

Gastric Mucin Phenotype Indicated Aggressive Biological Behavior in Early Differentiated Gastric Adenocarcinomas by Endoscopic Treatment

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Research

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Abstract

Background:

The distribution of mucin phenotypes and its relationship with clinicopathological features in early differentiated gastric adenocarcinomas among the Chinese cohort is unknown. We aim to investigate the mucin phenotypes and analyze the relationship between mucin phenotypes and clinicopathological features, especially the biological behavior, in early differentiated gastric adenocarcinomas from endoscopic specimens in a Chinese cohort.

Methods:

Immunohistochemical staining of CD10, MUC2, MUC5AC, MUC6 was performed in 257 patients with early differentiated gastric adenocarcinomas. Tumor location, gross type, tumor size, histological type, depth of invasion, lymphovascular invasion, mucosal background and other clinicopathological parameters were evaluated. Analyzed the relationship between mucin phenotypes and clinicopathological features through chi-square test.

Results

The incidence of gastric-, gastrointestinal-, intestinal- and null-phenotype was 21%, 56%, 20% and 3% respectively. The mucin phenotypes were related with histology classification ($P \leq 0.05$). The proportion of gastric-phenotype became greater during the transition from differentiated to undifferentiated ($P \leq 0.05$). Complete intestinal metaplasia in gastric- and intestinal-phenotype was higher than gastrointestinal-phenotype ($P \leq 0.05$). Those mixed with poorly differentiated adenocarcinoma were mainly of gastric-phenotype, which was significantly higher than that of purely differentiated tubular adenocarcinoma ($P < 0.05$), and the depth of infiltration of mixed type was deeper ($P < 0.05$). Neither recurrence nor metastasis were detected.

Conclusions

The mucin phenotype of early differentiated gastric adenocarcinoma is of clinical implication, and gastric-phenotype has aggressive biological behavior in early differentiated gastric cancers, especially in those mixed with poorly differentiated adenocarcinoma or papillary adenocarcinoma component.

Background

Gastric cancer (GC), one of the most common human cancers worldwide, is a sort of disease with multiple pathogenic factors, various prognoses and different responses to treatments. Thus, distinguishing the worse prognosis one from the better one properly seems significantly important. There are four different morphological-based classification systems, the World Health Organization (WHO/2019)[1], the Japanese Gastric Cancer Association (JGCA/2017)(1), Laurén(2) and Nakamura(3). According to the WHO classification, GCs are subclassified into papillary, tubular, poorly cohesive,

mucinous and mixed types. In JGCA classification, the subtypes are papillary(pap), tubular 1, tubular 2, poorly 1 (solid type), signet-ring cell, poorly 2 (non-solid type), mucinous, which are similar to those in WHO. GCs are divided into intestinal and diffuse type using the Laurén's classification, or as differentiated and undifferentiated type based on Nakamura's classification(2–4). Differentiated type contains pap, tub1, tub2 in JGCA classification and papillary, well/moderately differentiated adenocarcinoma in WHO classification. These different histological types exhibit distinct biological behaviors.

The mucus produced by cancers is one of the factors determining the nature of biological behavior. The main component is a high molecular weight glycoprotein called mucin(5). As the cancer progresses, the nature of mucus changes, which is related to the degree of biological malignancy. In the 1990s, with the progress of structural analysis of mucin and the widespread use of monoclonal antibodies to the core protein of mucin, mucin phenotype subclassification emerged. Mucin phenotype subclassification entirely based on the mucin expression mode, independent of histological features. Thus GCs are classified into gastric, intestinal, gastrointestinal and null mucin phenotype(4–6). Previous researches have reported the gastric-phenotype determined a higher potential of invasion and metastasis than the intestinal type, which resulted in a worse prognosis of GCs(7–11). But the researches were mostly focused on advanced gastric cancers, the early gastric cancers were rarely involved.

The early gastric cancer (EGC) is defined as the tumor invasion is confined to mucosa and submucosa, irrespective of the regional lymph nodes metastasis(12). Endoscopic mucosal resection(EMR) and endoscopic submucosal dissection(ESD) are used as the treatment of some intramucosal carcinoma and submucosal lesions, which have a very low possibility of lymph node metastasis(13,14).

To our knowledge, there is no research discussed mucin phenotypes in EGCs by EMR/ESD from China investigators. And there is little information on the effects of the mucin phenotypes on the clinicopathological features of EGCs among the Chinese cohort. To this end, we examined the mucin expressions and mucin phenotypes, and explored mucin phenotypes' clinicopathological characteristics and biological behavior.

Methods

Patients and tissue specimens

Our series consisted of 257 consecutive patients who underwent EMR/ESD for differentiated EGCs between January 2012 and June 2018 at The Second Affiliated Hospital of Zhejiang University, China. The group comprised 182 men and 75 women with an age ranged from 29 to 87 (mean 64) years old.

The location of lesion was classified in terms of upper (27 cases), middle (46 cases) and lower (184 cases) third of the stomach. The size of the lesion was measured by the maximum diameter, it ranged from 0.1 to 6.5 (mean 1.5) centimeters. The category of protruding (52 cases) included type 0-I and 0-IIa, depressed (102 cases) category contained type 0-IIc and III, and all the others went to the category of

protruding and depressed (103 cases). Based on the WHO and JGCA classification, the EGCs were subclassified into well differentiated tubular adenocarcinoma (tubular, well-diff / tub 1, 198 cases), moderately differentiated tubular adenocarcinoma (tubular, mod-diff / tub 2, 37 cases), papillary adenocarcinoma (3 cases) and mixed (tub-pap / sig / por, 19 cases). In the mixed cases, the undifferentiated components (sig / por) were less than 50%.

Immunohistochemistry

All the specimens were fixed with 10% buffered formalin, embedded in paraffin and cut into 4µm thick sections, in addition to hematoxylin and eosin (HE) staining. MUC2 was detected by mAb Ccp58 (Zsbio, 1:100), MUC5AC by mAb MRQ-19 (Zsbio, 1:100), MUC6 by mAb MRQ-20 (Zsbio, 1:100), and CD10 (Zsbio, 1:100) by immunohistochemistry(IHC). IHC was performed by the Ventana NexES Staining System. The marker CD10 exhibited both cytoplasmic and luminal membranous reactivity, whereas MUC5AC, MUC6 and MUC2 exhibited only cytoplasmic reactivity. A tumor was considered positive if at least one single tumor cell was stained correctly.

Classification of mucin phenotype

Based on MUC5AC, MUC6, MUC2 and CD10, the EGCs could be classified into gastric-phenotype (G-type), gastrointestinal-phenotype (GI-type), intestinal-phenotype (I-type) and null-phenotype (N-type)(4,6,15–17). The following criteria were used for the classification of mucin phenotypes: ☐ G-type presents positive to at least one of the MUC5AC and MUC6, while CD10 and MUC2 were both negative; ☐ I-type presents positive to CD10 and/or MUC2, while MUC5AC and MUC6 were both negative; ☐ GI-type presents positive to marker CD10 and/or MUC2 associated to one of the markers MUC5AC and MUC6 positive; ☐ None of the four markers was positive, the phenotype was classified as N-type.

In addition, in GI-type, we labelled the one expressed MUC5AC and/or MUC6 more than MUC2 and CD10 as gastric-predominant GI phenotype (GI-G type), otherwise, labelled as intestinal-predominant phenotype (GI-I type).

Statistical analysis

Associations between mucin expression profiles and clinicopathologic parameters were examined by chi-square test or Fisher's exact test. Statistical significance was used, and was established to be $P < 0.05$. Statistical calculations were performed with the IBM SPSS Statistics (version 23.0).

Results

Expression of mucin markers and mucin phenotype in early gastric cancers

The expression of CD10, MUC2, MUC5AC and MUC6 in all the EGCs were 43.58% (112/257), 63.81% (164/257), 64.98% (167/257) and 72.76% (187/257) respectively. (Fig. 1)

257 EGCs were classified into G-type(21%,54/257), GI-type(56%,144/257), I-type(20%,51/257) and N-type(3%,8/257). For the GI-type, it contained GI-G type (72%,103/144) and GI-I type (28%,41/144). The cases showing G-type and GI-G type (61%, 157/257) were more than that of I-type and GI-I type (36%, 95/257). (Fig. 2)

Relationship between mucin phenotype and clinicopathological features

The relationship between mucin phenotype and clinicopathological features were summarized in table 1. The mucin phenotypes were statistically related with JGCA and WHO classification ($P \leq 0.05$), but the parameters of gender, age, margin, color, tumor size, gross type, depth of invasion, lymphovascular invasion all did not significantly differ among those mucin phenotypes($P \geq 0.05$). The I-type had the highest proportion of differentiated EGCs among the four mucin phenotypes (100.0% vs.79.7% vs. 83.1% vs. 87.5%, $P=0.027$). While the G-type had more portion of tub-por/sig and pap/tub-pap cases than I-, GI- and N-type (20.4% vs.7.0% vs.0.0% vs.12.5%, $P=0.027$) in JGCA classification. In WHO classification, the G-type had more mixed histological components (18.5% vs. 5.6% vs. 0.0% vs. 12.5%, $P=0.006$) compared with other mucin phenotypes.

Relationship between mucin phenotypes and background mucosa

Intestinal metaplasia (IM) of background mucosa was observed in 199 of 249 (79.9%) cases (G-,GI- and GI-type), including 38 cases of incomplete IM and 161 cases of complete IM. IM did not significantly differ among mucin phenotypes ($P \geq 0.05$). But incomplete and complete IM were significantly differed in different mucin phenotypes ($P=0.004$, $P=0.018$). The expression of incomplete IM in GI-type was higher than that of G-type and I-type GC (21.5%vs. 9.3% vs. 3.9%). On the contrary, there were 77.8% (42/54) G-type cases and 70.6% (36/51) I-type GCs appeared complete IM, which was higher than GI-type 57.6% (83/114). The IM status of background mucosa and the relationship with mucin phenotypes were showed in Table 2.

Biological behavior of mucin phenotypes

In addition to tubular adenocarcinoma components, 22 of the 257 cases also contained components of papillary adenocarcinoma, or poorly differentiated carcinoma, or signet ring cell carcinoma. 15 cases (68.18%) contained por/sig components, and the other 7 (31.82%) contained pap components. And 11 cases showed G-type, 10 cases showed GI-type, only one case showed N-type, none of them showed I-type. Among the 10 GI-type cases, 9 cases showed GI-G type, one showed GI-I type . Almost all the 22 cases showed G- and GI-G type, which was significantly higher than I-type ($P=0.011$). In addition, the 22 cases got a higher proportion of infiltration into the submucosa layer ($P \leq 0.001$). (Table 3, Fig. 3, Fig. 4).

Follow-up

Six patients received additional gastrectomy, and there was no residual tumor or lymph node metastasis. All cases were under close follow-up, neither recurrence nor metastasis were detected.

Discussion

Mucin phenotype is a classification based on the mucin markers expression mode. After the year of 2000, the mucin phenotypes of gastric and intestinal were analyzed by IHC(15). The mucin markers of MUC5AC, MUC6, MUC2 and CD10 are considered necessary, though there is no consensus on the amount of markers which should be used to define a mucin phenotype, or the percentage of tumor cells must be stained(6–8,11,15,18). MUC5AC is a secreted mucin expressed in the surface mucous epithelium of normal gastric mucosa. High expression of MUC6 is observed in fundic neck mucous cells and pyloric glands of gastric mucosa. CD10 is a marker for the brush border on the luminal surface of small intestinal. In the normal adult intestine, MUC2 expression is observed in the perinuclear areas of goblet cells.

We showed the expression of MUC5AC, MUC6, MUC2 and CD10 demonstrated in 167 (64.98%), 187 (72.76%), 164 (63.81%), 112 (43.58%) of the 257 EGCs. In the previous researches, the expression of MUC5AC, MUC6, MUC2 and CD10 in GCs was 55.1%~67.5%, 44.9%~64%, 35.4%~49.3% and 20.6%~20.9% respectively[19, 20]. For EGCs, the expression of each mucin marker was 68.75%~96.8%, 19.6%~71.58%, 25%~62.10%, 0%~79% respectively(6,11,21).

Based on the combinations of expression of those markers, the 257 EGCs were classified into G-type (21%,54/257), GI-type (56%,144/257), I-type (20%,51/257) and N-type(3%,8/257). The incidence of each mucin phenotype in previous reports have been showed to be 15%~41.1%, 20.3%~60.1%, 18.5%~46.6%, 3.7%~31.6% in advanced GCs(3,13–14), and 7.9%~36.8%, 18.8%~41.2%, 15.4%~55.56%, 0%~11.1% in early stage GCs(7,11,19–22,24–26) respectively. Our data was consistent with them. As we notice, the reported expression ranges vary greatly among different investigators, different markers, antibodies and case groups may play a role in it. Koyama et al have reported the incidence of G-type was 19.3%(27), which was similar to ours (21%), but in his report, the incidence of I-type was much higher the G-type(43.8% vs. 19.3%), so as Fabio *et al.*(11). While Tajima *et al.*(22) reported an opposite result, in their study G-type was much higher than I-type (36.8% vs. 15.4%). In our research, the incidence of G-type is almost as same as I-type (21% vs. 20%). All in all, in our data, much more than half of these cases showed G- and GI-G type (61.09%,157/257), which is much higher than I- and GI-I type(36.96%,95/257), as a previous report revealed that almost all the intramucosal GC cases showed the gastric phenotype, including GI phenotype(28).

The relationship between mucin phenotypes and clinicopathological features was investigated. We found the histology classification (both the JGCA and WHO classification) were closely related with mucin phenotype. The I-type had a higher proportion than G-, GI- and N-type (100.0% vs.79.7%, 93.1%, 87.5%, $P=0.027$). The G-type was correlated significantly with the mixed type (with poorly differentiated/papillary carcinoma) histologically. Our data showed the proportion of G-type turned greater during the transition from solely differentiated type to mixed type carcinoma. Mixed type carcinoma in early stage more frequently expressed G-type mucin, and the G-type tumors were associated with a higher rate of undifferentiated-type as compared with the I-type tumors(7,22).

While there were no significant differences between mucin phenotypes and other parameters, including gender, age, margin, color, tumor size, gross type, depth of invasion, lymphovascular invasion ($P \geq 0.05$). These results perfectly shared the same viewpoint with some researchers(22,25,29), there was no clear correlation between phenotypes and clinicopathologic characteristics, including sex, age, tumor size, location, macroscopic features, lymphatic or venous invasion, or lymph node metastases in the case of the differentiated type(22,25,29). While Koseki *et al.*(7) and Oya *et al.*(30) have reported the incidence of lymphatic invasion, venous invasion and lymph node metastasis in gastric phenotype carcinomas was significantly more frequent than that in intestinal phenotype carcinomas. In addition, G-type GCs had a correlation with some distinguished macroscopic features: the smaller tumor diameter(28), the discolored surface and non-wavy tumor margins(31,32). G-type differentiated adenocarcinomas showed depressed type, indistinct margins and monotonous color tone across the mucosal layer, whereas I-type had an elevated, distinct margin and a red mucosa(3,33,34). The discrepancy of these results may have been due to heterogeneous components which contained poorly differentiated adenocarcinoma(29).

Intestinal metaplasia has been frequently observed surrounding the GC, especially differentiated adenocarcinomas. IM had a malignant potential and has been regarded as a precursor of gastric neoplasms. According to Laurén, The intestinal type adenocarcinoma preceded by metaplastic changes, while the diffuse type adenocarcinoma arised in non-IM gastric mucosa(2). In the current study, the background mucosa IM was observed in 79.9% cases (G-,GI- and I-type) and 87.0% in G-type. 25.5% cases of I-type arised from the normal mucosa without IM. IM did not significantly differ among mucin phenotypes ($P \geq 0.05$). But incomplete and complete IM were significantly differed in different mucin phenotypes ($P = 0.004$, $P = 0.018$). There were 77.78% (42/54) G-type and 70.6% (36/51) I-type appeared complete IM, which was higher than GI-type (83/114,57.6%). The expression of incomplete IM in GI-type was higher than that of G- and I-type (21.5%vs. 9.3% vs. 3.9%). Our results demonstrated a remarkable difference between mucin phenotypes and the background mucosa. Similar results have been repoted by Kabashima *et al.*(36)and Matsuoka(37). The mucin phenotype of the carcinoma was independent of mucin phenotypic changes in the surrounding mucosa, they might perform individual intestinalization. G-type might imitate the surrounding mucosa, the carcinomas and the background mucosa had an unstable status, then commonly possess the hybrid phenotype of the stomach and the small intestine(36,37).

Mucin phenotypes can indicate biological behavior in GCs. G-type GCs got increased potential for invasion and metastasis: infiltrating deeper layers or more surrounding structures, higher rate of lymph node metastasis, and poorer prognosis(3,12,18,21). Even, the differentiated adenocarcinoma with G-type had a similar outcome, focused on the prognosis, with undifferentiated adenocarcinoma(7–10). In our research, six patients received additional gastrectomy, and there was no residual tumor or lymph node metastasis. All cases were under close follow-up, neither recurrence nor metastasis were detected. The mixed type (mixed with poorly differentiated or papillary adenocarcinoma) were mainly of G-type, which was significantly higher than that of purely differentiated tubular adenocarcinoma ($P < 0.05$), and the depth of infiltration was deeper ($P < 0.05$). G-type had the highest portion (11/54,20.37%) with poorly differentiated/undifferentiated components, and almost all of them (19/22,86.36%) expressed G- and GI-

G type. The mixed type may represent a progressive loss of glandular structure during its progression from mucosal to advanced stage, and those with submucosal invasive was a risk factor for lymph node metastasis.(7,22,38) Differentiated GC with G-type often changed histologically into signet ring-cell carcinoma or poorly differentiated adenocarcinoma. Those imply the fact of more aggressive biological behavior and poorer prognosis.

Conclusion

Our study firstly reported the expression of mucin markers (MUC5AC, MUC6, MUC2 and CD10) and mucin phenotypes in differentiated EGC samples from the treatment of ESD/EMR among the Chinese population. Mucin phenotypes of early differentiated gastric cancer is of clinical significance, and G-type have aggressive biological behavior in early differentiated gastric cancers, especially in those mixed with poorly differentiated adenocarcinoma or papillary adenocarcinoma component.

Abbreviations

GC

gastric cancer, **EGC**:early gastric cancer

WHO

World Health Organization,

JGCA

Japanese Gastric Cancer Association,

EMR

endoscopic mucosal resection,

ESD

endoscopic submucosal dissection,

G-type

gastric-phenotype, **GI-type**:gastrointestinal-phenotype,

I-type

intestinal-phenotype, **N-type**:null-type,

GI-G type

gastric-predominant GI phenotype,

GI-I type

intestinal-predominant phenotype,

IM

Intestinal metaplasia

Declarations

Ethics approval and consent to participate

All study procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions.

Competing interests

The authors declare no personal, financial, or institutional interest in any of the materials described in this article.

Authors's contributions

Conception and design: Jinghong Xu

Acquisition and analysis of data: Jinghong Xu, Kai Song, Qi Yang and Yu Yan

Drafting the manuscript and figures: Kai Song and Jinhong Xu

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Tables

Table 1 Relationship between Mucin Phenotypes and Clinicopathological Features

	G-type	GI-type	I-type	N-type	c2 value	<i>P</i> value
Gender					0.671	0.906
Male	40(74.1%)	99(68.8%)	37(72.5%)	6(75.0%)		
Female	14(25.9%)	45(31.2%)	14(27.5%)	2(25.0%)		
Age					0.412	0.945
≤64 years	28(51.9%)	75(52.1%)	29(56.9%)	4(50.0%)		
≥64 years	26(48.1%)	69(47.9%)	22(43.1%)	4(50.0%)		
Tumor Size					2.356	0.502
≤2cm	34(63.0%)	99(68.8%)	32(62.7%)	7(87.5%)		
≥2cm	20(37.0%)	45(31.2%)	19(37.3%)	1(12.5%)		
Margin					4.269	0.224
Distinct	35(64.8%)	111(77.1%)	40(78.4%)	5(62.5%)		
Indistinct	19(35.2%)	33(22.9%)	11(21.6%)	3(37.5%)		
Color					3.423	0.337
Darken	31(57.4%)	75(52.1%)	34(66.7%)	5(62.5%)		
Faded	23(42.6%)	69(47.9%)	17(33.3%)	3(37.5%)		
Tumor Location					5.819	0.407
Upper	4(7.4%)	15(10.4%)	8(15.7%)	0(0.0%)		
Middle	12(22.2%)	21(14.6%)	12(23.5%)	1(12.5%)		
Low	38(70.4%)	108(75.0%)	31(60.8%)	7(87.5%)		
Tumor Location					0.787	0.842
EGJ	4(7.4%)	7(4.9%)	3(5.9%)	0(0.0%)		
No-EGJ	50(92.6%)	137(95.1%)	48(94.1%)	8(100.0%)		
Gross type					8.943	0.162
Protruding	10(18.5%)	32(22.2%)	10(19.6%)	0(0.0%)		
Depressed	16(29.6%)	57(39.6%)	26(51.0%)	3(37.5%)		
Protruding-Depressed	28(51.9%)	55(38.2%)	15(29.4%)	5(62.5%)		
JGCA Classification					16.870	0.027

tub1	34(62.9%)	112(77.8%)	45(88.2%)	7(87.5%)		
tub2	9(16.7%)	22(15.2%)	6(11.8%)	0(0.0%)		
tub-por/sig	7(13.0%)	7(4.9%)	0(0.0%)	1(12.5%)		
pap/tub-pap	4(7.4%)	3(2.1%)	0(0.0%)	0(0.0%)		
WHO Classification					18.052	0.017
Tubular, well-diff	34(62.9%)	112(77.8%)	45(88.2%)	7(87.5%)		
Tubular, mod-diff	9(16.7%)	22(15.2%)	6(11.8%)	0(0.0%)		
Papillary	1(1.9%)	2(1.4%)	0(0.0%)	0(0.0%)		
Mixed	10(18.5%)	8(7.0%)	0(0.0%)	1(12.5%)		
Depth of Invasion					1.360	0.681
M	47(87.0%)	132(91.7%)	46(90.2%)	8(100.0%)		
SM	7(13.0%)	12(8.3%)	5(9.8%)	0(0.0%)		
Lymphovascular invasion					2.541	0.687
(+)	1(1.9%)	1(0.7%)	0(0.0%)	0(0.0%)		
(-)	53(98.1%)	143(99.3%)	51(100.0%)	8(100.0%)		

+, present; - absent; EGJ, esophagogastric junction; well-diff, well differentiated; mod-diff, moderately differentiated; M, mucosa; SM, submucosa.

Table 2 Relationship between mucin phenotypes and background mucosa

	G-type	GI-type	I-type	c2 value	P value
Intestinal metaplasia				2.685	0.265
(+)	47(87.1%)	114(79.2%)	38(74.5%)		
(-)	7(12.9%)	30(20.8%)	13(25.5%)		
Incomplete intestinal metaplasia				10.948	0.004
(+)	5(9.3%)	31(21.5%)	2(3.9%)		
(-)	49(90.7%)	113(78.5%)	49(96.1%)		
Complete intestinal metaplasia				7.957	0.018
(+)	42(77.8%)	83(57.6%)	36(70.6%)		
(-)	12(22.2%)	61(42.4%)	15(29.4%)		

+, present; - absent.

Table 3 Relationship between Biological Behavior and Mucin Phenotypes

	tub	tub-por/sig	pap/tub-pap	c2 value	P value
Depth of Invasion				15.824	0.000
M	219(93.2%)	9(60.0%)	5(71.4%)		
SM	16(6.8%)	6(40.0%)	2(28.6%)		
Mucin Phenotype				14.743	0.011
G-type	43(18.3 %)	7(46.7%)	4 (57.1%)		
GI-type	134(57.0%)	7(46.7%)	3(42.9%)		
I-type	51(21.7%)	0(0.0%)	0(0.0%)		
N-type	7(3.0%)	1(6.6%)	0(0.0%)		

M, mucosa; SM, submucosa.

Figures

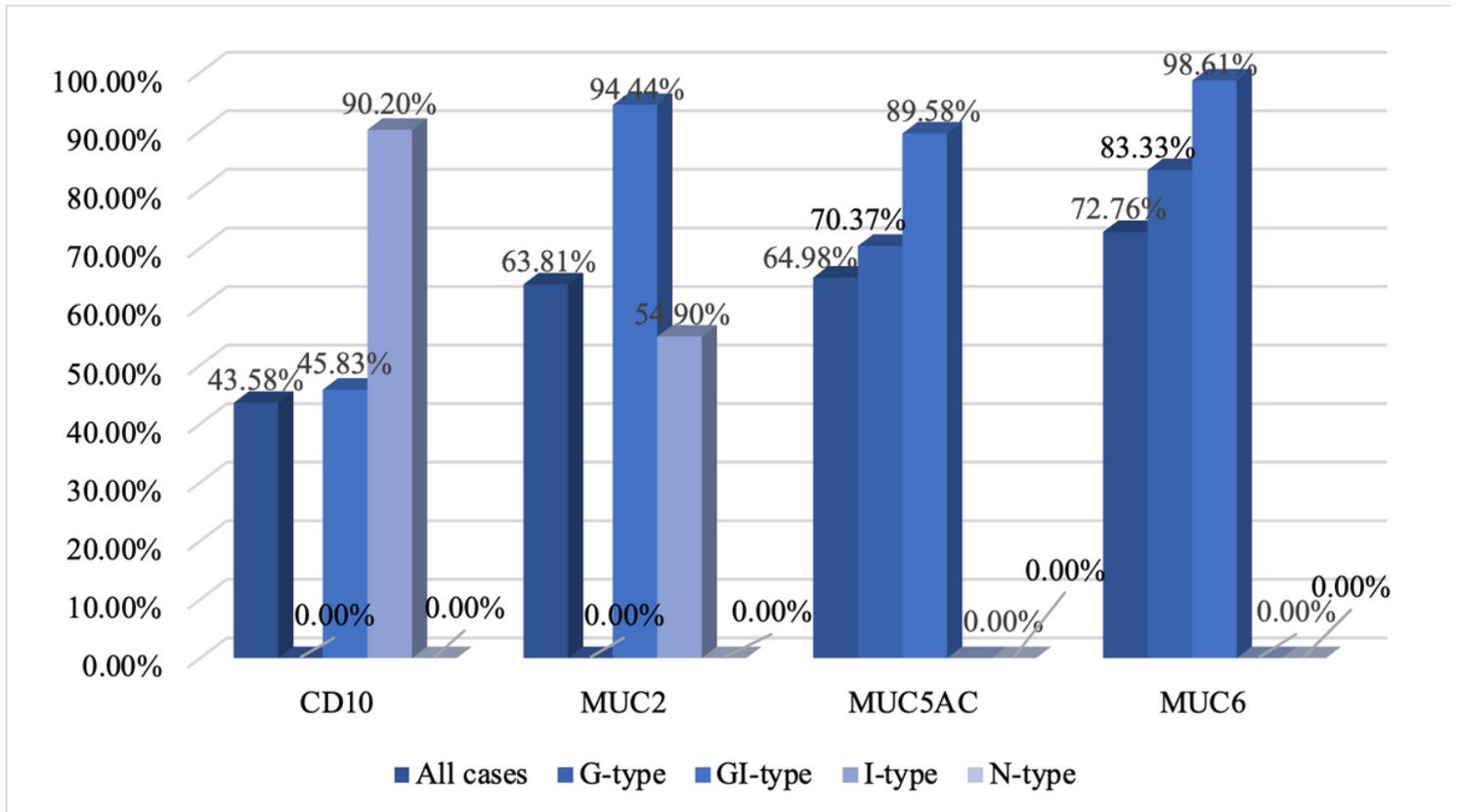


Figure 1

The proportion of CD10, MUC2, MUC5AC, MUC6 of each mucin phenotype

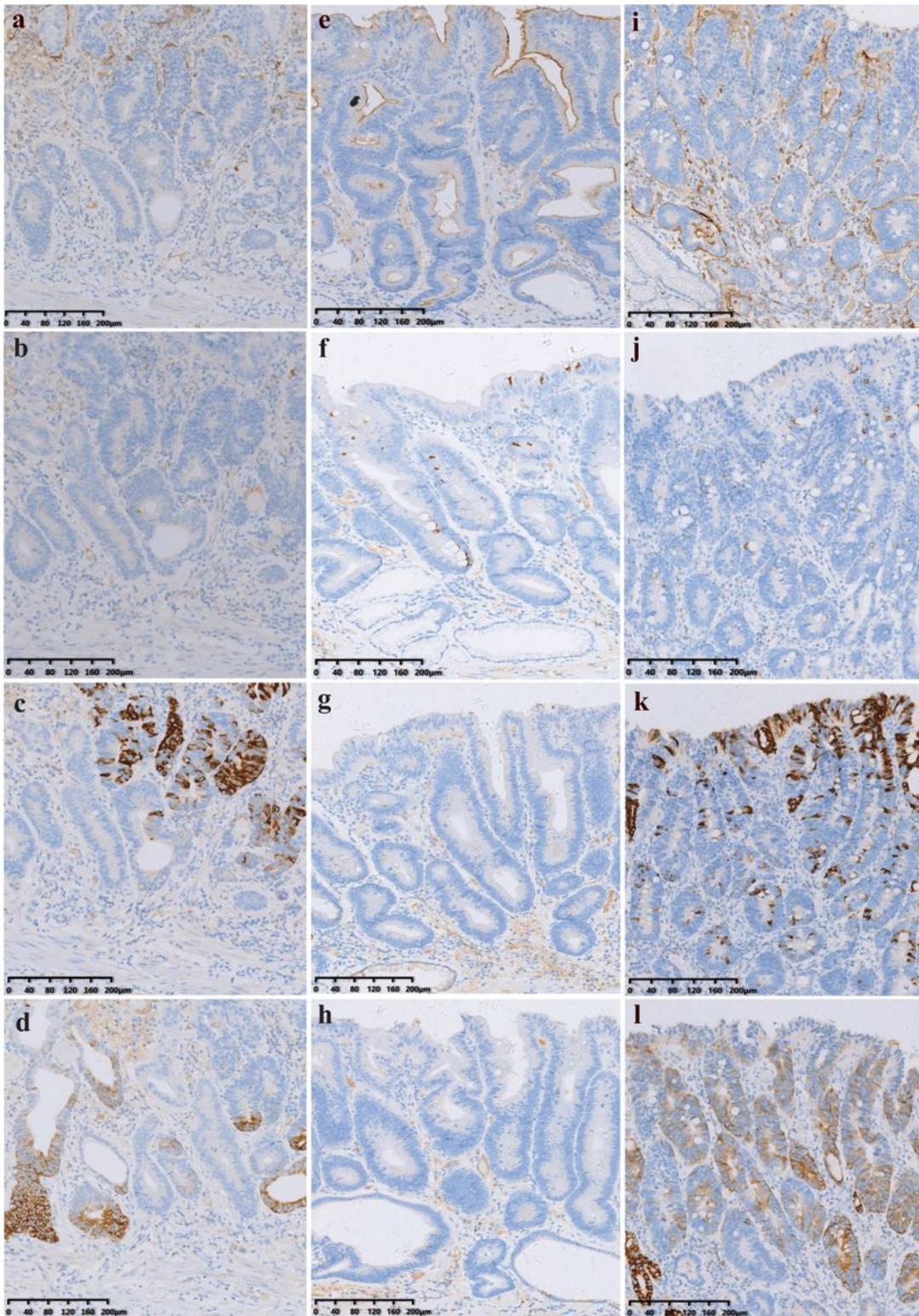


Figure 2

IHC staining of gastric phenotype, intestinal phenotype and gastrointestinal phenotype. G-type (a-d) shows negative staining of CD10 (a) and MUC2 (b), while MUC5AC (c) and MUC6 (d) positive. To the opposite, I-type (e-h) shows CD10 (e) and MUC2 (f) markers positive, while MUC5AC (g) and MUC6 (h)

negative. GI-type (i-l) shows positive staining of CD10 (i), MUC2 (j), MUC5AC (k) and MUC6 (l). (HE and IHC x10)

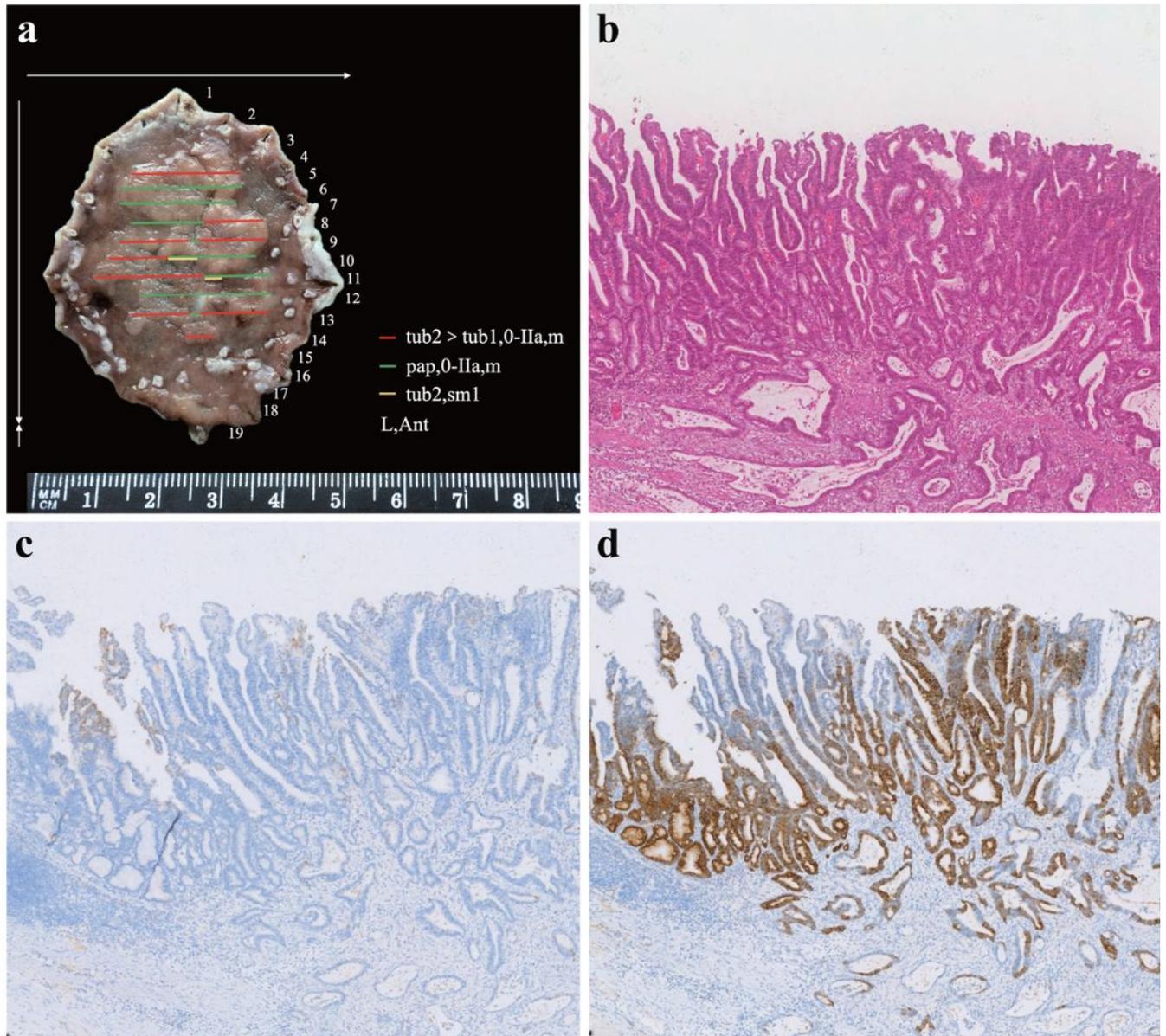
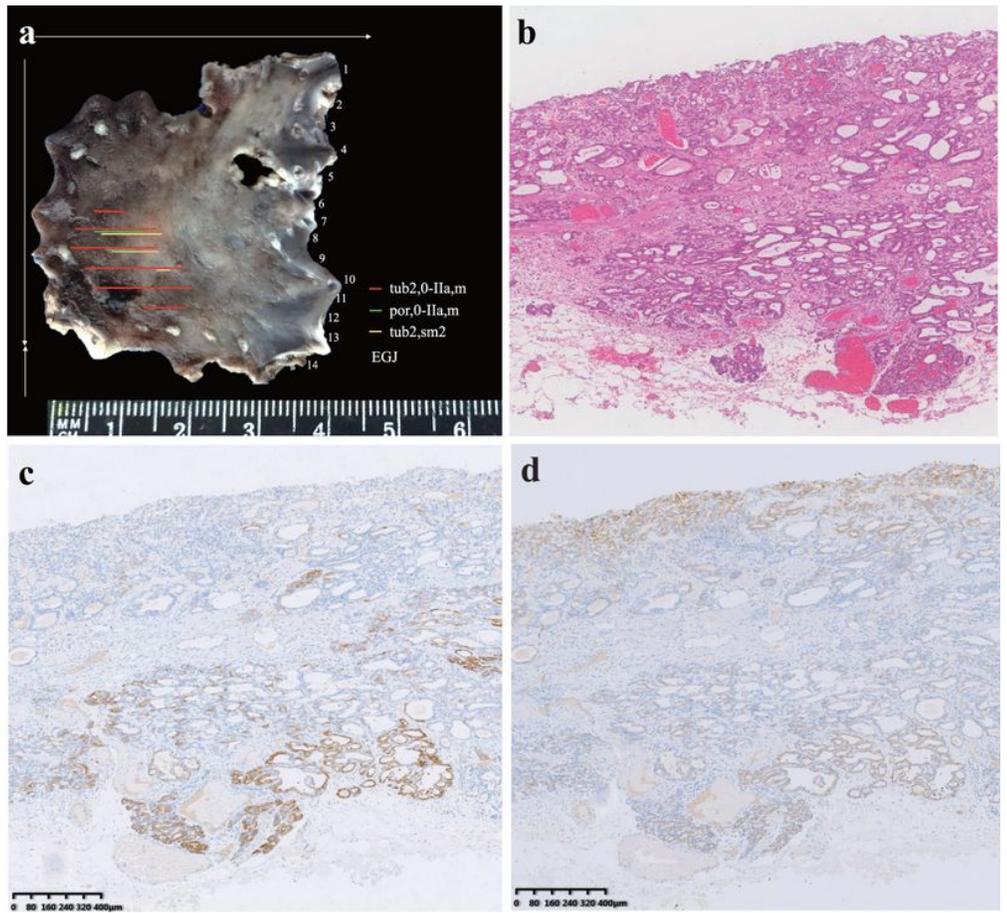


Figure 3

Tubular adenocarcinoma mixed with papillary adenocarcinoma showing gastric mucin phenotype. Reconstructive map (a). H&E staining showing papillary structure on the surface and tubular adenocarcinoma in the submucosa, invasive depth is 300 μ m (b). Focal positive staining for MUC5AC (c) and strongly positive staining for MUC6 (d), the invasive tubular adenocarcinoma showing MUC5AC negative (c) and MUC6 positive (d). (HE and IHC x10)



b

Figure 4

Tubular adenocarcinoma mixed with poorly differentiated carcinoma showing gastric mucin phenotype. Reconstructive map (a). H&E staining showing poorly differentiated carcinoma in the submucosa, invasive depth is 2500 μ m (b). Positive staining for MUC5AC (c) and MUC6 (d), the invasive tubular adenocarcinoma showing MUC5AC (c) and MUC6 (d) positive. (HE and IHC x10)