

Mucin Phenotypic Expression and Submucosal Invasion of Gastric Differentiated-type Adenocarcinoma with Minimal Intestinal Metaplasia

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ABSTRACT

AIM: The gastric differentiated-type adenocarcinoma, which basically arises from intestinal metaplasia (IM), is occasionally derived from the ordinary gastric mucosa without intestinal metaplasia. However, in the clinical endoscopic field, it is still difficult to diagnose the invasion depth of differentiated-type adenocarcinoma surrounded by the ordinary gastric mucosa. The aim of our research was to clarify the prediction method of submucosal carcinoma invasion of these lesions.

MATERIALS AND METHODS: The study population consisted of 282 early gastric cancer (EGC) lesions. The degree of histological IM in the background mucosa was classified according to The Updated Sydney System, moreover cases with normal and mild grades of IM were considered to have minimal IM. Mucin immunohistochemical staining was performed on 27 EGCs of the differentiated-type with background mucosa minimal IM, followed by classification into four mucin-phenotypes according to the expression of MUC5AC, MUC6, MUC2, and CD10.

RESULTS: The differentiated-type of EGC with minimal IM accounted for 9.6% of all EGCs. Among those with a gastric phenotype, the rate of submucosal invasion was 62.5%, which was significantly higher than that in the incomplete intestinal-type cases (12.5%).

CONCLUSION: Our results indicated that information regarding the mucin phenotypic expression is beneficial for predicting submucosal invasion of differentiated-type carcinoma surrounded by minimal IM.

Key words: Early gastric cancer; Mucin phenotype; Tubular adenocarcinoma; Intestinal metaplasia; Submucosal invasion

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INTRODUCTION

Gastric carcinoma is categorized into a number of diverse histological types, but there have been attempts to simplify the classification into just two groups based on the presence or absence of gland formation. Western countries have adopted Lauren's categorization system whereby gastric carcinomas are divided into diffuse-type and intestinal-type^[1], while Nakamura's classification into differentiated and undifferentiated-types has been mainly used in Japan^[2]. The latter system divides gastric carcinomas into two groups based on not only gland formation, but also histogenesis, i.e., differentiated-type adenocarcinoma arises from gastric mucosa with intestinal metaplasia (IM), while undifferentiated-type adenocarcinoma is derived from the ordinary gastric mucosa without IM. This classification of the

histological types that is based on the background mucosa has contributed to the improvement of gastric cancer diagnosis that aims toward precise clinical image diagnosis and minimally invasive treatment of early gastric cancer (EGC)^[3].

Although it is generally accepted that most EGCs conform to Nakamura's histogenesis theory, some pathologists have reported that differentiated-type carcinoma is occasionally derived from the ordinary gastric mucosa. Further, they believe that this type of lesion can be classified as gastric-type based on the mucin phenotypic expression and specific biological behavior^[4-7]. By contrast, among the clinical endoscopic field, it is still difficult to assess the lateral extent and invasion depth of differentiated-type adenocarcinoma derived from the ordinary gastric mucosa before endoscopic treatment^[8-10].

This study was performed to analyze the clinicopathological findings, mucin phenotypic expression, and endoscopic images of differentiated-type carcinoma those were surrounded by gastric mucosa with minimal histological IM. The aim of our research was to clarify the prediction method of submucosal carcinoma invasion of these lesions.

MATERIALS AND METHODS

Materials

The study population consisted of 225 EGC patients and 282 gastric carcinoma lesions identified by histopathological search in cases archived by the Foundation for Detection of Early Gastric Carcinoma between April 2002 and March 2013. Carcinomas confined to the mucosa or submucosa were selected for the present study. Treatment methods were endoscopic excision of 159 lesions and surgical resection of 123 lesions.

Methods

EGC was classified into two groups: differentiated- and undifferentiated-types. The clinicopathological findings included those described in the "Japanese Classification of Gastric Carcinoma 3rd English Edition"^[11], and "7Th edition of the American Joint Committee on Cancer cancer staging manual"^[12]. Tubular adenocarcinoma well differentiated (tub1), tubular adenocarcinoma moderately differentiated (tub2), and papillary adenocarcinoma (pap) were classified as differentiated-type, and poorly differentiated adenocarcinoma (por), signet-ring cell carcinoma (sig), and mucinous adenocarcinoma (muc) were classified as undifferentiated-type. In this study, background mucosa was defined as gastric mucosa located on the non-tumorous side in contact with the outer border of the EGC lesion. The degree of histological IM in the background mucosa was classified into 4 groups according to The Updated Sydney System^[13]: normal, 0%; mild, less than 30%; moderate, less than 60%; and marked, more than 60%. The cases with normal and mild grades of histological IM were regarded as minimal IM background mucosa in this article, because it can be considered that there is no practical effect on the clinicopathological characteristics of EGCs, if the histological degree of IM is confined to less than 30%.

EGC in each group was assessed based on the patient's age at the time of treatment, maximum diameter of the lesion, incidence of differentiated-type carcinoma, incidence of intramucosal carcinoma (confined to the mucosa T1a), and macroscopic type of EGC. Endoscopic findings of EGC were assessed based on white light images and chromoendoscopic images with indigo carmine (GIF XQ 260, GIF Q 260, and GIF H 260; Olympus Optical Co., Tokyo, Japan).

Mucin immunohistochemical staining

Mucin immunohistochemical staining was performed on 27 EGC lesions of the differentiated-type with normal and mild IM in background mucosa, it was assessed by The Updated Sydney system, and then the mucin-phenotypic expression was examined. These 27 EGCs were taken from the study population of 282 EGC lesions.

Four mouse monoclonal antibodies all purchased from Leica Microsystems (Tokyo, Japan) were used as primary antibodies: MUC5AC (CLH2), MUC6 (CLH5), MUC2 (Cep58), and CD10 (56C6). For immunohistochemical staining, 4 µm-thick paraffin sections were used. Xylene was used for deparaffinization, ethanol was used for dehydration, and endogenous peroxidase was blocked by 3% hydrogen peroxidase. Antigen retrieval was performed for 5 min at 121°C in 10 mmol/L citric acid buffer (pH 6.0) for MUC5AC, MUC6, and MUC2, and 60 min with Cell Conditioner (CC1; 1 mmol/L, pH 8.5, EDTA) for CD10. After blocking background staining, slides were incubated overnight with 1:500 dilution of MUC5AC, MUC6 or MUC2 primary antibody, or 1:50 dilution of CD10 primary antibody in a humid chamber. After reaction with primary antibody, slides were labeled by the avidin-biotinylated peroxidase complex method and visualized with 3-3 diaminobenzidine. The cytoplasm of foveolar epithelium and neck cells were positively-stained for MUC5AC, and the cytoplasm of pyloric gland cells were positive for MUC6 staining. MUC2 staining was demonstrated around the nuclei of goblet cells in the intestinal epithelium, while CD10 staining was detected in the brush border of IM. For evaluation of the immunohistochemical staining, slides were determined as positive if over 10% of tumor cells showed staining^[4-5].

Classification of mucin phenotypic expression

The mucin phenotypes of EGC were classified into four groups based on the combination of CD10, MUC2, MUC5AC, and MUC6 expression. Complete intestinal-type was positive for CD10, and negative for MUC6 and MUC5AC. Incomplete intestinal-type was positive for CD10 and MUC5AC or MUC6, or only MUC2. Gastric-type was positive for MUC5AC or MUC6, and negative for CD10 and MUC2^[4-5]. Figure 1 shows the classification method.

Of 27 lesions subjected to immunohistochemical staining, the incidence of submucosal cancer (invaded the submucosa, T1b), macroscopic type and color tone on endoscopy, and mucin phenotypic expressions were compared between 16 incomplete intestinal-type lesions and 8 gastric-type lesions. Macroscopic type was classified into 3 groups using indigo carmine chromoendoscopic images: Type 0-IIb and Type 0-IIc lesions were classified as depression type, Type 0-I and Type 0-IIa lesions as elevated type, and a mixture of elevated and depression types as mixed type. Color tone of EGC was determined on endoscopic images under white light, and classified into 2 groups: red and white/normal.

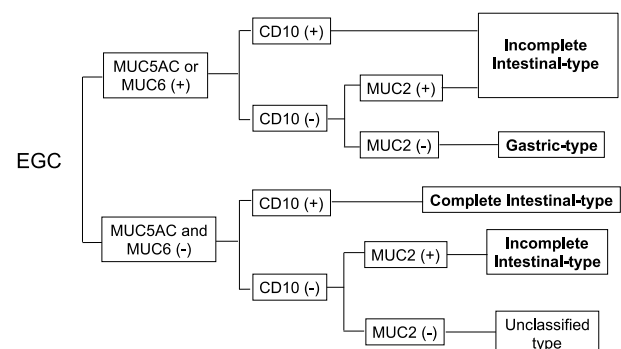


Figure 1 Algorithm for classification by mucin phenotypic expression.

Statistical Analysis

The relationship between phenotypic expression and clinicopathological factors were examined by F test and Fisher's exact test. The level of significance was defined as less than 0.05.

RESULTS

The Table 1 shows Clinicopathological findings of EGCs. There were 28 lesions with normal grade of IM in the background mucosa (9.0%), 31 lesions in the mild group (10.1%), 76 lesions in the moderate group (27.0%), and 147 lesions in the marked group (52.1%). Then number of differentiated-type carcinoma was 8 lesions, 19 lesions, 56 lesions, and 133 lesions in the normal, mild, moderate, and marked groups, respectively. There was no relationship between incidence of intramucosal carcinoma (T1a) and degree of IM. Depression type lesions were the most common regardless of the degree of IM.

Analysis of mucin phenotypic expression of 27 differentiated-type lesions with normal or mild IM in the background mucosa showed 16 incomplete intestinal-type lesions (59.2%), which was the most common type, followed by 8 gastric-type lesions (29.6%) (Figure 2).

The average diameters of incomplete-type and gastric-type lesions were 15.6 mm ± 9.5 (mean ± SD) and 13.1 mm ± 7.7, respectively. The incidence of T1b (submucosal carcinoma) in the gastric-type was 62.5% (Figure 3), while it was 12.5% in the incomplete intestinal-type (Fisher's exact test; *P* < 0.05).

Color tone and macroscopic type of incomplete intestinal-type and gastric-type lesions are shown in Figure 4 and Figure 5. Statistical analysis indicated no relationship between mucin phenotypic expression of EGC and color tone or macroscopic type.

Figure 6 shows a differentiated-type EGC with normal grade IM in the background mucosa. The lesion was located in the greater curvature of the middle portion of the stomach, the macroscopic type was Type 0-IIc, the diameter was 1.2 cm, and invasion depth was T1b (invaded the submucosa) (Figure 6a and Figure 6b). By mucin immunohistochemical analysis, the lesion was found to be positive for MUC5AC and MUC6, and negative for MUC2 and CD10, thus, it was determined to be of the gastric-type (Figure 6c and Figure 6d).

Table 1 Clinicopathological findings of EGCs (*n* = 282).

Background mucosa IM grade	normal	mild	moderate	marked
Lesion number	<i>n</i> = 28	<i>n</i> = 31	<i>n</i> = 76	<i>n</i> = 147
Age (mean)	57.8	58.1	62.6	65.3
Size (mean, mm)	15.6	19.1	20.6	17.6
Number of differentiated type	8	19	56	133
Incidence of T1a	78.5%	64.5%	76.3%	78.9%
Macroscopic type				
0-IIc, 0-IIb	26	25	59	105
0-I, 0-IIa	1	3	12	31
mixed	1	3	5	11

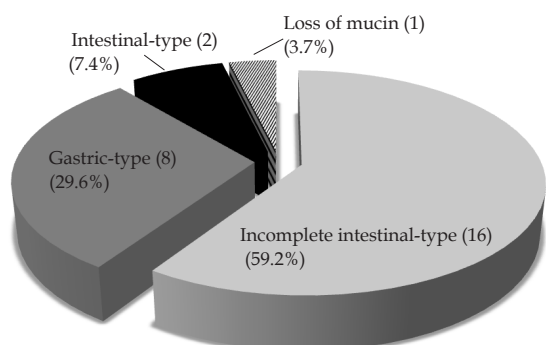
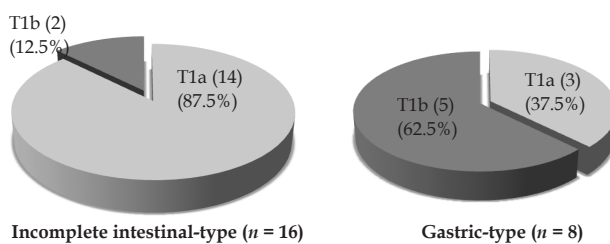


Figure 2 Mucin phenotypic expression in differentiated-type EGC with background gastric mucosa containing normal or mild intestinal metaplasia (*n* = 27).

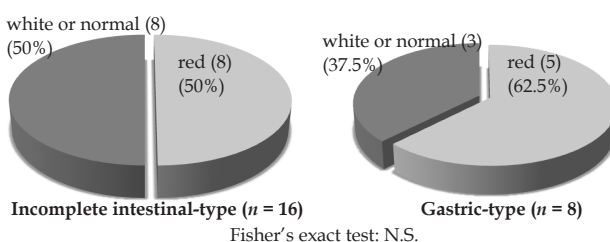
DISCUSSION

Clinicopathological findings of EGC are closely related to the background mucosa, i.e., differentiated-type carcinoma is derived from IM, while undifferentiated-type carcinoma arises from the ordinary gastric mucosa^[2-3]. However, some EGCs do not match this histogenesis theory in clinical settings; some differentiated-type carcinomas have been found to occur from the ordinary gastric gland mucosa without IM, in which it is especially difficult to diagnose invasion depth of the carcinoma^[4-9]. Endoscopists predict submucosal invasion of EGC from the macroscopic findings of focal thickness and ulceration in the cancer lesion^[14-15]. However, in the case of differentiated-type carcinoma occurring from the ordinary gastric gland mucosa with no IM and no atrophy, as shown in Figure 6, it is difficult to diagnose not only the focal thickness, but also ulcer formation. Therefore, we should establish additional way to predict submucosal invasion of these cases. In the present study, we retrospectively analyzed the frequency of submucosal invasion of differentiated-type adenocarcinomas that were surrounded by the ordinary gastric gland mucosa with minimal IM, based on the mucin phenotypic expression and endoscopic images. The aim of our research was to clarify the prediction method of submucosal carcinoma invasion of these lesions.



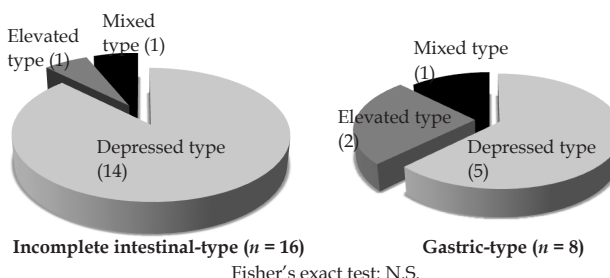
Fisher's exact test; *P* < 0.05

Figure 3 Invasion depth of EGC with gastric-type and incomplete intestinal-type of mucin expression. Gastric-type shows significantly higher incidence of submucosal invasion (T1b) by Fisher's exact test. F test shows equal variances of lesion sizes between incomplete intestinal-type and gastric-type.



Fisher's exact test: N.S.

Figure 4 Endoscopic color tone of EGC with gastric-type and incomplete intestinal-type of mucin expression. No significant differences are observed by Fisher's exact test.



Fisher's exact test: N.S.

Figure 5 Macroscopic findings of gastric-type and incomplete intestinal-type of mucin expression. There are no significant differences by Fisher's exact test.

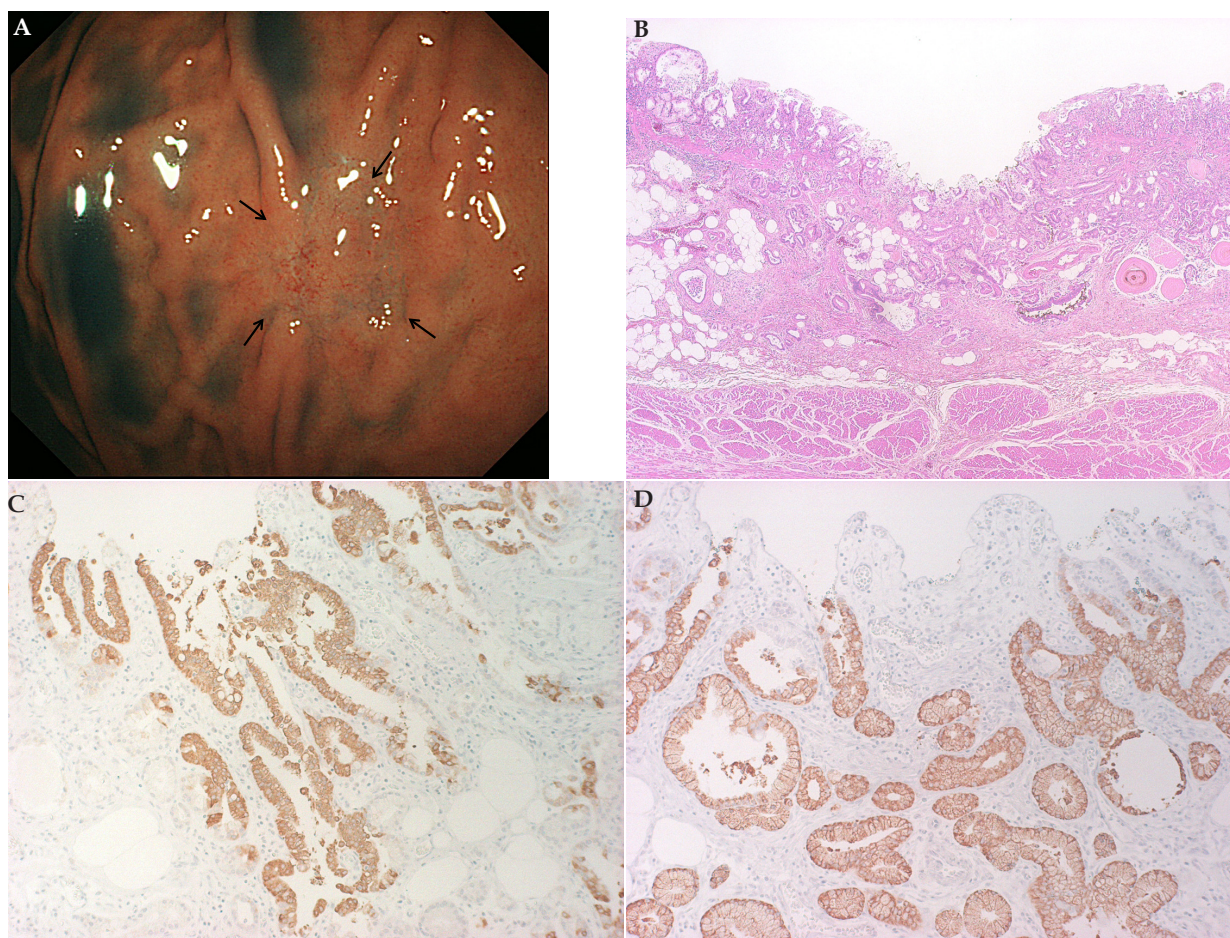


Figure 6 Differentiated-type EGC with background gastric mucosa with normal grade intestinal metaplasia. A, Endoscopic image of a case of differentiated-type EGC with background gastric mucosa with no intestinal metaplasia (57-year-old male). Black arrows indicate the border of EGC 1.2 cm in diameter (chromoendoscopy image with indigo carmine). B, Pathological findings reveal submucosal invasion of the carcinoma (hematoxylin-eosin staining). C, MUC5AC staining is detected in the cytoplasm of the gastric carcinoma cells. D, MUC6 staining is also observed in the cytoplasm of the gastric carcinoma cells. The mucin phenotypic expression is considered to be of the gastric-type.

The number of differentiated-type carcinoma was 8 lesions and 19 lesions in the normal and mild IM groups, respectively (Table 1). The result indicated that differentiated-type EGC derived from background mucosa with minimal IM accounted for 9.6% of all EGCs in this study. Then, we investigated the mucin phenotypic expression in 27 EGC lesions in the normal and mild IM groups, and compared incidence of submucosal invasion, color tone, and macroscopic type between incomplete intestinal-type and gastric-type EGCs. In gastric-type, the rate of invasion into the submucosal layer was 62.5%, which was significantly higher than that in incomplete intestinal-type (12.5%) (Figure 3). However, we also found that even when assessing endoscopic images under white light retrospectively, it is difficult to distinguish between incomplete intestinal-type and gastric-type based on the change in color tone or macroscopic type of EGCs (Figure 4 and Figure 5).

The above observations indicated that examining the mucin phenotypic expression of carcinomas by immunohistochemical staining was effective as a differentiation tool.

For lesions diagnosed as differentiated-type EGC derived from background mucosa with minimal IM based on biopsy findings with hematoxylin-eosin staining, the mucin phenotypic expression should be analyzed for confirmation. Then, cases identified with gastric-type of mucin phenotypic expression, which are predicted to have increased invasion into the submucosal layer, should be prepared for additional treatment.

In conclusion, our results suggest that information regarding the mucin phenotypic expression is beneficial for predicting invasion depth of differentiated-type EGCs that are surrounded by background mucosa with minimal IM.

CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

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