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Immunohistochemical Mucin Expression of Short-Segment Barrett's Esophagus

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Barrett's mucosa consists of metaplastic columnar epithelium (specialized columnar epithelium) of the esophagus. Recently, "short-segment Barrett's esophagus (SSBE)" was proposed. In the present study, we examined immunohistochemical mucin expression and the Ki-67 labeling index (LI) of SSBE, in 5-15 mm lengths. All 27 SSBE cases showed gastric mucin (MUC5AC, HGM, MUC6). CD10 and MUC2, which were markers of intestinal phenotypes, were detected in 13 (48.1 %) and 14 (51.9 %) of the 27 SSBE cases. Ki-67 LI of SSBE positive cases for CD10 was 23.6 %, while that of SSBE negative cases for CD10 was 14.4 % ($p < 0.05$). SSBE cases were divided into two groups: one was gastric epithelium type with low Ki-67 LI, and the other was metaplastic epithelium with intestinal metaplasia and high Ki-67 LI. The latter group was suggested to be more important as a premalignant lesion of esophageal adenocarcinoma.

Key words : esophagus, Barrett's mucosa, mucin, Ki-67, immunohistochemistry

INTRODUCTION

Barrett's mucosa is inflammatory and reactive conditions in which the normal squamous mucosa of the esophagus is replaced by metaplastic columnar epithelium [1, 2]. The Barrett's mucosa may be caused by esophageal reflux of digestive fluid including gastric juice and/or bile. Ordinary Barrett's mucosa is lined by gastric-type epithelium with or without intestinal metaplasia. Several studies have reported a high incidence of adenocarcinoma arising in Barrett's mucosa and analyzed morphological characteristics of the esophageal adenocarcinoma with Barrett's mucosa. Recently, "short-segment Barrett's esophagus (SSBE)" was proposed, and is defined as short segments of columnar epithelium with intestinal metaplasia of esophagus [3, 4]. However, SSBE has not been characterized extensively.

Mucins are high-molecular weight glycoproteins and are widely distinguished in areo-digestive and genital organs [5-8]. Recent studies have demonstrated that the mucins are associated with cell-to-cell adhesion, cell migration including tumor metastasis and immunological defense. Nine distinct epithelial mucin genes have been identified, and are divided into two groups; one is extracellular secreted mucins (MUC2, MUC5AC, MUC5B and MUC6) and the other is membrane-associated mucins which have membrane anchors and are smaller than the secreted mucins (MUC1, MUC3 and MUC4). CD10 is expressed on the surface of a variety of normal and neoplastic cells [9-11]. It was initially identified as the common acute lymphoblastic leukemia antigen (CALLA) and considered to be tumor-specific. CD10 is also

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expressed in various non-lymphoid cells and tissues, such as breast myoepithelial cells, bile canaliculi, fibroblasts, with especially high expression on the brush border of kidney and gut epithelial cells. Ki-67 is a monoclonal antibody that reacts with a nuclear antigen expressed in all active phases of the cell cycle (G1, S, G2, and M), but is absent in quiescent cells (G0) [12-15]. The development of the Ki-67-equivalent monoclonal antibody MIB-1 has allowed immunohistochemical detection of proliferating cells using routine formalin-fixed paraffin-embedded tissues.

In this study, we analyzed the immunohistochemical distribution of mucin and CD10, and evaluated proliferation activity using the Ki-67 (MIB-1) labeling index (LI) in SSBE, and examined the relation between mucin expression and Ki-67 LI of SSBE.

MATERIALS AND METHODS

Definition of short-segment Barrett's esophagus (SSBE) and tissue specimens

All tissue specimens were obtained at surgical resection of esophageal squamous carcinoma at Tokai University Hospital. Twenty-seven cases of SSBE were examined in this study, and were histopathologically defined as short segments (0.5-1.5 cm in length) of columnar epithelium of the esophagus adjacent to the esophago-gastric junction, using routine hematoxylin-eosin sections. The diagnosis of SSBE was confirmed by the existence of submucosal esophageal glands covered by columnar mucosa, in addition to double muscularis mucosae of the esophagus (Fig. 1).

Eleven cases of gastric mucosa with intestinal metaplasia were taken from surgical resections of gastric cancer.

Immunohistochemical staining

Each tissue specimen was fixed with 10 % buffered formalin. Immunohistochemical staining was performed on 5-μm-thick sections [16-20]. As primary antibodies, the following mouse monoclonal antibodies were used: MUC2 (Ccp58, Novocastra Laboratories Ltd., Newcastle upon Tyne, UK), MUC5AC (CLH2, Novocastra Laboratories Ltd.), MUC6 (CLH5, Novocastra Laboratories Ltd.), HGM (45M1, Novocastra Laboratories Ltd.), CD10 (56C6, Novocastra Laboratories Ltd.) and MIB-1 (for detecting Ki-67, Dako A/S, Copenhagen, Denmark).

Deparaffinized and dehydrated sections were immersed in 0.3 % hydrogen peroxide (H_2O_2) in methanol for 30 min to abolish endogenous peroxidase activities. For detecting mucin glycoproteins and Ki-67, the sections were pretreated with autoclave heating (ES-215, High-pressure steam sterilizer, TOMY, Japan) at 121 ˚C for 4 min for antigen retrieval. Nonspecific binding was abolished with diluted normal sheep serum (Cosmo Bio Co. Ltd., Tokyo, Japan). Next, the sections were overlayed with primary monoclonal antibodies at 1:100 optimally diluted with 1 % bovine serum albumin containing phosphate-buffered saline (PBS) and left overnight at 4 ˚C in a moist chamber. Immunoreactivites were detected by the peroxidase-labeled streptavidine biotin (LSAB) method (Dako A/S) with modifications. After being washed with PBS, the secondary biotinylated antibodies, anti-mouse Ig $(Fab)_2$ antibody at 1:100 (Amersham International plc., Buckinghamshire, UK) were applied for 60 min at room temperature. The sections were then treated with the streptavidine-conjugated horseradish peroxidase for 30 min at room temperature. The reaction products were visualized using diaminobenzidine tetrahydrochloride (DAB) action for 4 min in Tris buffer. Counter staining was performed using hematoxylin.

Evaluations of immunohistochemical staining

Immunohistochemical expression patterns of mucins were divided into four groups according to the percentage of epithelial cells positive for mucin expression, as follows: − , negative; + 1, less than 30 %; + 2, 30 %-50 %; and + 3, greater than 50 %. Labeling index (LI) of Ki-67-positive cells was expressed by counting a minimum of 300 epithelial cells. The Ki-67 LI was expressed as the mean ± standard deviation (SD) and was tested for significance by Student's t test.

RESULTS

Mucin expression patterns and Ki-67 LI are summarized in SSBE cases (Table 1), in addition to cases of gastric intestinal metaplasia (control, Table 2). Of the 27 cases of SSBE, incomplete intestinal metaplasia was found in 13 cases (48.1 %) on the hematoxylin-eosin sections (Fig. 1). All 13 metaplastic cases (cases 15-27) showed incomplete intestinal metaplasia, and none of SSBE exhibited

Table 1. Mucin expression and Ki-67 labeling index (LI) of short segment Barrett's esophagus (SSBE)

Case No	Metaplasia	MUC5AC	HGM	MUC6	MUC2	CD10	Ki-67 LI (%)	
1	negative	++	++	++	-	-	5.0	
2	negative	+++	+++	++	-	-	14.4	
3	negative	++	++	+++	-	-	5.0	
4	negative	+++	+++	++	-	-	29.1	
5	negative	++	++	+++	-	-	6.6	
6	negative	+++	+++	+++	+	-	10.6	
7	negative	+++	+++	+++	-	-	35.0	
8	negative	++	++	++	-	-	3.8	
9	negative	++	+++	+++	-	-	2.9	
10	negative	++	+++	+++	-	-	17.1	
11	negative	+++	+++	+++	-	-	38.2	
12	negative	+++	++	+++	-	-	18.9	
13	negative	++	++	+++	-	-	9.4	
14	negative	+++	+++	++	-	-	5.9	
							14.4±11.9	(cases 1-14)
15	positve	++	++	+++	+	+	6.2	
16	positve	+++	+++	+++	+	+	12.5	
17	positve	+++	++	++	++	+	12.0	
18	positve	+++	++	++	+	+	21.4	
19	positve	++	+++	+++	++	+	25.6	
20	positve	+++	+++	+++	+	+	38.1	
21	positve	++	++	+++	++	+	44.1	
22	positve	++	++	+++	+	+	34.5	
23	positve	++	+++	+++	++	+	44.9	
24	positve	+++	+++	++	+++	+	19.9	
25	positve	+++	+++	+++	++	+	20.1	
26	positve	+++	+++	+++	++	+	11.0	
27	positve	++	++	+++	+	+	16.6	
							23.6±12.9	(cases 15-27)
							18.8±13.0	(Total, cases 1-27)

All metaplastic cases (cases15-27) showed incomplete intestinal metaplasia.
Ki-67 LI, labeling index of Ki-67

Table 2. Mucin expression and Ki-67 labeling index (LI) of gastric intestinal metaplasia

Case No	Metaplasia	MUC5AC	HGM	MUC6	MUC2	CD10	Ki-67 LI (%)	
1	C	+++	+++	+++	++	+	0.0	
2	C	+++	+++	+++	+++	++	33.2	
3	C	+++	+++	+++	+++	++	27.7	
4	C	+++	+++	+++	++	++	19.0	
5	C	+++	+++	++	+++	+++	8.7	
							17.7±13.6	(cases 1-5)
6	I	+++	+++	+++	+	+	14.4	
7	I	+++	+++	+++	+++	-	33.8	
8	I	+++	+++	+++	+++	+	17.9	
9	I	+++	+++	++	+++	+++	15.6	
10	I	+++	+++	+++	+++	+	11.6	
11	I	+++	+++	+++	+	+	37.7	
							21.8±11.0	(cases 6-11)
							19.9±11.8	(Total, cases 1-11)

C, Complete intestinal metaplasia (all brush border, goblet cell, and Paneth cell) ;
I, Incomplete intestinal metaplasia;
Ki-67 LI, labeling index of Ki-67

complete intestinal metaplasia.

All 27 SSBE cases revealed MUC5AC, HGM and MUC6 which were markers of the gastric phenotype (Fig. 2). CD10 and MUC2, which were markers of the intestinal phenotype, were detected in 13 (48.1 %) and 14 (51.8 %) of the 27 SSBE cases, respectively. CD10 expression was correlated with intestinal metaplasia on the hematoxylin-eosin sections.

Ki-67 LI of SSBE was 18.8 ± 13.0 %, which was similar to that of gastric incomplete intestinal metaplasia (21.8 ± 11.0 %), while Ki-67 LI of gastric complete intestinal metaplasia was 17.7 ± 13.6 %. Ki-67 LI of SSBE positive

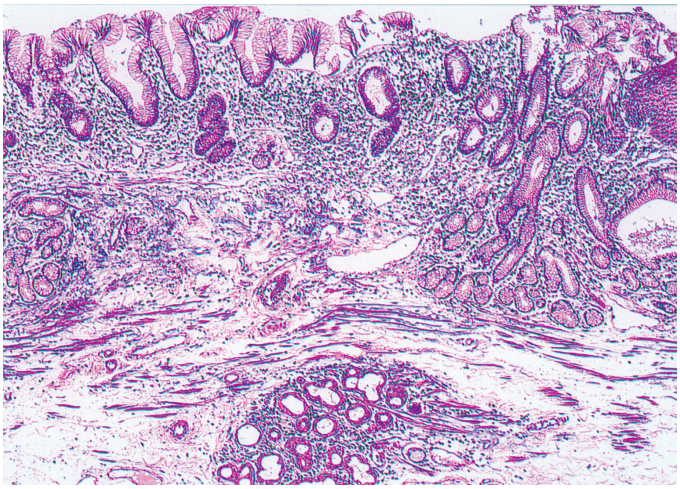

Fig. 1 Microscopic findings of short-segment Barrett's mucosa (SSBE). SSBE was characterized by the existence of submucosal esophageal glands covered by columnar mucosa, in addition to double muscularis mucosae of the esophagus (hematoxylin and eosin, × 100).

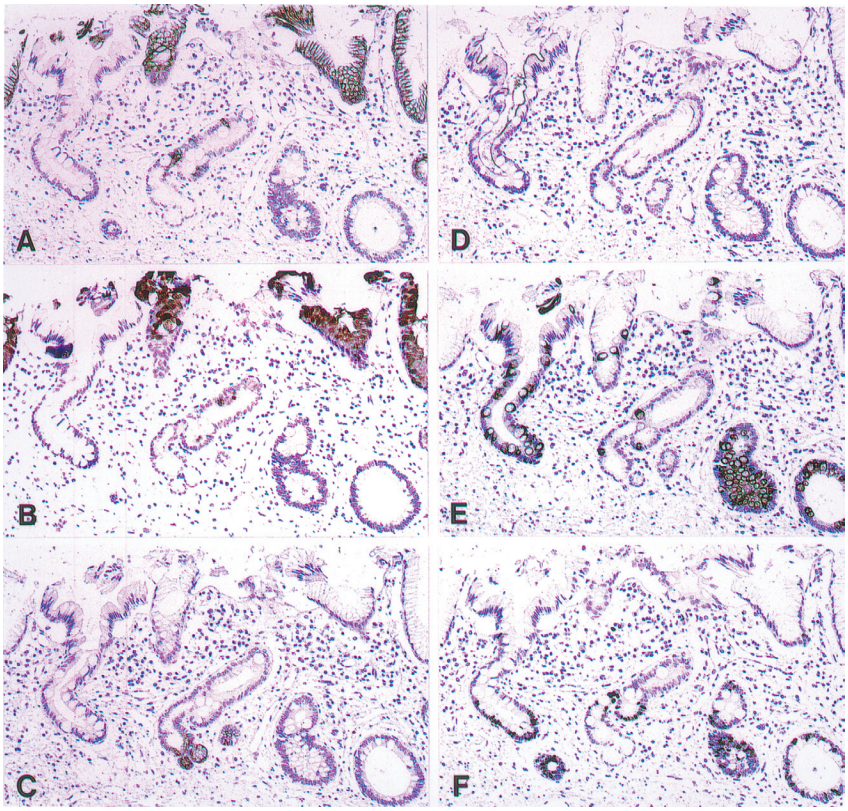

Fig. 2 Immunohistochemical findings of short-segment Barrett's mucosa (SSBE). MUC5AC (A, left, top; LSAB method, × 125); HGM (B, left, middle; LSAB method, × 125); MUC6 (C, left, bottom; LSAB method, × 125); CD10 (D, right, top; LSAB method, × 125); MUC2 (E, right, middle; LSAB method, × 125) and Ki-67 (F, right, bottom; LSAB method, × 125).

cases for CD10 was 23.6 ± 12.9 %, while that of SSBE negative cases for CD10 was 14.4 ± 11.9 % ($p = 0.032$, statistical significance).

DISCUSSION

Immunohistochemically, we examined 27 cases of SSBE. SSBE cases were divided into two groups: one was gastric epithelium with low Ki-67 LI (14.4 %), and the other was metaplastic epithelium with intestinal metaplasia and high Ki-67 LI (23.6 %). This study is the first to examine mucin and CD10 expression, as well as Ki-67 LI of SSBE.

Barrett first described the columnar epithelium of the esophagus in 1950, and subsequentry the columnar lined esophagus has been called Barrett's esophagus (or Barrett' s mucosa). Barrett's mucosa has been shown to be inflammatory and reactive, and is lined by gastric epithelium with or without intestinal metaplasia [1, 2, 21-23].

Current advances in mucin analysis have demonstrated characteristics of mucins and several investigations reported specific clinical mucin expression of Barrett's esophagus (Barrett's mucosa) [24-28]. They concluded that intestinal metaplasia was a significant phenotype of Barrett's mucosa using analysis of both histopathology and mucin expression [29-32]. However, it has not been clarified whether early stages of Barrett' s mucosa show intestinal metaplasia; i.e., whether the intestinal phenotype is the primary phenotype or a subsequent phenotype. Therefore, we examined the mucin expression of SSBE because SSBE was suggested to be an early stage of traditional Barrett's esophagus.

Recently, although SSBE was proposed, a detailed definition of SSBE has not been established because only a few studies reported a histopathological definition of SSBE [3, 4]. We defined SSBE as short segments (0.5-1.5 cm in length) of columnar epithelium of the esophageal mucosa, adjacent to the esophago-gastric junction. We also confirmed submucosal esophageal glands and double muscularis mucosae below the esophageal columnar epithelium. A previous study reported intestinal metaplasia at the esophago-gastric junction without clinical (traditional) Barrett's esophagus, i.e., SSBE with intestinal metaplasia [4]. However, while no extensive studies demonstrated mucin expression of SSBE, a number of studies examined mucin expression of clinical Barrett' s esophagus, in addition to adenocarcinoma arising from Barrett's esophagus. We examined the mucin expression and Ki-67 LI of SSBE [33]. CD10 expression, as a marker of the intestinal phenotype, was well correlated with intestinal metaplasia on the hematoxylin-eosin sections [9-11]. Interestingly, SSBE cases were divided into two groups: one was gastric epithelium type with low Ki-67 LI which showed no apparent intestinal metaplasia, and the other group was metaplastic epithelium type with incomplete intestinal metaplasia which exhibited high Ki-67 LI. It is suggested that the latter group with high Ki-67 was more important as a premalignant lesion of esophageal adenocarcinoma near the esophago-gastric junction. Several studies reported that the esophageal adenocarcinomas frequently expressed intestinal mucin phenotypes, and speculated that the adenocarcinomas arose from intestinal metaplasia of the Barrett's esophagus [25-28]. These results were thought to support our conclusion, i.e., significance of incomplete intestinal metaplasia with high Ki-67 LI as a premalignant lesion of Barrett's adenocarcinoma.

REFERENCES

1) Paull A, Trier JS, Dalton MD, Camp RC, Loeb P, Goyal RK.: The histologic spectrum of Barrett's esophagus. N Engl J Med 295: 476-480, 1976
2) Nishimaki T, Holscher AH, Schuler M, Becker K, Muto T, Siewert JR.: Chronic esophagitis and subsequent morphological changes of the esophageal mucosa in Barrett's esophagus: a histological study of esophagectomy specimens. Surg Today 24: 203-209, 1994
3) Spechler SJ.: Short and ultrashort Barrett's esophagus-what does it mean? Semin Gastrointest Dis 8: 59-67, 1997
4) Nakamura M, Kawano T, Endo M, Iwai T.: Intestinal metaplasia at the esophagogastric junction in Japanese patients without clinical Barrett's esophagus. Am J Gastroenterol 94: 3145-3149, 1999
5) Dekker J, Rossen JW, Buller HA, Einerhand AW.: The MUC family: an obituary. Trends Biochem Sci 27: 126-131, 2002
6) Wittel UA, Goel A, Varshney GC, Batra SK.: Mucin antibodies - new tools in diagnosis and therapy of cancer. Front Biosci 6: D1296-1310, 2001
7) Moniaux N, Escande F, Porchet N, Aubert JP, Batra SK.: Structural organization and classification of the human mucin genes. Front Biosci 6: D1192-1206, 2001
8) Fowler J, Vinall L, Swallow D.: Polymorphism of the human muc genes. Front Biosci 6: D1207-1215, 2001
9) McIntosh GG, Lodge AJ, Watson P, Hall AG, Wood K,

Anderson JJ, Angus B, Horne CH, Milton ID.: NCL-CD10-270: a new monoclonal antibody recognizing CD10 in paraffin-embedded tissue. Am J Pathol 154: 77-82, 1999
10) Haralambidou S, Melo JV, Catovsky D.: Different reactivity of monoclonal antibodies against common acute lymphoblastic leukaemia antigen (CD10). J Clin Pathol 40: 490-493, 1987
11) Mechtersheimer G, Moller P.: Expression of the common acute lymphoblastic leukemia antigen (CD10) in mesenchymal tumors. Am J Pathol 134: 961-965, 1989
12) Ng IO, Na J, Lai EC, Fan ST, Ng M.: Ki-67 antigen expression in hepatocellular carcinoma using monoclonal antibody MIB1. A comparison with proliferating cell nuclear antigen. Am J Clin Pathol 104: 313-318, 1995
13) Ueda T, Aozasa K, Tsujimoto M, Ohsawa M, Uchida A, Aoki Y, Ono K, Matsumoto K.: Prognostic significance of Ki-67 reactivity in soft tissue sarcomas. Cancer 63: 1607-1611, 1989
14) Gerdes J, Lemke H, Baisch H, Wacker HH, Schwab U, Stein H.: Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. J Immunol 133: 1710-1715, 1984
15) Gerdes J, Schwab U, Lemke H, Stein H.: Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. Int J Cancer 31: 13-20, 1983
16) Kashiwagi H, Kijima H, Dowaki S, Ohtani Y, Tobita K, Yamazaki H, Nakamura M, Ueyama Y, Tanaka M, Inokuchi S, Makuuchi H.: MUC1 and MUC2 expression in human gallbladder carcinoma: a clinicopathological study and relationship with prognosis. Oncol Rep 8: 485-489, 2001
17) Kashiwagi H, Kijima H, Dowaki S, Ohtani Y, Tobita K, Tsukui M, Tanaka Y, Matsubayasi H, Tsuchida T, Yamazaki H, Nakamura M, Ueyama Y, Tanaka M, Tajima T, Makuuchi H.: DF3 expression in human gallbladder carcinoma: significance for lymphatic invasion. Int J Oncol 16: 455-459, 2000
18) Kijima H, Chino O, Oshiba G, Tanaka H, Kenmochi T, Kise Y, Shimada H, Abe Y, Tokunaga T, Yamazaki H, Nakamura M, Tanaka M, Makuuchi H, Ueyama Y.: Immunohistochemical MUC1 (DF3 antigen) expression of human esophageal squamous cell carcinoma. Anticancer Res 21: 1285-1289, 2001
19) Matsubayashi H, Kuraoka K, Kobayashi Y, Yokota T, Iiri Y, Shichijo K, Tada T, Satoh K, Kijima H.: Ruptured epidermoid cyst and haematoma of spleen: a diagnostic clue of high levels of serum carcinoembryonic antigen, carbohydrate antigen 19-9 and Sialyl Lewis x. Dig Liver Dis 33: 595-599, 2001
20) Nagata J, Kijima H, Hasumi K, Suzuki T, Shirai T, Mine T.: Adenocarcinoma and multiple adenomas of the large intestine, associated with Cronkhite-Canada syndrome. Dig Liver Dis 35: 434-438, 2003
21) Glickman JN, Chen YY, Wang HH, Antonioli DA, Odze RD.: Phenotypic characteristics of a distinctive multilayered epithelium suggests that it is a precursor in the development of Barrett's esophagus. Am J Surg Pathol 25: 569-578, 2001
22) van Sandick JW, van Lanschot JB, van Felius L, Haringsma J, Tytgat GN, Dekker W, Drillenburg P, Offerhaus GJ, ten Kate FJ.: Intestinal metaplasia of the esophagus or esophagogastric junction: evidence of distinct clinical, pathologic, and histochemical staining features. Am J Clin Pathol 117: 117-125, 2002
23) Oberg S, Johansson J, Wenner J, Walther B.: Metaplastic columnar mucosa in the cervical esophagus after esophagectomy. Ann Surg 235: 338-345, 2002
24) Arul GS, Moorghen M, Myerscough N, Alderson DA, Spicer RD, Corfield AP.: Mucin gene expression in Barrett's oesophagus: an in situ hybridisation and immunohistochemical study. Gut 47: 753-761, 2000
25) Guillem P, Billeret V, Buisine MP, Flejou JF, Lecomte-Houcke M, Degand P, Aubert JP, Triboulet JP, Porchet N.: Mucin gene expression and cell differentiation in human normal, premalignant and malignant esophagus. Int J Cancer 88: 856-861, 2000
26) Warson C, Van De Bovenkamp JH, Korteland-Van Male AM, Buller HA, Einerhand AW, Ectors NL, Dekker J.: Barrett's esophagus is characterized by expression of gastric-type mucins (MUC5AC, MUC6) and TFF peptides (TFF1 and TFF2), but the risk of carcinoma development may be indicated by the intestinal-type mucin, MUC2. Hum Pathol 33: 660-668, 2002
27) Chinyama CN, Marshall RE, Owen WJ, Mason RC, Kothari D, Wilkinson ML, Sanderson JD.: Expression of MUC1 and MUC2 mucin gene products in Barrett' s metaplasia, dysplasia and adenocarcinoma: an immunopathological study with clinical correlation. Histopathology 35: 517-524, 1999
28) Endo T, Tamaki K, Arimura Y, Itoh F, Hinoda Y, Hareyama M, Irimura T, Fujita M, Imai K.: Expression of sulfated carbohydrate chain and core peptides of mucin detected by monoclonal antibodies in Barrett's esophagus and esophageal adenocarcinoma. J Gastroenterol 33: 811-815, 1998
29) Reis CA, David L, Correa P, Carneiro F, de Bolos C, Garcia E, Mandel U, Clausen H, Sobrinho-Simoes M.: Intestinal metaplasia of human stomach displays distinct patterns of mucin (MUC1, MUC2, MUC5AC, and MUC6) expression. Cancer Res 59: 1003-1007, 1999
30) Inada K, Nakanishi H, Fujimitsu Y, Shimizu N, Ichinose M, Miki K, Nakamura S, Tatematsu M.: Gastric and intestinal mixed and solely intestinal types of intestinal metaplasia in the human stomach. Pathol Int 47: 831-841, 1997
31) Teixeira A, David L, Reis CA, Costa J, Sobrinho-Simoes M.: Expression of mucins (MUC1, MUC2, MUC5AC, and MUC6) and type 1 Lewis antigens in cases with and without Helicobacter pylori colonization in metaplastic glands of the human stomach. J Pathol 197: 37-43, 2002
32) Allen A, Hutton DA, Pearson JP. The MUC2 gene product: a human intestinal mucin.: Int J Biochem Cell Biol 30: 797-801, 1998
33) Terasaki-Fukuzawa Y, Kijima H, Suto A, Takeshita T, Iezumi K, Sato S, Yoshida H, Sato T, Shimbori M, Shiina Y.: Decreased nm23 expression, but not Ki-67

labeling index, is significantly correlated with lymph node metastasis of breast invasive ductal carcinoma. Int J Mol Med 9: 25-29, 2002