and tumours with high CD44v9 levels have an increased risk of multiple recurrences. On the other hand, CD44 high CD44v9 levels have an increased risk of multiple recurrences. It is also possible that CD44v9-positive cells may not necessarily represent true CSCs.

However, the CD44v9 cell surface marker has the potential to identify CSCs in primary gastric cancer, whereas the general CD44 cell surface marker does not always identify gastric CSCs. It was completely resected in all patients included in the study, and we excluded patients whose endoscopic resected specimens had positive margins (cases of incomplete resection). According to the criteria used in the studies by Nakajima et al. (2006) and Nasu et al. (2005), nine patients developed synchronous recurrence and four patients developed metachronous cancer in the present study. The development of metachronous multiple cancers was identified in 1 of 45 cases (2.2%) in the CD44v9-negative group and in 3 of 6 cases (50.0%) in the CD44v9-positive group (P<0.001; Supplementary Figure 3).

In a previous report, the severity of gastric atrophy was shown to be a predictive factor for multiple gastric cancer (Shiotani et al., 2008; Hanaoka et al., 2010). The present study showed that the recurrence rate was higher in CD44v9-positive tumours than in CD44v9-negative tumours, even when applying the multivariate Cox proportional hazard model adjusted for the type of gastric atrophy. Therefore, CD44v9 expression is an independent predictive factor, irrespective of the severity of gastric atrophy. In addition, the recurrence rate was significantly higher for samples with open-type gastric atrophy than for samples with closed-type gastric atrophy, and a significant difference was identified in the multivariate Cox proportional hazard model adjusted for CD44v9 positivity. The HR for CD44v9 positivity was greater than that for the type of gastric atrophy, suggesting that CD44v9 expression may represent a more powerful predictive recurrence marker than the type of gastric atrophy.

Although H. pylori infection clearly delineated EGC development when H. pylori-naive-negative and H. pylori-positive patients were compared (Hanaoka et al., 2010), H. pylori eradication only reduced the risk of EGC recurrence to one-third (Fukase et al., 2008). In the present study, CD44v9 positivity could predict EGC recurrence in a smaller population than was required for H. pylori infection status to predict recurrence. With regard to the infection status of H. pylori at the initial ESD in all of the present cases (Supplementary Figure 4), 32 of 52 patients in the CD44v9-negative group and 7 of 13 patients in the CD44v9-positive group were H. pylori positive (Supplementary Table 2). After ESD, the success of H. pylori eradication therapy was confirmed in 21 of 32 (65.6%) patients in the CD44v9-negative group (5.6 ± 6.1 months after ESD) and in all 7 patients (100%) in the CD44v9-positive group (6.1 ± 3.0 months after ESD). Therefore, H. pylori positivity as a confounding factor could only have affected the CD44v9-negative group, suggesting that the present results were reliable even after accounting for the H. pylori-associated effects. Thus, CD44v9 positivity can predict the development of multiple EGC even after accounting for the presence or absence of H. pylori infection status.

Although the incidence of gastric mucin-producing tumours was significantly higher in cases of tumours with recurrence than in cases of tumours without recurrence of advanced gastric cancer (Tajima et al., 2004), a correlation between the subclassification of mucin phenotypes and the intragastric recurrence rate was not found in the present study. Furthermore, $miR$-21 and PDCD4 expression did not predict the risk of developing multiple EGCs.

Table 2. HRs using univariate and multivariate Cox proportional hazard model

| Factor          | Class               | Univariate analysis | Multivariate analysis |
|-----------------|---------------------|---------------------|-----------------------|
|                 |                     | HR                  | 95% CI                | P-value | HR                  | 95% CI                | P-value |
| CD44v9          | Positive            | 19.7                | 5.37–72.4             | <0.001  | 21.8                | 5.71–83.1             | <0.001  |
|                 | Negative            | Reference           | Reference             |         |                     | Reference             |         |
| Mucin phenotype | Gastric phenotype   | 0.87                | 0.21–3.64             | 0.85    |                     | Reference             |         |
|                 | Mixed phenotype     | 1.09                | 0.32–3.76             | 0.89    |                     | Reference             |         |
|                 | Intestinal phenotype| Reference           | Reference             |         |                     | Reference             |         |
| mR-21           | Higher              | 0.82                | 0.27–2.43             | 0.72    |                     | Reference             |         |
|                 | Lower               | Reference           | Reference             |         |                     | Reference             |         |
| PDCD4           | Higher              | 0.79                | 0.27–2.35             | 0.67    |                     | Reference             |         |
|                 | Lower               | Reference           | Reference             |         |                     | Reference             |         |
| Type of gastric atrophy |                |                     |                       |         |                     |                       |         |
|                 | Open-type           | 4.17                | 1.23–14.1             | 0.022   | 4.95                | 1.30–18.8             | 0.019   |
|                 | Closed-type         | Reference           | Reference             |         |                     | Reference             |         |

Abbreviations: CD44v9 – CD44 variant 9; CI – confidence interval; HR – hazard ratio; $miR$-21 – microRNA-21; PDCD4 – programmed cell death protein 4. Bold values indicate significant differences.
A limitation of this study is that the cut-off values for CD44v9 and the other biomarkers were determined on the basis of their optimal performance in this specific population of 65 patients. Risk prediction tools are known to suffer from optimism bias, and their performance in new populations does not always match the success of that in the derivation population. Therefore, prospective validation of these biomarkers using separate patient populations is required.

In conclusion, the data presented in our study suggest that the recurrence rate of EGC following ESD is higher in cases of CD44v9-positive tumours than in cases of CD44v9-negative tumours. CD44 variant 9 expression in primary EGC represents a useful biomarker for predicting patients’ potential risk of developing multiple EGCs after curative resection with ESD.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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