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Intestinal metaplasia in Barrett's oesophagus may be an epiphenomenon rather than a pre-neoplastic condition, and CDX2-positive cardiac-type epithelium is associated with minute Barrett's tumour

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Intestinal metaplasia in Barrett's oesophagus may be an epiphenomenon rather than a pre-neoplastic condition, and CDX2-positive cardiac-type epithelium is associated with minute Barrett's tumour

Aims: Although intestinal-type epithelium in Barrett's oesophagus has been traditionally recognised as having a distinct malignant potential, whether this also holds true for cardiac-type epithelium remains controversial. The aim of this study was to identify highly associated type of epithelium with Barrett's tumour.

Methods and results: We analysed tumours and the corresponding background mucosa with special regard to tumour size in 40 cases of superficial Barrett's tumour by using immunohistochemical staining for CDX2, CD10, MUC2, MUC5AC, and MUC6. Intestinal metaplasia in tumour-adjacent mucosa was not associated with tumour size, but was significantly correlated with the extent of Barrett's oesophagus ($P < 0.001$). Majority (69.2%, 9/13) of small tumours (≤10 mm in size) had no intestinal metaplasia in adjacent non-neoplastic mucosae. Minute (≤5 mm) tumours were significantly associated with gastric immunophenotype ($P < 0.001$). Purely gastric-immunophenotype tumour cells expressed CDX2, and cardiac-type epithelium adjacent to small tumours also displayed low-level CDX2 expression.

Conclusions: Our data suggest that intestinal metaplasia in Barrett's oesophagus is an epiphenomenon rather than a pre-neoplastic condition and that CDX2-positive cardiac-type epithelium is highly associated with minute Barrett's tumour. Further prospective studies are needed to evaluate the risk of malignancy of cardiac-type epithelium with regard to sub-morphological intestinalisation.

Keywords: Barrett's oesophagus, cardiac-type epithelium, CDX2, intestinal metaplasia, tumour immunophenotype

Introduction

Barrett's oesophagus describes the condition in which metaplastic columnar epithelium replaces the stratified squamous epithelium that normally lines the distal oesophagus, thereby implying malignant potential.[1] However, the definition of Barrett's oesophagus varies among countries and even among researchers within

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the same country.[2–4] The non-conformity comes from whether or not intestinal metaplasia (defined by the presence of goblet cells) must be confirmed in columnar-lined oesophagus to establish the diagnosis of Barrett's oesophagus. Intestinal metaplasia found in Barrett's oesophagus has been traditionally recognised as the most common and distinctive epithelial type that predisposes patients to cancer development;[5–12] however, recent studies[13–17] have challenged this traditional concept. It remains controversial whether the cardiac-type epithelium (devoid of goblet cells) also has a distinct malignant potential.[1,18]

We can examine tumour histogenesis by investigating the background tissue or the tumour itself. It is reasonable to suppose that tumour-origin tissue is more preserved when the tumour is smaller, as it is more likely that tumour-origin tissue has not been completely overgrown by the tumour. In addition, the phenotypic expression of tumour cells is widely thought to resemble that of tumour-origin tissue,[19] especially in small tumours. As far as we know, no previous reports have precisely investigated the relationship between tumour size and background mucosal type or tumour phenotype by whole-section analysis, although several reports have considered background mucosa of Barrett's tumour[5,10,15,20–23] and the tumour phenotype.[23–26] In the present study, we examined both background mucosa and tumour phenotype with special regard to tumour size by whole-section analysis to consider the histogenesis of Barrett's tumour. Tumour phenotype was determined by immunohistochemical staining with MUC5AC, MUC6, MUC2, and CD10.

CDX2 is a caudal-related homeobox transcription factor that plays critical roles in regulating intestinal epithelial development, maintenance, and proliferation.[27] CDX2 expression in cardiac-type epithelium, which we refer to as sub-morphological intestinalisation, has been documented;[28–30] however, its malignant potential is uncertain. Thus, we also evaluated CDX2 expression in cardiac-type epithelium as well as in tumour cells with a gastric mucin phenotype to elucidate the histogenesis of Barrett's tumour.

Materials and methods

CLINICOPATHOLOGIC CHARACTERISTICS

We studied a retrospectively identified consecutive series of 40 specimens of superficial (mucosal or submucosal) oesophageal adenocarcinoma registered in the files of the Division of Molecular and Diagnostic Pathology, Niigata University Graduate School of Medical and Dental Sciences, from 1992 through 2013. Thirty-one tumours were resected endoscopically, and the remaining nine tumours were resected surgically at Niigata University General Hospital and its affiliated institutions in Niigata Prefecture, Japan. Prior patients' consent to the use of resected specimens for research purposes had been obtained, and the specimens were coded to protect patient confidentiality. The current study protocol was approved by the Ethics Committee of Niigata University School of Medicine (approval number 1892). All tumours were located predominantly in the oesophagus above the gastro-oesophageal junction (GOJ), defined endoscopi-

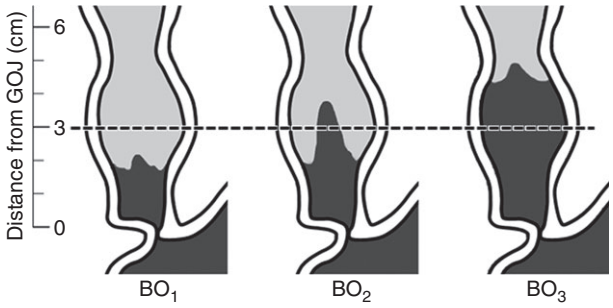

Figure 1. Classification of Barrett's oesophagus (BO) categorised by maximum and circumferential extent of columnar-lined mucosa. BO with maximum extent <3 cm was designated BO_1; BO with maximum extent 3 cm or more and circumferential extent <3 cm was designated BO_2; and BO with circumferential extent 3 cm or more was designated BO_3. GOJ, gastro-oesophageal junction.

Table 1. Tumour size and histologic features of 40 cases of superficial Barrett's tumour

	Endoscopically resected (n = 31)	Surgically resected (n = 9)	Total (n = 40)
Tumour size (mm)			
Mean ± SD	15.3 ± 11.6	20.0 ± 9.3	16.4 ± 11.2
Range	1–51	11–34	1–51
Histologic grade (dominant)			
Well differentiated	31	8	39
Moderately differentiated	0	1	1
Depth of tumour invasion			
M	24	2	26
SM1	2	5	7
SM2	5	2	7

SD, standard deviation; M, intramucosal; SM1, submucosal tumour invasion limited to 0.2 mm in vertical; SM2, submucosal tumour invasion more than 0.2 mm in vertical.

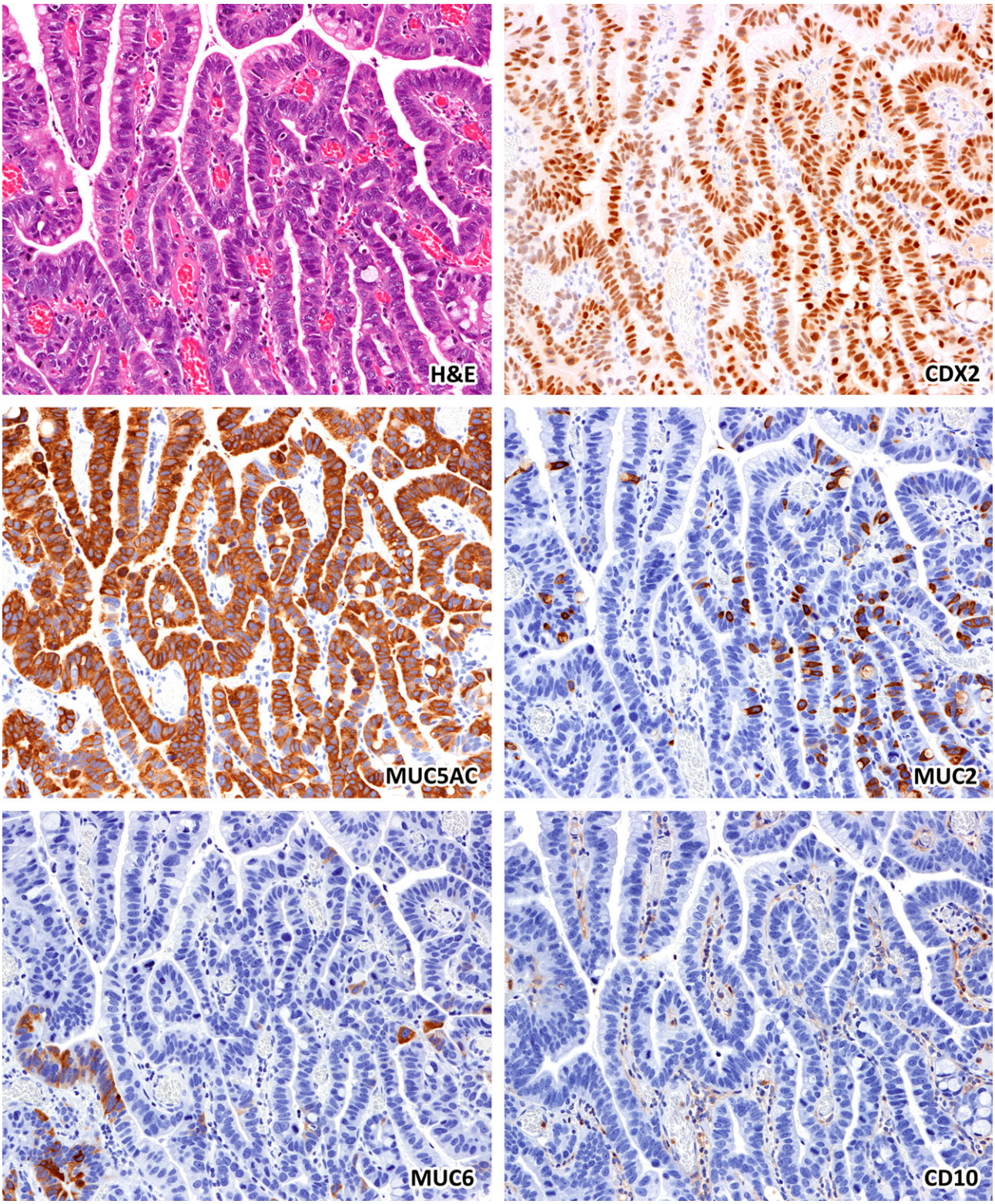

Figure 2. Intramucosal well-differentiated adenocarcinoma according to the Japanese classification, with a mixed immunophenotype (gastric predominant). The tumour cells are diffusely positive for MUC5AC and CDX2, but the intensity of CDX2 positive staining is heterogeneous. Some tumour cells are positive for MUC2 and MUC6. No tumour cells are positive for CD10.

cally as the distal end of the palisade vessels[31,32] or the proximal limit of the gastric mucosal folds.[33] All 31 endoscopically resected specimens, ranging in size from 2.3 to 10.0 cm, contained GOJ and squamocolumnar junction (SCJ). The 9 surgically resected specimens were obtained by distal esophagectomy with proximal or total gastrectomy, and all 9 contained SCJ. All patients were Japanese (38 men and 2 women; mean age, 70.4 years), and none had received adjuvant therapy.

CLASSIFICATION OF BARRETT'S OESOPHAGUS

The 40 cases were divided into three groups based on the extent of columnar-lined mucosa in the oesophagus (Figure 1). Cases in which the maximum extent was <3 cm were designated BO_1 (n = 25); cases with a maximum extent of 3 cm or more and a circumferential extent of <3 cm were designated BO_2 (n = 10); and cases with a circumferential extent of 3 cm or more were designated BO_3 (n = 5).

SPECIMEN PROCESSING AND HISTOLOGIC FEATURES OF TUMOURS

All specimens were serially sectioned parallel to the longitudinal axis of the oesophagus. In endoscopically resected specimens, whole tissues were embedded in paraffin blocks. In surgically resected specimens, whole oesophageal and GOJ tissues and distal-margin tissue were embedded in paraffin blocks. They were then routinely processed and stained with hematoxylin and eosin (H&E). Each specimen was assessed for the presence of oesophageal glands proper or their ducts and squamous islands, and the findings confirmed that the tissue was derived from the tubular oesophagus.[32] There was histologic evidence that the tumour was located within or predominantly within the oesophagus in 39 of 40 cases.

The histologic features of the 40 tumours were interpreted according to the Japanese Classification of Esophageal Cancer, tenth edition,[34] and summarised in Table 1. None of the tumours were ulcerated. Well-differentiated intramucosal adenocarcinoma (Figure 2) according to the Japanese classification would have been diagnosed as high-grade dysplasia in Western countries.[2,35]

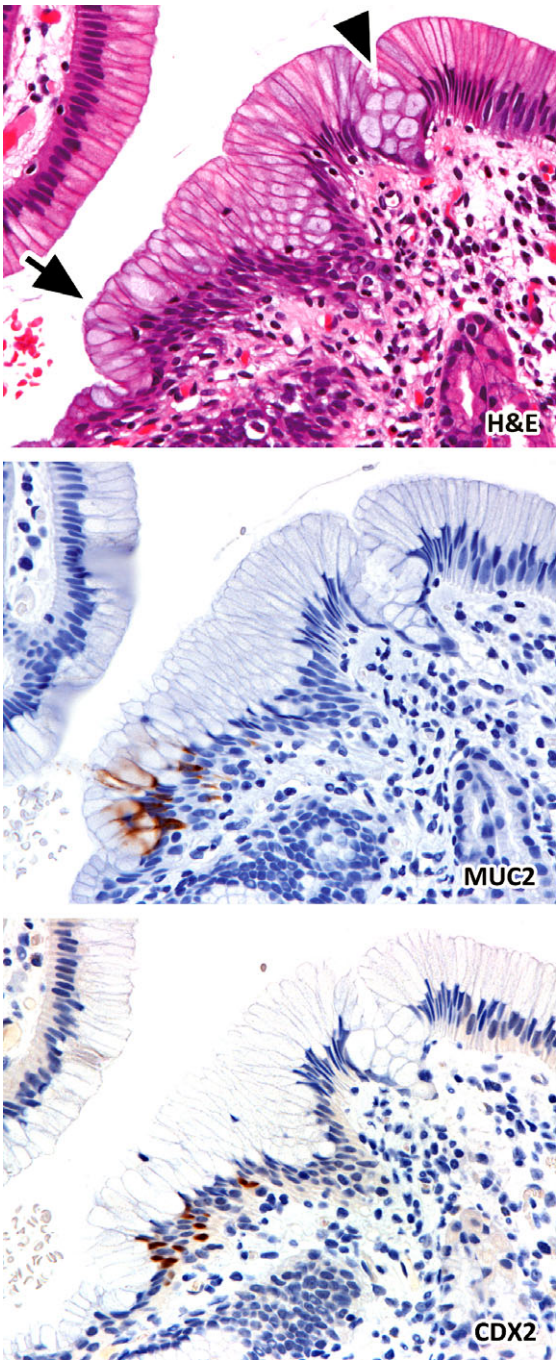

Figure 3. Goblet and pseudogoblet cells. In some cases, it is difficult to distinguish goblet from pseudogoblet cells. Pseudogoblet cells (arrowhead) are negative for both MUC2 and CDX2, whereas goblet cells (arrow) are positive for MUC2 and CDX2.

IMMUNOHISTOCHEMISTRY

The paraffin blocks containing each tumour were consecutively cut into 3-μm sections for H&E staining and immunohistochemical staining with CDX2 (clone AMT28, 1:00 dilution; Novocastra Laboratories Ltd., Newcastle, UK), CD10 (clone 56C6, 1:200; Novocastra), MUC2 (clone Ccp58, 1:300; Novocastra), MUC5AC (clone CLH2, 1:100, Novocastra), and

Table 2. Tumour-adjacent mucosal type of 206 tumour-containing sections from 40 cases of Barrett's tumour

	Sections (n = 206)		Cases (n = 40)			
			Dominant mucosa*		Mucosa adjacent to tumour end	
	Proximal side	Distal side	Proximal side	Distal side	Proximal end	Distal end
Squamous	150	9	31	0	32	0
Intestinal	43	42	9	9	8	10
Cardiac	7	155	0	31	0	30
NA	6	0	0	0	0	0

NA, not assessed (due to erosion).
*If both the cardiac-type and intestinal-type mucosae were adjacent to the tumour equally in number, the intestinal-type mucosa was regarded as the dominant type.

MUC6 (clone CLH5, 1:100; Novocastra). The immunohistochemical techniques were performed as previously described in detail.[36] Two independent pathologists (G.W. and Y.A.), who were blinded to the clinical data, assessed each section.

EVALUATION OF BACKGROUND MUCOSA

Mucosae immediately adjacent to each tumour on both the proximal and distal sides of all tumour-containing sections were evaluated as the background mucosae and classified into three types: (i) squamous mucosa; (ii) cardiac-type mucosa with or without parietal cells; and (iii) intestinal-type mucosa with goblet cells. Mucosa with atrophic fundic glands and no intestinal metaplasia was included in the cardiac-type mucosa group. Background mucosae were classified on the basis of the findings of H&E plus MUC2 staining to distinguish goblet cells from pseudogoblet cells[15,37] (Figure 3). In each case, the most frequently observed type of tumour-adjacent mucosa on both the proximal and distal sides was recorded as the dominant type. If both cardiac-type and intestinal-type mucosae were adjacent to the tumour equally in number, we regarded the intestinal-type mucosa as the dominant type.

The expression level of CDX2 was evaluated in background mucosa adjacent to the most proximal and distal ends of the tumour in a field of vision under a magnification of 200× (×20 objective lens). The extent of CDX2 expression was scored semiquantitatively (min. 0, max. 16) as previously described.[23,38]

EVALUATION OF TUMOUR IMMUNOPHENOTYPE

MUC5AC and MUC6 were defined as gastric phenotype markers, and MUC2 and CD10 were defined as intestinal phenotype markers. Immunoreactivity of at least 5% of the tumour cell population was considered positive, and each tumour was classified as gastric immunophenotype, intestinal immunophenotype, mixed immunophenotype, or null immunophenotype as previously described.[36] Mixed immunophenotype tumours were further characterised as gastric predominant (G > I) or intestinal predominant (I > G).

In addition to the phenotype assessed throughout the tumour, the extent of positive staining for the five markers (CDX2, CD10, MUC2, MUC5AC, and MUC6) at two small fields at the most proximal end and the most distal end of the tumour was independently evaluated in a field of vision under a magnification of 200× (×20 objective lens) in each case. The extent of positive staining for mucin core peptide and CD10 was scored as follows: 0, no positive cells (0%); 1, only a few positive cells (>0%, <5%); 2, some positive cells (5% to 30%); 3, well-defined areas of positive cells (30% to 60%); and 4, extensive areas of positive cells (>60%). The extent of CDX2 positive staining in the most proximal and distal ends of the tumour was scored in the same way as for background mucosa.

STATISTICAL ANALYSIS

The Fisher exact test was used to compare proportions. An adjusted residual was used to compare the significance of observation frequency among the individual cells. Paired variables were compared with the Wilcoxon signed-rank test. CDX2 scores were compared in three groups with the Kruskal-Wallis test, and comparisons between individual groups were performed by using the Mann–Whitney U test with Bonferroni correction. Logistic regression analysis was used to identify factors independently associated

Table 3. Association of tumour-adjacent mucosal type with different clinicopathologic parameters

		Dominant type of tumour-adjacent mucosa (Proximal/Distal)					Presence of IM in tumour-adjacent mucosae (Proximal/Distal)				
	n	Sq/CM	Sq/IM	IM/CM	IM/IM	*P* value	−/−	−/+	+/−	+/+	*P* value
Age (y)											
≤70	17	11	3	2	1	1.000	6	6	3	2	0.686
>70	23	14	3	4	2		10	6	2	5	
Sex											
Men	38	23	6	6	3	1.000	14	12	5	7	0.754
Women	2	2	0	0	0		2	0	0	0	
Tumour size (X, mm)											
X ≤ 5	4	2	1	1	0	0.459	2	1	1	0	0.327
5 < X ≤ 10	9	8	0	0	1		7	1	0	1	
10 < X ≤ 20	17	10	3	2	2		5	5	3	4	
X > 20	10	5	2	3	0		2	5	1	2	
Tumour location (centre of the tumour)											
≤1 cm from GOJ	21	17**	3	1	0	**0.030**	12*	5	3	1†	**0.045**
>1 cm from GOJ	19	8††	3	5	3		4†	7	2	6*	
Extent of Barrett's oesophagus											
BO_1	25	20**	4	1†	0†	**<0.001**	14**	7	3	1††	**<0.001**
BO_2	10	5	2	3	0		2	5	2	1	
BO_3	5	0††	0	2	3***		0	0	0	5***	

Sq, squamous epithelium; CM, cardiac-type mucosa; IM, intestinal-type mucosa; GOJ, gastro-oesophageal junction; BO_1, Barrett's oesophagus with maximum extent <3 cm; BO_2, BO with maximum extent 3 cm or more and circumferential extent <3 cm; BO_3, BO with circumferential extent 3 cm or more. Bold values indicate statistical significance ($P < 0.05$).
*, **, and *** significantly over-represented based on adjusted residual analysis ($P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively); † and †† significantly under-represented based on adjusted residual analysis ($P < 0.05$ and $P < 0.01$, respectively).

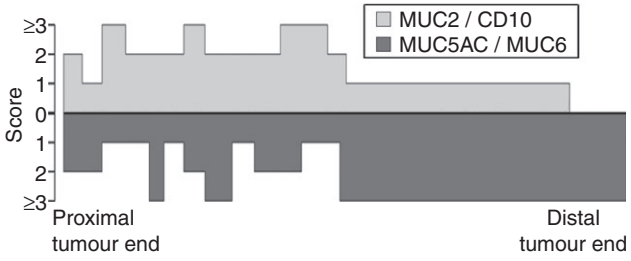

Figure 4. Diagram demonstrating a representative case of intramucosal tumour, 51 mm in size, with a mixed immunophenotype showing immunostaining heterogeneity.

with CDX2 expression in cardiac-type mucosa. All statistical analyses were performed with IBM SPSS statistics 22 for Windows (IBM Japan Inc., Tokyo, Japan). All tests were 2-tailed, and *P* values <0.05 were considered statistically significant.

Results

BACKGROUND MUCOSA AND CLINICOPATHOLOGIC PARAMETERS

There were a total of 206 tumour-containing sections from all 40 cases, ranging from 1 to 13 sections per case. Types of tumour-adjacent mucosa on both the proximal and distal sides are shown in Table 2. On the proximal side, the most frequently observed mucosa (dominant mucosa) was squamous, the second most dominant was intestinal, and the least dom-

inant was cardiac in both section-based and case-based analyses. On the distal side, the most dominant mucosa was cardiac, the second most dominant was intestinal, and the least dominant was squamous.

Associations of tumour-adjacent mucosal type with age, sex, tumour size, tumour location, and extent of tumour-containing Barrett's oesophagus are presented in Table 3. The type of tumour-adjacent mucosa was not associated with age, sex, or tumour size, but was significantly correlated with tumour location and extent of Barrett's mucosa ($P < 0.05$, $P < 0.001$, respectively). Tumours mainly located 1 cm above the GOJ frequently had adjacent mucosa with intestinal metaplasia as compared to tumours mainly located within 1 cm of the GOJ. The frequency of intestinal-type tumour-adjacent mucosa was increased as the extent of tumour-containing Barrett's mucosa increased.

TUMOUR IMMUNOPHENOTYPE AND CLINICOPATHOLOGIC PARAMETERS

Immunostaining heterogeneity was observed for all five markers, especially CDX2, MUC2, and MUC6 (Figure 2). A representative case showing the heterogeneous distribution of mucin expression is presented in Figure 4.

Of the 40 tumours, 3 (7.5%) were gastric, 34 (85.0%) were mixed (29 gastric predominant; 5 intestinal predominant), and 3 (7.5%) were intestinal immunophenotypes. There were no tumours demonstrating the null immunophenotype. Associations of tumour immunophenotype with tumour-adjacent mucosal type, age, sex, tumour size, and extent of tumour-containing Barrett's oesophagus are presented in Table 4. Tumour immunophenotype was not associated with tumour-adjacent mucosal type, age, sex, tumour location, or extent of Barrett's mucosa, but was significantly correlated with tumour size ($P = 0.022$). Gastric immunophenotype was more commonly seen in minute tumours (≤5 mm in size) than expected ($P < 0.001$), whereas the majority (77.8%, 28/36) of tumours more than 5 mm in size were of gastric-predominant mixed immunophenotype (Figure 2).

IMMUNOEXPRESSION IN BOTH TUMOUR ENDS

Immunohistochemical scores of five markers (CDX2, CD10, MUC2, MUC5AC, and MUC6) in both tumour ends are shown in Table 5. In all 40 tumours, expression of intestinal markers (CDX2, CD10, and MUC2) was significantly higher in the proximal ends

Table 4. Association of tumour immunophenotype with different clinicopathologic parameters

		Tumour immunophenotype				
			Mixed			
	n	G	G > I	I > G	I	*P* value
Dominant type of tumour-adjacent mucosa (Proximal/Distal)						
Sq/CM	25	1	16	5	3	0.407
Sq/IM	6	2	4	0	0	
IM/CM	6	0	6	0	0	
IM/IM	3	0	3	0	0	
Presence of IM in tumour-adjacent mucosae (Proximal/Distal)						
−/−	16	1	11	2	2	0.953
−/+	12	2	7	2	1	
+/−	5	0	5	0	0	
+/+	7	0	6	1	0	
Age (y)						
≤70	17	2	13	2	0	0.481
>70	23	1	16	3	3	
Sex						
Men	38	3	29	4	2	0.071
Women	2	0	0	1	1	
Tumour size (X, mm)						
X ≤ 5	4	2***	1†	0	1	**0.022**
5 < X ≤ 10	9	0	7	1	1	
10 < X ≤ 20	17	0	15	1	1	
X > 20	10	1	6	3	0	
Tumour location (centre of the tumour)						
≤1 cm from GOJ	21	1	15	2	3	0.450
>1 cm from GOJ	19	2	14	3	0	
Extent of Barrett's oesophagus						
BO_1	25	1	18	3	3	0.502
BO_2	10	2	6	2	0	
BO_3	5	0	5	0	0	

G, gastric; I, intestinal; Sq, squamous epithelium; CM, cardiac-type mucosa; IM, intestinal-type mucosa; GOJ, gastro-oesophageal junction; BO_1, Barrett's oesophagus with maximum extent <3 cm; BO_2, BO with maximum extent 3 cm or more and circumferential extent <3 cm; BO_3, BO with circumferential extent 3 cm or more. Bold values indicate statistical significance ($P < 0.05$).

***significantly over-represented based on adjusted residual analysis ($P < 0.001$).

†significantly under-represented based on adjusted residual analysis ($P < 0.05$).

Table 5. Immunohistochemical scores of CDX2, CD10, MUC2, MUC5AC, and MUC6 in both proximal and distal ends of tumours stratified by tumour size

	Proximal end (P)		Distal end (D)		*P* value
	Mean	Median	Mean	Median	
Any tumour size (n = 40)					
CDX2	9.25	10.00	7.65	8.00	**<0.001** (P > D)
CD10	0.58	0.00	0.30	0.00	**0.036** (P > D)
MUC2	2.03	2.00	1.40	1.00	**0.001** (P > D)
MUC5AC	2.20	2.00	2.73	3.00	**0.011** (P < D)
MUC6	1.85	2.00	2.30	2.50	**0.020** (P < D)
<15 mm in tumour size (n = 20)					
CDX2	9.20	9.00	8.05	7.50	0.088
CD10	0.40	0.00	0.15	0.00	0.500
MUC2	2.35	2.00	1.80	2.00	0.109
MUC5AC	2.35	3.00	2.85	4.00	0.113
MUC6	2.20	2.00	2.40	3.00	0.547
≥15 mm in tumour size (n = 20)					
CDX2	9.30	10.50	7.25	8.50	**0.007** (P > D)
CD10	0.75	0.00	0.45	0.00	0.148
MUC2	1.70	2.00	1.00	1.00	**0.003** (P > D)
MUC5AC	2.05	2.00	2.60	2.00	0.087
MUC6	1.50	2.00	2.20	2.00	**0.020** (P < D)

P > D, Scores of proximal end were significantly higher than those of distal end; P < D, Scores of proximal end were significantly lower than those of distal end (Wilcoxon signed-rank test). Bold values indicate statistical significance ($P < 0.05$).

than in the distal ends ($P < 0.001$, $P = 0.036$, and $P = 0.001$, respectively), whereas expression of gastric markers (MUC5AC and MUC6) was significantly higher in the distal ends than in the proximal ends ($P = 0.011$, $P = 0.020$, respectively). In the 20 tumours <15 mm in size, there were no significant differences in immunoexpression between the proximal and distal ends of the tumours. However, in the 20 tumours 15 mm or more in size, expression levels of CDX2, MUC2, and MUC6 were significantly different between the proximal and distal ends ($P = 0.007$, $P = 0.003$, and $P = 0.020$, respectively).

Immunophenotype in both tumour ends is shown in Table 6. The incidence of gastric immunophenotype with no positive cells for both CD10 and MUC2 was significantly higher in the distal ends (17.5%, 7/40) than in the proximal ends (none of 40) ($P = 0.012$).

CDX2 EXPRESSION IN GASTRIC-IMMUNOPHENOTYPE TUMOUR CELLS

CDX2 scores in the distal tumour ends showing a gastric immunophenotype (Figure 5) and in tumour-adjacent cardiac-type mucosa are shown in Figure 6. The CDX2 score was 3 or less in all tumour-adjacent cardiac-type mucosa. The CDX2 score was significantly higher in gastric-immunophenotype tumour ends than in cardiac-type mucosa, even if there were no tumour cells positive for both CD10 and MUC2 intestinal markers ($P = 0.006$).

CDX2 EXPRESSION IN TUMOUR-ADJACENT MUCOSA

All CDX2 scores were zero in squamous epithelium ($n = 32$), four or more in intestinal-type mucosa (dis-

Table 6. Immunophenotype in both tumour ends

	Proximal end (n = 40)	Distal end (n = 40)
Gastric		
I = 0%	0	7
0<I<5%	8	11
Mixed		
G > I	19	15
I > G	5	1
Intestinal		
0<G<5%	5	3
G = 0%	3	2
Null		
0<I<G<5%	0	1

I, expression of MUC2 and/or CD10; G, expression of MUC5AC and/or MUC6.

tal side: n = 10, mean score 8.4, median 8.5; proximal side: n = 8, mean score 10.5, median 11.5), and three or less in cardiac-type mucosa (n = 30, mean score 0.77, median 1.00).

Associations of CDX2 expression in distal tumour-adjacent cardiac-type mucosa with different clinicopathologic parameters are presented in Table 7. Univariate analysis identified two factors significantly associated with a CDX2 expression score of 2 or 3 (Figure 7): tumour size and tumour immunophenotype (P = 0.007, P = 0.008, respectively). Logistic regression analysis revealed that tumour size was independently associated with CDX2 expression score of 2 or 3 in tumour-adjacent cardiac-type mucosa (P = 0.018). Age, sex, tumour location, extent of Barrett's oesophagus, and immunophenotype in distal tumour ends were not associated with a CDX2 expression score of 2 or 3 in cardiac-type mucosa.

Discussion

It is widely accepted that the majority of tumours in Barrett's oesophagus arise from intestinal-type epithelium.[6–12] Although various tumours from different hosts may differ in proliferation rates and tumour doubling times, it is reasonable to suppose that tumour-origin mucosa would not be completely overgrown or concealed by minute tumours.[10] Therefore, intestinal metaplasia in tumour-adjacent mucosa would be more common when the tumour size was smaller if intestinal-type epithelium was the major origin of tumours in Barrett's oesophagus. In the present study, however, neither the dominant type of tumour-adjacent mucosa nor the presence of intestinal metaplasia in tumour-adjacent mucosa was correlated with tumour size (Table 3). In addition, our results of whole-section analysis showed that majority (69.2%, 9/13) of small tumours <10 mm in size had no intestinal metaplasia in adjacent non-neoplastic mucosae (Table 3), as was previously reported (70.8%, 80/113) by Takubo *et al.* using single-section analysis.[15] Vieth and Barr[3] and Takubo *et al.*[39] reviewed the same eight articles[10,15,40–45] on the background mucosa of Barrett's or GOJ adenocarcinoma with regard to tumour size. Although neither conclusion was based on statistical analyses, Vieth and Barr concluded that larger tumours are frequently associated with goblet cells, whereas Takubo *et al.* concluded that no relationship between tumour size and background is evident. Those findings accompanied by our results indicate that intestinal-type epithelium is unlikely to be the major origin of tumours in Barrett's oesophagus.

Intestinal metaplasia in tumour-adjacent mucosa was significantly correlated with tumour location and extent of Barrett's oesophagus (Table 3). The prevalence of intestinal metaplasia occurring within cardiac-type mucosa increases as the length or extent of Barrett's mucosa increases.[14,46,47] Considering that the presence of intestinal metaplasia in tumour-adjacent mucosa was dependent on or parallel to the extent of Barrett's mucosa but independent of tumour size, it could be speculated that intestinal metaplasia in tumour-adjacent mucosa might be an epiphenomenon of Barrett's oesophagus extension rather than a reflection of tumour-origin tissue.

The phenotypic expression of tumour cells is widely thought to resemble that of tumour-origin tissue,[19] especially in early-stage or small tumours. Therefore, assessment of phenotypic expression of superficial Barrett's tumour should reveal its histogenesis. In the present study, tumour immunophenotype was significantly correlated with tumour size, and tumours with a gastric immunophenotype were significantly more common (50%, 2/4) than expected in the group of minute tumours (≤5 mm in size), whereas the majority (85.0%, 34/40) of all tumours were of mixed immunophenotype (Table 4). Our results are compatible with the results of Khor *et al.*,[23] who reported that the majority (64.5%, 20/31) of minute intramucosal carcinomas (mean size, 4.8 mm) in Barrett's oesophagus were of gastric immunophenotype, and with the results of Tajima et al.,[24] who reported that

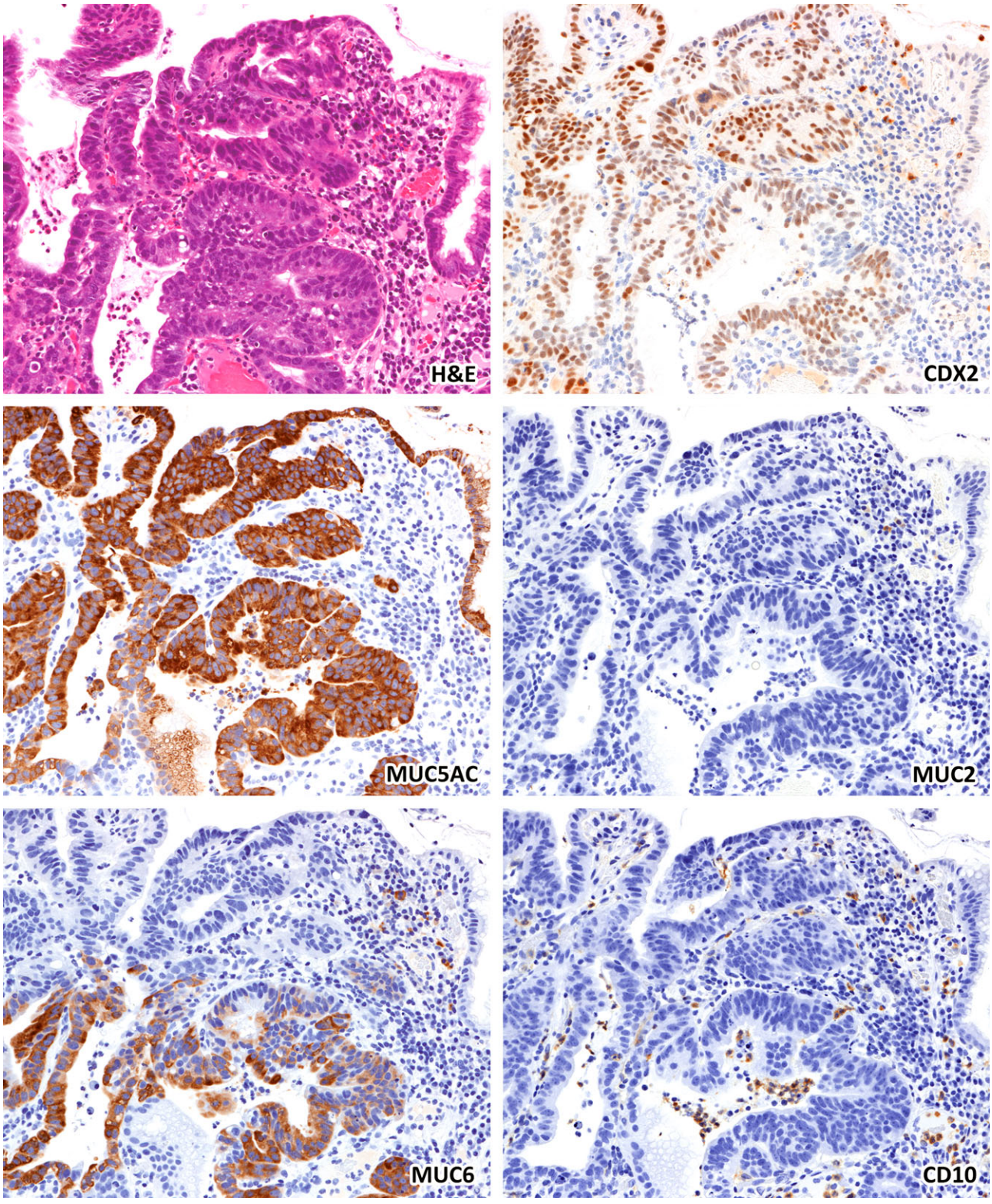

Figure 5. Gastric-immunophenotype tumour end with CDX2 expression. Tumour cells are negative for intestinal markers (MUC2 and CD10) and positive for gastric markers (MUC5AC and MUC6) plus CDX2.

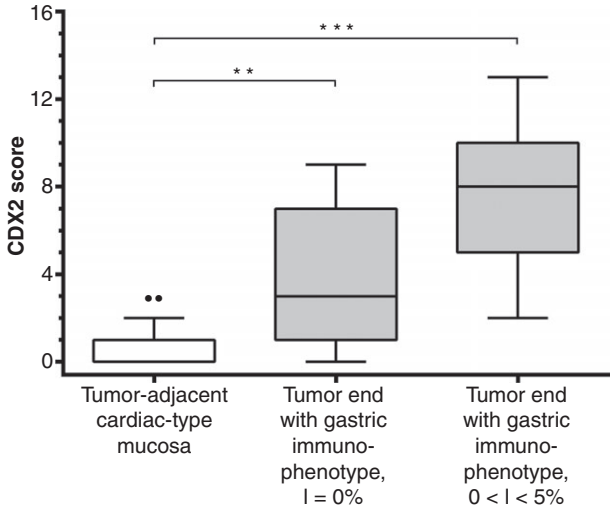

Figure 6. Box plots of CDX2 scores in tumour-adjacent cardiac-type mucosa (n = 30) and tumour ends showing gastric immunophenotype with (n = 11) or without (n = 7) a few positive tumour cells (<5%) for intestinal (MUC2 and/or CD10) markers. CDX2 expression was significantly different in the three groups ($P < 0.001$, Kruskal–Wallis test). The closed circles indicate outliers. ** Significant at $P = 0.006$, *** Significant at $P < 0.001$ (Mann–Whitney U test with Bonferroni correction).

the majority (85.7%, 6/7) of superficial Barrett's adenocarcinomas (mean size, 38.5 mm) were of mixed immunophenotype. These data suggest a phenotypic shift from gastric to mixed (both gastric and intestinal) phenotypic expression during tumour extension. This phenotypic shift from gastric to intestinal has also been described in gastric carcinoma.[19,48,49] These data led to our speculation that a certain amount of Barrett's tumours, especially gastric type-predominant tumours (gastric and gastric-predominant mixed immunophenotype tumours, 80.0%, 32/40 in the present study), might arise in cardiac-type epithelium. This speculation is supported by genetic studies[16,17] demonstrating similar molecular abnormalities in cardiac and intestinal type mucosa and by clinical studies[13,14] showing a similar cancer risk in patients with or without intestinal-type mucosa in columnar-lined oesophagus.

Immunophenotypic markers, especially CDX2, were heterogeneously expressed in Barrett's tumours, as determined by immunostaining.[23,28,38] We also confirmed the immunophenotypic heterogeneity in mucin core protein (Figure 4), but demonstrated the tendencies in immunophenotypic expressions between proximal and distal ends of tumours (Table 5). Gastric immunophenotypic expressions were lower in proximal ends than in distal ends, while intestinal immunophenotypic expressions were higher in proximal ends than in distal ends. These immunophenotypic differences between both ends of tumours were related to tumour size (Table 5). These tendencies of immunophenotypic expressions in Barrett's tumours seem to resemble those in background Barrett's mucosa, which contains a mosaic of different types of columnar mucosa;[50] additionally, there is a proximal-to-distal gradient of mucosal phenotype (Table 2), especially in long-segment Barrett's oesophagus.[12,51] Our results that distal tumour ends with a purely gastric immunophenotype showed high-level expression of CDX2 compared with non-neoplastic cardiac-type mucosa (Figure 6) might imply the pathway of tumourigenesis from CDX2-positive cardiac-type epithelium. Indeed, low-level CDX2 expression (score 3 or 4) in cardiac-type epithelium confined to small (<10 mm) tumour borders (Table 7, Figure 7) (not observed in larger tumour borders and not associated with the extent of Barrett's oesophagus) could be a reflection of tumour-origin mucosa. Although it was reported that CDX2 has both tumour-suppressor and oncogenic activity,[27] CDX2 appears to be up-regulated in early tumourigenesis in Barrett's oesophagus.

In conclusion, we demonstrated that the presence of intestinal metaplasia depended on the extent of Barrett's mucosa but was independent of tumour size, suggesting that intestinal metaplasia in Barrett's oesophagus might be an epiphenomenon rather than a pre-neoplastic condition. We also demonstrated that gastric-immunophenotype tumours were associated with minute tumour size, and our result of purely gastric-phenotypic expression together with CDX2 expression in both tumours and tumour-adjacent cardiac-type mucosa suggest that CDX2-positive cardiac-type epithelium might be a precursor of Barrett's tumour. We could not examine bioptic specimens of earlier lesions or precursor mucosae (low-grade dysplasia, non-neoplastic mucosa with or without intestinal metaplasia) in the same patients, as our studied cases were not follow-up cases. Further prospective studies are needed to evaluate the risk of malignancy of cardiac-type epithelium with particular regard to sub-morphological intestinalisation.

Author contributions

G. Watanabe and Y. Ajioka designed the study and assessed the H&E and immunohistochemical sections. M. Takeuchi reviewed endoscopic findings. A. Annenkov, T. Kato, and K. Watanabe collected and assembled clinical data. G. Watanabe, Y. Ajioka, A. Annenkov, T. Kato, K. Watanabe, Y. Tani, K. Ikegami, Y. Yokota, and M. Fukuda were participated in

Table 7. CDX2 expression in tumour-adjacent cardiac-type mucosa in association with different clinicopathologic parameters

		CDX2 score in tumour-adjacent CM			Logistic regression analysis		
	n	0 or 1	2 or 3	*P* value	Odds ratio	95% CI	*P* value
Age (y)							
≤70	12	11	1	0.632			
>70	18	15	3				
Sex							
Men	28	25	3	0.253			
Women	2	1	1				
Tumour size (X, mm)							
X ≤ 5	3	1	2	**0.007**	0.069	0.008–0.626	**0.018**
5 < X ≤ 10	8	6	2				
10 < X	19	19	0				
Tumour location (centre of the tumour)							
≤1 cm from GOJ	17	14	3	0.613			
>1 cm from GOJ	13	12	1				
Extent of Barrett's oesophagus							
BO_1	20	17	3	1.000			
BO_2	8	7	1				
BO_3	2	2	0				
Tumour immunophenotype							
Gastric	1	0	1	**0.008**			0.190
Mixed, G > I	21	20	1				
Mixed, I > G	5	5	0				
Intestinal	3	1	2				
Immunophenotype in distal tumour end							
Gastric	15	14	1	0.397			
Mixed, G > I	8	7	1				
Mixed, I > G	1	1	0				
Intestinal	5	3	2				
Null	1	1	0				

CM, cardiac-type mucosa; CI, confidence interval; BO_1, Barrett's oesophagus with maximum extent <3 cm; BO_2, BO with maximum extent 3 cm or more and circumferential extent <3 cm; BO_3, BO with circumferential extent 3 cm or more. Bold values indicate statistical significance ($P < 0.05$).

the discussion of data analysis and interpretation. G. Watanabe performed statistical analysis and wrote the manuscript, and all the authors were involved in revising the manuscript.

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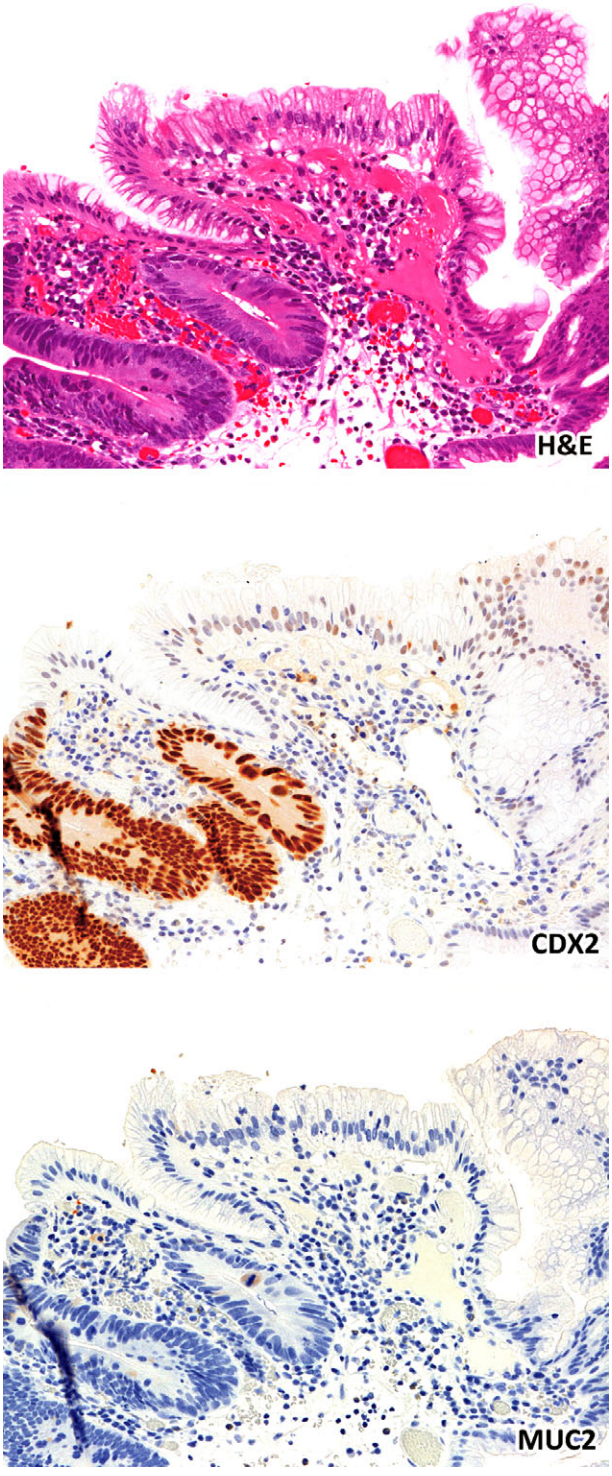

Figure 7. Tumour-adjacent cardiac-type mucosa with low-level CDX2 expression. While tumour cells are strongly positive for CDX2, cardiac-type epithelium is weakly positive for CDX2. No MUC2 positive staining is observed.

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Conflict of interest

No commercial interest or potential interest concerning this article exists.

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