Re-evaluation of phenotypic expression in undifferentiated-type early gastric adenocarcinomas using mucin core protein and CDX2

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Abstract

Background Undifferentiated-type early gastric adenocarcinomas are generally classified into two groups: pure undifferentiated-type adenocarcinomas, which naturally develop as undifferentiated-type without a glandular component; and mixed differentiated/undifferentiated-type adenocarcinomas, which are associated with some vestigial glandular component and presumably develop from differentiated-type adenocarcinoma. The differences in phenotypic expression between these two groups were examined using mucin core protein and CDX2.

Methods A total of 210 lesions of undifferentiated-type early gastric adenocarcinoma less than 25 mm in diameter were classified into four categories (gastric type, gastrointestinal type, intestinal type, and null type) based on their MUC5AC, MUC6, MUC2, and CDX2 immunoprofiles.

Results Gastric type was significantly ($p < 0.01$) decreased and gastrointestinal type was significantly ($p < 0.01$) increased both in pure undifferentiated-type

adenocarcinomas and in mixed differentiated/undifferentiated-type adenocarcinomas when CDX2 was applied to mucin core protein. In the pure undifferentiated-type adenocarcinomas, gastric type decreased and gastrointestinal type increased as tumor size increased ($p < 0.05$). In contrast, in the mixed differentiated/undifferentiated-type adenocarcinomas, gastrointestinal type was most common even in small-sized (≤ 10 mm) carcinomas and was generally stable regardless of tumor size. In submucosal carcinomas, gastrointestinal type decreased and gastric type and intestinal type increased during carcinoma invasion from the intramucosal to submucosal parts ($p < 0.05$). The positivity rates for all phenotypic markers, especially gastric markers, tended to decrease during submucosal invasion.

Conclusions CDX2 is a sensitive marker for assessing intestinal phenotypic expression, and it is likely that there are two different pathways of tumor progression in undifferentiated-type adenocarcinoma of the stomach, according to phenotypic expression.

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CDX2

Introduction

Histologically, human gastric adenocarcinomas have been classified into two major groups by Lauren [1]—the intestinal type and the diffuse type (depending on the degree of glandular formation of the cancer cells)—a classification which roughly corresponds to the differentiated type and undifferentiated type, respectively [2]. It has been considered that the differentiated types arise from

intestinalized mucosa and take an intestinal phenotype, while the undifferentiated types develop from atrophic gastric mucosa without intestinal metaplasia and take a gastric phenotype [3, 4]. This hypothesis is based on morphological similarities between cancers and intestinal metaplasia, as well as being based on the results of comparisons between carcinomas and the surrounding mucosa. However, recent advances in mucin immunohistochemistry, including mucin core protein (MUC), have revealed that differentiated-type adenocarcinomas are composed of both intestinal and gastric phenotypes [5–8].

Previous investigations of the phenotypic expression of undifferentiated-type adenocarcinomas are limited, and it has been reported that there are some undifferentiated-type adenocarcinomas of intestinal phenotype [9, 10], and that the intestinal phenotype in gastric signet-ring cell carcinomas appears to occur during tumor progression [11–13] or during tumor spread in the mucosa [14]. However, how frequent and when phenotypic alteration occurs during the development of undifferentiated-type adenocarcinomas remain controversial.

There are several commercial markers that are useful for distinguishing gastric carcinoma phenotypes. The caudal-related homeobox gene CDX2 is important for the maintenance of intestinal epithelial cells [15], and there have been several reports of CDX2 expression in human gastric carcinomas and intestinal metaplasia [16–20]. It has previously been shown that CDX2 is closely associated with intestinal phenotypic expression in gastric carcinomas [17–19, 21]. However, few studies have evaluated phenotypic expression in undifferentiated-type adenocarcinoma and/or signet-ring cell carcinoma using CDX2 as a phenotypic marker [16, 17, 22, 23].

It has been reported that, as gastric adenocarcinoma invades deeply or increases in size, the incidence of differentiated-type decreases and that of undifferentiated-type increases [24–26], which raises the possibility of histological transformation from differentiated-type to undifferentiated-type during tumor progression. Furthermore, differentiated-type adenocarcinomas of the gastric phenotype are considered to have higher rates of histological transformation into undifferentiated-type adenocarcinomas, as well as higher biological malignancy, such as high invasiveness and high metastatic potential, compared with those of the intestinal phenotype [27]. In contrast, even minute gastric adenocarcinomas, up to 5 mm in size and considered to be incipient in carcinogenesis, contain a certain amount of pure undifferentiated-type adenocarcinoma. These findings allow us to postulate different development pathways for undifferentiated-type adenocarcinoma: one that naturally develops as undifferentiated-type without a glandular component (pure undifferentiated-type: PURE-type); and one that develops with some

rudimentary glandular component (mixed undifferentiated-type and differentiated-type: MIXED-type), presumably from differentiated-type adenocarcinoma.

The aim of this study was to clarify the differences in phenotypic expression between these two groups of undifferentiated-type adenocarcinomas: PURE-type carcinoma and MIXED-type carcinoma. For this purpose, (1) two different criteria of phenotypic classification were compared (immunoprofile based on MUC only and that based on MUC and CDX2 combined), and (2) differences in phenotypic expression between PURE-type carcinoma and MIXED-type carcinoma were examined according to the background mucosa, depth of tumor invasion, and tumor size.

Materials and methods

Patients, lesions, and tissue sampling

From our pathological files of gastric carcinomas from July 1979 through March 2006, 210 lesions of undifferentiated-type early gastric adenocarcinomas measuring less than 25 mm in diameter were selected for this study. All tumors had been surgically resected, from 203 Japanese patients who had had no systemic adjuvant therapy, at Niigata University General Hospital and its affiliated institutions. Each surgically resected specimen was fixed in 10 % buffered neutral formalin solution and serially cut into 5-mm-thick sections throughout each lesion and embedded in paraffin. All sections were stained with hematoxylin–eosin (HE) for histological examination. Paraffin blocks corresponding to the selected HE sections were cut into 3- μ m-thick consecutive sections for immunohistochemical staining.

The histological classification was based on the *Japanese classification of gastric carcinoma* [28]. Undifferentiated-type adenocarcinomas were defined as those with indistinct or no glandular structure, including solid-type, poorly differentiated adenocarcinoma (por1); non-solid-type, poorly differentiated adenocarcinoma (por2); and signet-ring cell carcinoma (sig). The undifferentiated-type adenocarcinomas were further subclassified into two groups: (1) PURE-type, without any glandular formation; and (2) MIXED-type, with a mixture of non-glandular (POR) and some vestigial glandular (TUB) components.

Immunohistochemical staining

For antigen retrieval, the deparaffinized sections were microwave-treated at approximately 98 °C for 20 min (sections for MUC5AC/HGM, MUC6, MUC2, and CDX2) in 10 mmol/L citrate buffer (pH 6.0, except for

sections for CDX2), and in 1 mmol/L tris–ethylenediamine tetraacetic acid buffer (pH 9.0, sections for CDX2). Endogenous peroxidase activity was blocked by 20 min of incubation with 0.3 % hydrogen peroxidase in absolute methanol, and then the sections were washed in tap water. Non-specific binding was blocked with normal serum (Nichirei, Tokyo, Japan). The sections were incubated overnight with monoclonal antibodies as follows: (1) MUC5AC (CLH2, 1:100 dilution; Novocastra Laboratories, Newcastle, UK) and/or HGM (45M1, 1:50; Novocastra) as a marker for gastric foveolar epithelium; (2) MUC6 (CLH5, 1:100; Novocastra) and/or M-GGMC-1 (HIK 1083, 1:50; Kanto Chemical, Tokyo, Japan) as a marker for pyloric glands; and (3) MUC2 (Ccp58, 1:300; Novocastra) and CDX2 (AMT28, 1:100; Novocastra) as markers for goblet cells and intestinal epithelial cells. The sections were then incubated with Histofine Simple Stain MAX-PO (MULTI) (Nichirei Biosciences, Tokyo, Japan) for 30 min at room temperature. Antibody-antigen

reactivity was visualized using diaminobenzidine and light counterstaining with hematoxylin.

Definition of phenotypes of carcinomas

MUC5AC, HGM, MUC6, and M-GGMC-1 were defined as gastric phenotype markers, whereas MUC2 and CDX2 were selected as intestinal phenotype markers. It has been reported that MUC5AC and MUC6 recognize core proteins, whereas HGM and M-GGMC-1 recognize carbohydrate side-chains [8, 29–31]. Cytoplasmic reactivity was judged as positive for MUC5AC/HGM, MUC6/M-GGMC-1, and MUC2, and nuclear reactivity was judged as positive for CDX2. Samples were classified as positive if 5 % or more of the tumor cells were stained; otherwise they were considered negative [8, 16, 22]. Using these markers, the phenotypes were classified into four categories: (1) gastric type (G type), demonstrating positive expression for gastric markers but negative expression for intestinal markers (Fig. 1); (2) intestinal type (I type), demonstrating positive

Fig. 1 Pure undifferentiated-type (PURE-type) carcinoma with gastric type (G type). **a** HE staining. MUC5AC (**b**) and MUC6 (**c**) are positive in the cytoplasm. MUC2 (**d**) and CDX2 (**e**) are negative (**a–e**, $\times 200$)

Fig. 2 PURE-type carcinoma with intestinal type (I type). **a** HE staining. MUC5AC (**b**) and MUC6 (**c**) are negative. MUC2 (**d**) is positive in the cytoplasm, and CDX2 (**e**) is positive in the nucleus (**a–e**, $\times 200$)

expression for intestinal markers but negative expression for gastric markers (Fig. 2); (3) gastrointestinal type (GI type), demonstrating positive expression both for gastric and intestinal markers (Fig. 3); and (4) null type (N type), with negative expression for all markers. Furthermore, to compare our findings with previous reports that did not use CDX2 as a phenotypic marker, the carcinoma phenotypes were separately evaluated in two steps: phenotype judged by MUC only, and that judged by MUC and CDX2 combined. In the MIXED-type group, phenotypes in the POR component and in the TUB component were analyzed separately. Similarly, in submucosal invasive tumors, phenotypes of the intramucosal part and of the submucosal part were analyzed separately (Fig. 4).

Evaluation of surrounding mucosa

The surrounding non-neoplastic mucosa within 5 mm of the margins of the carcinoma cells was evaluated with respect to degree of chronic inflammation,

polymorphonuclear neutrophil activity, degree of glandular atrophy, and degree of intestinal metaplasia, based on the updated Sydney system [32].

Statistical analysis

Statistical analysis was performed using the χ^2 test, Fisher's exact test, and the unpaired *t*-test. Bonferroni's method was used for multiple comparisons. All statistical analyses were performed using the PASW statistics 17 software package (SPSS Japan, Tokyo, Japan). A two-tailed *p* value of less than 0.05 was considered significant.

Results

Clinicopathological findings

The clinicopathological findings of the 106 PURE-type carcinomas and the 104 MIXED-type carcinomas are

Fig. 3 Mixed differentiated/undifferentiated-type (MIXED-type) carcinoma with gastrointestinal type (GI type). The non-glandular (POR) component is seen in the *right upper side*, and the glandular (TUB) component is seen in the *left lower side* of the lesion. **a** HE

staining. In both the POR and TUB components, MUC5AC (**b**), MUC6 (**c**), and MUC2 (**d**) are positive in the cytoplasm, and CDX2 (**e**) is positive in the nucleus (**a–e**, $\times 200$)

summarized in Table 1. Submucosal invasive carcinomas were more frequent in the MIXED-type than in the PURE-type ($p < 0.001$). The proportion of male subjects tended to be higher in the MIXED-type than in the PURE-type, but there were no significant differences in age, sex, tumor location, macroscopic type, or tumor size between these two types.

Histopathological findings of surrounding mucosa

The histology of the non-neoplastic surrounding mucosa is summarized in Table 2. The number of PURE-type carcinomas was greater in intermediate-type mucosa than in pyloric gland-type mucosa. Conversely, the number of MIXED-type carcinomas was greater in pyloric gland-type mucosa than in intermediate-type mucosa ($p = 0.017$). MIXED-type carcinomas demonstrated higher intestinal metaplasia scores and significantly higher

glandular atrophy scores ($p = 0.035$) than PURE-type carcinomas.

Correlation between histological type and phenotypic expression

The distributions of phenotypes and expressions of phenotypic markers are summarized in Tables 3 and 4. Comparing the two phenotypic classifications (MUC only vs. MUC and CDX2), G type was significantly decreased in the latter (MUC and CDX2) among the three groups (61.3 vs. 17.9 % in PURE-type carcinomas; 59.8 vs. 13.7 % in POR components of MIXED-type carcinomas; 51.0 vs. 11.5 % in TUB components of MIXED-type carcinomas; $p < 0.01$), whereas GI type was significantly increased in the latter (MUC and CDX2) among the three groups (34.9 vs. 78.3 % in the PURE-type carcinomas; 29.4 vs. 75.5 % in the POR components of MIXED-type carcinomas; 44.2

Table 1 Clinicopathological findings of the 210 cases

	PURE-type (n = 106)	MIXED-type (n = 104)	p value
Age, years, mean $\pm$ SD (range)	60.1 $\pm$ 11.1 (35–86)	60.1 $\pm$ 12.5 (26–84)	0.981
Sex (male:female)	51:55	62:42	0.099
Location			0.117
U	16	16	
M	65	49	
L	25	39	
Macroscopic type			0.117
Elevated	8	14	
Flat	16	8	
Depressed	82	82	
Size, mm, mean $\pm$ SD (range)	15.7 $\pm$ 6.4 (0.4–25.0)	16.7 $\pm$ 5.9 (4.0–25.0)	0.226
Depth			<0.001
Intramucosal	76	47	
Submucosal	30	57	

PURE-type pure undifferentiated-type adenocarcinoma, *MIXED-type* mixed differentiated/undifferentiated-type adenocarcinoma, *U* upper third of the stomach, *M* middle third of the stomach, *L* lower third of the stomach

Table 2 Histopathological findings of the surrounding mucosa of undifferentiated-type adenocarcinomas

	PURE-type (n = 106)	MIXED-type (n = 104)	p value
Type of mucosa			0.017
Fundic-gland type	46	49	
Intermediate-gland type	33	16	
Pyloric-gland type	27	39	
Chronic inflammation (score ^a), mean $\pm$ SD	2.45 $\pm$ 0.68	2.40 $\pm$ 0.63	0.589
Neutrophil activity (score ^a), mean $\pm$ SD	1.33 $\pm$ 0.88	1.11 $\pm$ 0.90	0.070
Atrophy (score ^a), mean $\pm$ SD	1.88 $\pm$ 0.83	2.12 $\pm$ 0.80	0.035
Intestinal metaplasia (score ^a), mean $\pm$ SD	1.36 $\pm$ 1.11	1.66 $\pm$ 1.22	0.059

PURE-type pure undifferentiated-type adenocarcinoma, *MIXED-type* mixed differentiated/undifferentiated-type adenocarcinoma

^a Updated Sydney system

vs. 83.7 % in the TUB components of MIXED-type carcinomas; $p < 0.01$). Thus, G type was most common based on MUC only, though GI type was most common based on MUC and CDX2 in each group ($p < 0.01$). However, in both classifications, there was no significant difference in phenotype distribution among the three groups (Table 3).

As for the expression of each phenotypic marker, the positivity rate for MUC5AC was significantly higher in

Table 3 Phenotypic expression of undifferentiated-type adenocarcinomas

	PURE-type (n = 106)	MIXED-type POR (n = 102*)	TUB (n = 104)
Phenotype (MUC only) (%)	a	b	c
G type	65 (61.3)	61 (59.8)	53 (51.0)
GI type	37 (34.9)	30 (29.4)	46 (44.2)
I type	1 (0.9)	3 (2.9)	4 (3.8)
N type	3 (2.8)	8 (7.8)	1 (1.0)
Phenotype (MUC and CDX2) (%)	d	e	f
G type	19 (17.9)	14 (13.7)	12 (11.5)
GI type	83 (78.3)	77 (75.5)	87 (83.7)
I type	2 (1.9)	8 (7.8)	5 (4.8)
N type	2 (1.9)	3 (2.9)	0

PURE-type pure undifferentiated-type adenocarcinoma, *MIXED-type* mixed differentiated/undifferentiated-type adenocarcinoma, *POR* non-glandular component, *TUB* glandular component, *MUC* mucin core protein, *G type* gastric type, *GI type* gastrointestinal type, *I type* intestinal type, *N type* null type

* No intramucosal part in two tumors

^a vs. ^d and ^c vs. ^f, $p < 0.01$ (Fisher's exact test); ^b vs. ^e, $p < 0.01$ (χ^2 test)

Table 4 Expression of phenotypic markers in undifferentiated-type adenocarcinomas

	PURE-type (n = 106)	MIXED-type POR (n = 102*)	TUB (n = 104)
Phenotypic marker (%)			
MUC5AC or HGM	100 (96.2) ^a	80 (78.4) ^b	90 (86.5)
MUC6 or M-GGMC-1	73 (70.2)	67 (65.7)	79 (76.0)
MUC2	38 (36.5)	33 (32.4)	50 (48.1)
CDX2	85 (81.7)	85 (83.3)	92 (88.5)

PURE-type pure undifferentiated-type adenocarcinoma, *MIXED-type* mixed differentiated/undifferentiated-type adenocarcinoma, *POR* non-glandular component, *TUB* glandular component, *MUC* mucin core protein

* No intramucosal part in two tumors

^a vs. ^b, $p < 0.01$ (χ^2 test)

PURE-type carcinomas than in the POR component of MIXED-type carcinomas ($p < 0.01$). The positivity rate for MUC2 tended to be lower in the POR component than in the TUB component of MIXED-type carcinomas. CDX2 expression was detected not only in MUC2-positive cells but also in MUC2-negative cells (Figs. 2, 3), and there were no significant differences in CDX2 expression among the three groups (Table 4).

Fig. 4 PURE-type carcinoma with submucosal invasion. The mucosal part is seen in the *upper part*, and the submucosal part is seen in the *lower part* of the lesion. **a** HE staining. MUC5AC (**b**) and MUC6

(**c**) are positive in the mucosal part, but negative in the submucosal part. In contrast, MUC2 (**d**) and CDX2 (**e**) are positive in both the mucosal and submucosal parts (**a–e**, $\times 200$)

Correlation between carcinoma size and phenotypic expression

Figure 5 shows the correlation between the phenotypes and the size of carcinomas (≤ 10 mm and >10 mm) in each of the three groups. In PURE-type carcinomas, G type decreased significantly and GI type increased significantly as carcinoma size increased ($p < 0.05$; Fig. 5a, b). In contrast, both in the POR component and in the TUB component of MIXED-type carcinomas, the prevalence of phenotypes did not change with carcinoma size (Fig. 5d, e, g, h).

In PURE-type carcinomas, the positivity rates for gastric markers were generally stable, whereas the positivity rates for intestinal markers increased as carcinoma size increased; the positivity rate for CDX2 was significantly higher in the >10 -mm group than in the ≤ 10 -mm group ($p < 0.05$), and MUC2 expression showed similar changes (Fig. 5c). In contrast, in the POR and TUB components of

MIXED-type carcinomas, the positivity rates for gastric and intestinal markers were generally stable regardless of carcinoma size; there was no significant difference in the positivity rate for each phenotypic marker by tumor size (Fig. 5f, i).

Correlation between depth of carcinoma invasion and phenotypic expression

Figure 6 shows the phenotype relationship between the intramucosal and submucosal parts in submucosal carcinoma. In PURE-type carcinomas, the GI type decreased and both the G type and I type increased during carcinoma invasion from the intramucosal to submucosal parts, and the difference was significant when the phenotype was judged by MUC and CDX2 ($p < 0.05$). In the TUB component of MIXED-type carcinomas, there was also a decrease of GI type and an increase of G type and I type during submucosal carcinoma invasion, and the differences

Fig. 5 Differences in phenotypic expression by carcinoma size. Phenotype prevalence using mucin core protein (MUC) only and using MUC and CDX2 combined, and the positivity rate for

phenotypic markers in the PURE-type (a–c), the POR component of the MIXED-type (d–f), and the TUB component of the MIXED-type (g–i). N.S. not significant

Fig. 6 Differences in phenotypic expression according to depth of carcinoma invasion. *M* intramucosal part, *SM* submucosal part. Phenotype prevalence using MUC only and using MUC and CDX2

combined, and positivity rates for phenotypic markers in PURE-type (a–c), the POR component of the MIXED-type (d–f), and the TUB component of the MIXED-type (g–i)

Fig. 7 Schema of assumed pathways in tumorigenesis and development of gastric adenocarcinomas

were significant regardless of the use of CDX2 ($p < 0.05$; Fig. 6a, b, g, h).

The positivity rate for each phenotypic marker, especially gastric markers, tended to decrease during carcinoma invasion from the intramucosal to submucosal parts in all three groups (Fig. 4); this tendency was significant for MUC5AC in the TUB component of MIXED-type ($p < 0.05$; Fig. 6c, f, i).

Discussion

A total of 210 lesions of undifferentiated-type early gastric adenocarcinoma less than 25 mm in diameter were immunohistochemically investigated based on mucin core proteins and CDX2 to clarify the differences between two groups of different development pathways (PURE-type carcinoma and MIXED-type carcinoma) (Fig. 7).

As previously reported [8, 29–31], the distribution and positivity of MUC5AC and HGM, as well as the distribution and positivity of MUC6 and M-GGMC-1, are different. In brief, of the foveolar-type mucins, MUC5AC is detected more often than HGM mucin in gastric carcinoma, because MUC5AC is basically limited to being within the cytoplasm, while HGM is confined to the luminal surface coat, as well as the cytoplasm, of gastric carcinomas. Of the pyloric gland-type mucins, MUC6 mucin is more abundant than M-GGMC-1 mucin and is restricted to the cytoplasm, whereas M-GGMC-1 mucin is sometimes found on the luminal surface coat, as well as in the cytoplasm. Therefore, in the present study, MUC5AC, HGM, MUC6, and M-GGMC-1 stains were all used to accurately determine the mucin phenotypes of gastric carcinomas. Furthermore, CDX2 was used as an intestinal phenotype marker along with MUC2. The positivity rate was higher

for CDX2 than for MUC2, and CDX2 expression was detected in cancerous areas not only where MUC2 expression was apparent, but also in tissues exhibiting only gastric markers or null phenotypic lesions. Previous reports have shown that there is a positive correlation between CDX2 expression and MUC2 [21, 33], and that CDX2 expression might precede intestinal phenotypic expression during a shift from gastric to intestinal phenotype with the progression of gastric carcinomas [17]. Thus, CDX2 appears to be a more sensitive marker than MUC2 for assessing the presence of intestinal phenotypic expression.

Based on MUC only, PURE-type carcinomas were classified as 61.3 % (65/106) G type, 34.9 % (37/106) GI type, 0.9 % (1/106) I type, and 2.8 % (3/106) N type. Previous reports have shown the rates of the G, GI, I, and N types of early undifferentiated-type adenocarcinoma, including signet-ring cell carcinoma, to be 27.8–62.4 %, 24.7–68.5 %, 0–23.1 %, and 0–10.8 %, respectively [9, 10, 13, 14, 17, 26]. In contrast to the above findings based on MUC only, PURE-type carcinomas in the present study were classified into 17.9 % (19/106) G type, 78.3 % (83/106) GI type, 1.9 % (2/106) I type, and 1.9 % (2/106) N type based on MUC and CDX2. The present results clearly showed that there was an unexpectedly larger population of undifferentiated-type adenocarcinomas having the intestinal phenotype or gastrointestinal phenotype as compared with previously reported phenotypes based on MUC without the use of CDX2.

On the other hand, there are some reports [17–19, 33] suggesting a possible tumor suppressor role for CDX2, but the significance of CDX2 expression in gastric neoplasms is still under debate. The present study targeted the expression of mucin phenotype, and the materials used were limited to early-stage gastric carcinomas sized 25 mm or less, which made it difficult to evaluate biological

behavior or patient prognosis. Further studies with larger numbers of lesions, including advanced-stage lesions, are required to clarify the relationship between phenotype expression and biological significance.

We found that PURE-type carcinomas more frequently arose in fundic glands and intermediate-type mucosa than in pyloric gland-type mucosa. The background mucosa of the MIXED-type carcinoma demonstrated a higher intestinal metaplasia score and a significantly higher glandular atrophy score than that of PURE-type carcinoma. These findings seem to suggest that PURE-type carcinoma arises from ordinary gastric mucosa with mild glandular atrophy and intestinal metaplasia, while MIXED-type carcinoma arises from intestinalized mucosa with much glandular atrophy, regardless of phenotypic expression.

PURE-type carcinomas showed the gastric phenotype in 32.1 % of cases when their size was ≤ 10 mm, considered to be a very early stage of carcinogenesis. Furthermore, they also demonstrated a size-dependent increase in intestinal phenotypic markers (CDX2 and MUC2) but no correlation for gastric phenotypic markers, resulting in increased GI phenotype as tumor size increased (Fig. 5). In contrast, in MIXED-type carcinomas, the intramucosal part showed intestinal phenotypic expression even in smaller-sized carcinomas (Fig. 5). These results may allow us to consider that not a small number of PURE-type carcinomas arise with pure gastric phenotype and acquire the intestinal phenotype during mucosal development; on the other hand, a large number of MIXED-type carcinomas have the intestinal phenotype from the very early stage of carcinogenesis, and intestinal phenotypic expression tends to be reduced with morphological alteration (Fig. 7). Bamba et al. [14] reported time- (size-) dependent expression of the intestinal phenotype in signet-ring cell carcinomas. Natsagdorj et al. [22] reported that the undifferentiated-type gastric carcinomas derived from signet-ring cell carcinomas and the undifferentiated-type gastric carcinomas derived from differentiated-type carcinomas showed differences in intestinal phenotypic marker expressions.

In the present study, the proportion of GI type decreased and that of G and I type increased as carcinoma developed from the intramucosal to submucosal parts, and phenotypic marker expression, especially gastric markers, tended to decrease (Fig. 6). Each case, except for one (data not shown), individually showed phenotypic alteration with reduction of gastric and intestinal markers during submucosal invasion. These findings may suggest that the increased proportion of I type during submucosal invasion is caused not by the acquisition of intestinal marker expression, but by a reduction of gastric marker expression. In contrast, previous reports have shown that the phenotype of signet-ring cell carcinomas alters from gastric phenotype to intestinal phenotype during submucosal invasion [11–

13]. It may be argued that reduced gastric phenotypic expression in the submucosal part could be caused by the selective submucosal invasion of constitutively non-gastric-type cells. However, this seems unlikely, because the pattern of phenotypic expression in tumor cells right under the muscularis mucosae was the same as that in the intramucosal part, and the submucosal part showed gradual loss of phenotypic expression. Similarly, Nakamura et al. [34] reported the loss of gastric and intestinal phenotypic expression during tumor progression in differentiated-type early gastric carcinomas. Natsagdorj et al. [22] reported that intestinal phenotypic expression was reduced with deeper extramucosal invasion, due to loss of induction by the tissue environment, in undifferentiated-type gastric carcinomas.

In conclusion, CDX2 was a more sensitive marker than MUC2 for assessing the presence of intestinal phenotypic expression from the early stage in undifferentiated-type gastric adenocarcinomas. The present data suggest that there are two different groups of undifferentiated-type gastric adenocarcinomas, PURE-type carcinomas and MIXED-type carcinomas, according to phenotype expression. It is possible that these two groups develop via different tumorigenesis pathways with differences in phenotypic alteration during tumor progression.

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Conflict of interest The authors have no conflicts of interest to disclose.

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