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Mucin Histochemistry by Paradoxical Concanavalin A Staining in Early Gastric Carcinomas

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Phenotypic expression of tumor cells was investigated in 33 early gastric carcinomas by mucin histochemistry using paradoxical concanavalin A staining. This staining method had been developed to differentiate 3 classes of mucins located at various sites of the alimentary tract. Twenty-five (76%) tumors contained mixtures of neutral or acid class Ⅱ mucin and class Ⅲ mucin, suggesting the origin of multipotential stem cells. The surface mucous cell expression was more dominant than the pyloric gland or intestinal phenotypes in the well-and poorly differentiated adenocarcinomas. The intestinal properties of the tumor cells were noted not only in the well-differentiated but also in the poorly differentiated or signet ring cell carcinomas, not closely being related to the presence of background intestinal metaplasia. Signet ring cell carcinomas revealed a distinct pattern of mucin histochemistry compared with the other types.

Key Words : *Paradoxical Con A staining, Mucin histochemistry, Early gastric carcinoma, Intestinal metaplasia*

INTRODUCTION

Katsuyama and Spicer first reported that various treatments prior to concanavalin A (Con A) staining had permitted differentiation of 3 main classes of complex carbohydrates in the rat alimentary tract (Katsuyama and Spicer, 1978), and this paradoxical Con A (PCA) staining has recently been applied to various experimental and human gastric lesions. In general, Con A-reac-tive mucins lose their affinities for Con A when they are oxidized with periodate (class Ⅰ). The name-paradoxical Con A staining-was given because of the paradoxical enhancement of Con A reactivities of some mucins, which are prevalent in the gastric mucosa, disappearing (class Ⅱ) or persisting (class Ⅲ) with interposing reduction steps. By classifying the gastric mucins into class Ⅰ, Ⅱ, and Ⅲ, it is possible to determine the phenotype of the mucous cells in neoplastic or non-neoplastic gastric tissue as either gastric or intestinal, or more precisely (Sugunuma et al., 1981 ; T-atematsu et al., 1980). Thus PCA staining m-ay be a crucial tool in gastric carcinogenesis study. We performed PCA staining on 33 early gastric carcinomas to investigate their histogenesis and the possible role of the intestinal metaplasia in the carcinogenesis.

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MATERIALS AND METHODS

Thirty-three consecutive cases of early gastric carcinoma diagnosed at Korea Cancer Center Hospital during the period from January to June, 1989 were selected for this study. Their hematoxylin-eosin stained sections were reviewed, and the cases were classified into well-differentiated or poorly

differentiated adenocarcinomas, according to the degree of glandular differentiation. Tumors consisting of signet ring cells in more than a half portion were designated as signet ring cell carcinomas. The presence and type (complete or incomplete) of intestinal metaplasia were estimated in the adjacent mucosa, depending on the presence or absence of the goblet cells, columnar cells and Paneth cells. Representative sections of each case were subjected to paradoxical concanavalin A staining with two modified procedures. The deparaffinized sections were digested with diastase for removal of glycogen. They were oxidized with 1% periodic acid for 1 hour and then subjected to amine blockage with leukofuchsin (Schiff's solution) for 10 minutes (PAS-ConA-HRP method), or reduced with 0.2% sodium borohydride for 2 minutes (PA-Re-ConA-HRP method) before staining with concanavalin A and horseradish peroxidase. The sections were treated with Con A (Sigma) solution for 30 minutes and horseradish peroxidase (Sigma) solution for 20 minutes after washing with phosphate buffered saline and phosphate buffer. An H_2O_2-diaminobenzidine (Sigma) solution was applied for coloration, followed by alcian-blue staining in the latter procedure.

RESULTS

Among 33 cases of early gastric carcinoma subjected to this study, 20 carcinomas were confined to the mucosa, and 13 carcinomas had invaded the mucosa and submucosa. Four of the latter cases had regional lymph node metastases. All tumors were located in the antrum or pylorus, occasionally extending to the lower body of the stomach.

Histologically, 15 cases showed good glandular differentiation of tumor cells and were classified as well-differentiated adenocarcinoma. Ten cases consisted of diffusely infiltrating tumor cells forming abortive glandular structures, being classified as poorly differentiated adenocarcinoma. Eight carcinomas were predominantly composed of signet ring cells and designated signet ring cell carcinoma.

The results of mucin histochemistry by PCA staining is summarized in Table 1. In 25 cases (76%), the tumors contained a mixture of mucins of more than one type, and 8 carcinomas contained a single type mucin. Neutral class Ⅱ mucin, staining purple with PAS-ConA-HRP and unstaining with PA-Re-ConA-HRP, which is normally present in the surface mucous cells of the gastric mucosa, was the most prevalent, being detected in 28 cases (85%) in either pure or mixed from (Fig. 1). Blue acid class Ⅱ mucin, staining with PA-Re

Table 1. Results of Paradoxical Concanavalin A Staining on 33 Early Gastric Carcinomas

Mucin class	W/D	P/D	SRC	Total
Ⅱn	5	2		7
Ⅱn+Ⅱa	5			5
Ⅱn+Ⅲ	2	4		6
Ⅱn+Ⅱa+Ⅲ	3	4	2	9
Ⅱa			1	1
Ⅱa+Ⅲ			3	3
Ⅲ+Ⅱn			1	1
Ⅲ+Ⅱa			1	1
Total	15	10	8	33

W/D : well-differentiated adenocarcinoma, P/D : poorly differentiated adenocarcinoma, SRC : signet ring cell carcinoma, Ⅱn : neutral class Ⅱ mucin, Ⅱa : acid class Ⅱ mucin, Ⅲ : class Ⅲ mucin

-ConA-HRP, which is of the intestinal type, was observed in 19 cases, mostly in a mixed pattern with other types (Fig. 2). Class Ⅲ mucin, reddish brown on PAS-ConA-HRP, and brown or bluish-brown on PA-Re-ConA-HRP, characteristic of pyloric gland, cardiac gland, Brunner's gland and mucous neck cells, was noted in 20 cases as a minor participant (Fig. 3).

Considering the histologic type of tumors and the mucin histochemistry, cells of all well-differentiated adenocarcinomas showed major phenotypic expression of surface mucous cells. Tumor cells having intestinal or pyloric gland type mucin were found in small numbers in 8 and 5 cases, respectively. Poorly differentiated adenocarcinomas showed a similar pattern of mucin histochemistry to that of well-differentiated adenocarcinomas. They contained mucin of the surface mucous cell type as a major component, with occasionally associated pyloric gland and/or intestinal type mucin. PCA staining of signet ring cell carcinomas revealed three distinct types of signet ring cells; cells containing acid class Ⅱ mucin, cells containing neutral class Ⅱ mucin, and cells containing class Ⅲ mucin. Four cases consisted predominantly of intestinal type cells (Fig. 4), 2 carcinomas were predominantly of the pyloric gland cell type (Fig. 5), and the remaining 2 were composed of mixtures of cells having class Ⅱ or Ⅲ mucins (Fig. 6).

As shown in Table 2, complete or incomplete intestinal metaplasia of non-neoplastic mu-

Table 2. Incidence of Intestinal Metaplasia and Intestinal Expression by Tumor Cells

	W/D(15*)	P/D(10)	SRC(8)	Total
IM Ⅰ	5	2	3	10
IM Ⅱ	7	3	3	13
Total	12(80%)	5(50%)	6(75%)	23(70%)
Ⅱa mucin	8(53%)	4(40%)	7(90%)	19(58%)
with IM Ⅰ	2	2	2	6
with IM Ⅱ	3		3	6

W/D : well-differentiated adenocarcinoma, P/D : poorly differentiated adenocarcinoma, SRC : signet ring cell carcinoma, IM Ⅰ : complete intestinal metaplasia, IM Ⅱ : incomplete intestinal metaplasia, Ⅱa : acid class Ⅱ mucin

*Value represents total cases.

EXPLANATION OF FIGURES

Fig. 1. A poorly differentiated adenocarcinoma shows tumor cells with neutral class Ⅱ mucin stained purple with PAS-ConA-HRP staining (x50).

Fig. 2. A mixed staining pattern in a well-differentiated adenocarcinoma for mucins of blue intestinal type, unstained surface mucous cell type, and brown pyloric gland type on PA-Re-ConA-HRP staining (x50).

Fig. 3. Tumor cells of pyloric gland type containing bluish brown class Ⅲ mucin. Unstained areas represent surface mucous cell phenotype (PA-Re-ConA-HRP, x50).

Fig. 4. Signet ring cells, containing acid class Ⅱ mucin, staining blue on PA-Re-ConA-HRP staining. Adjacent normal pyloric glands show class Ⅲ mucin (x50).

Fig. 5. A signet ring cell carcinoma is predominantly composed of pyloric gland type cells (PA-Re-ConA-HRP, x50).

Fig. 6. urface mucous cell and pyloric gland cell type mucins in signet ring cells stain purple and brown on PAS-ConA-HRP staining (x50).

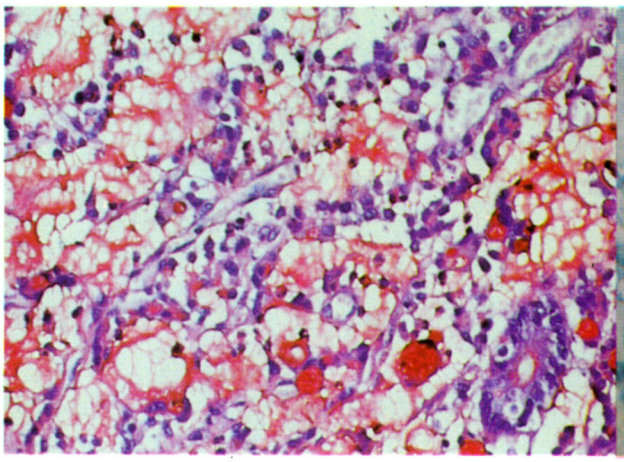

Fig. 1.

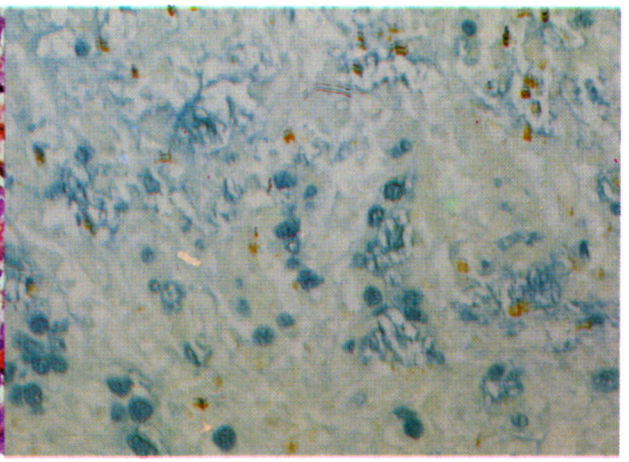

Fig. 2.

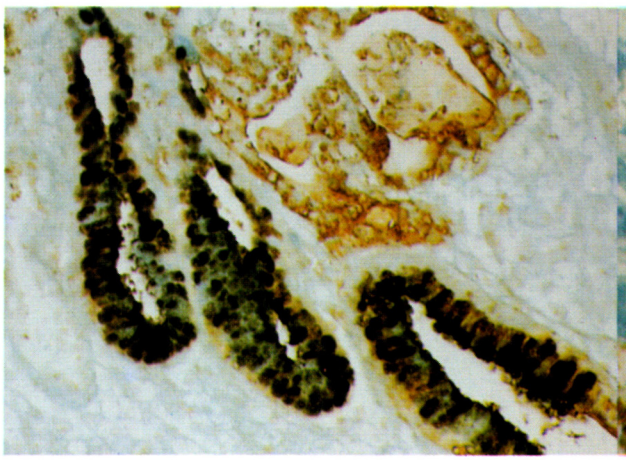

Fig. 3.

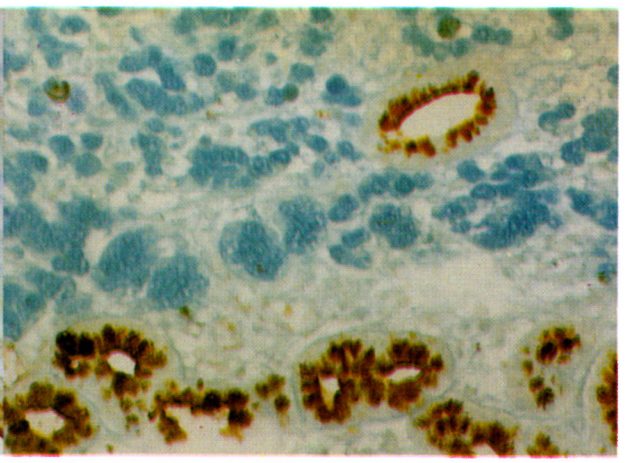

Fig. 4.

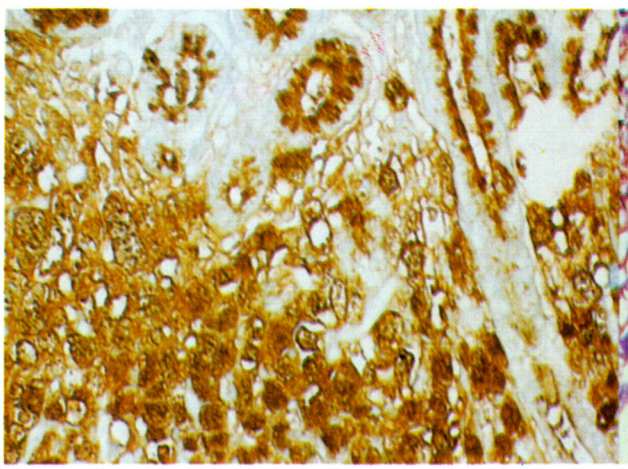

Fig. 5.

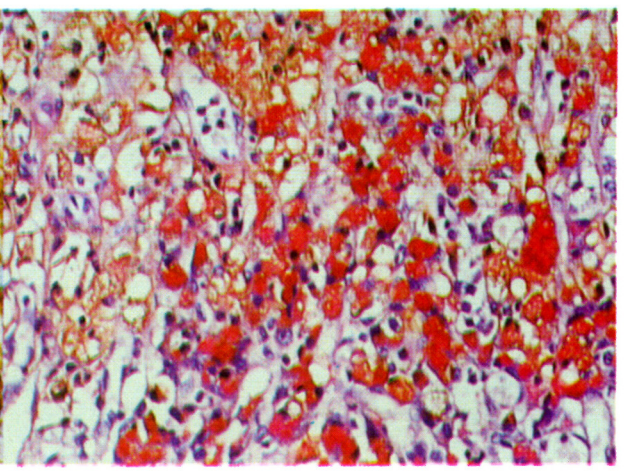

Fig. 6.

cosa, and intestinal expression of tumor cells, represented by acid class II mucin, were observed in 23 (70%) and 19 (58%) cases, respectively, with 12 overlapping cases. Intestinal metaplasia was found with 12/15 well-differentiated, 5/10 poorly differentiated, and 6/8 signet ring cell carcinomas, showing no significant association with a histologic type. Intra-tumoral intestinal expression was not preferentially detected in a histologic type, either, but occurred most frequently in signet ring cell carcinomas. There was no demonstrable difference in the mucin histochemistry according to the depth of invasion.

DISCUSSION

The present study demonstrated that the majority (76%) of early gastric carcinomas showed mixed phenotypic expression by mucin histochemistry using paradoxical Con A staining. The phenotypic expression of tumor cells is thought to resemble, but not absolutely represent, the tissue of origin of the tumor cells. We selected cases of early gastric cancer assuming that the tumor cells had less altered in their phenotypes from those of the origin of the tumors than in advanced carcinomas. Thus the various mixed pattern of phenotypic expression shown in this study is considered to better reflect the multidirectional differentiation of the tumor cells which might originate from the multipotential stem cells rather than different origins. Almost every component of gastric and intestinal mucosa, including parietal cells and Paneth cells, have been identified in gastric carcinomas, possibly as a result of cell differentiation (Capella et al., 1984; Lev and DeNucci, 1989). However, the surface mucous cells of the gastric mucosa seem to be the most important contributor to gastric carcinoma since neutral type II mucin was detected in the largest amount and in 85% of the cases included in this study. Similar results have recently been reported (Fiocca et al.,1990; Tatematsu et al.,1990), with an increased tendency of foveolar cell component in the intramucosal tumors (Fiocca et al.,1987).

Lauren (1963) classified gastric cancers histologically into two main groups: the intestinal type and the diffuse type. A close correlation between the presence of intestinal metaplasia, especially of the incomplete type, and the well-differentiated intestinal type adenocarcinoma has been observed in human gastric cancers (Filipe et al.,1985; Kawachi et al., 1976: Segura and Montero, 1983; Sipponen et al., 1980), while Tatematsu et al. (1983, 1987, 1989, 1990) repeatedly emphasized that intestinal metaplasia might not necessarily be a preneoplastic change in the gastric carcinogenesis. Using PCA staining, they observed the independent occurrence of intestinal metaplasia and intestinal expression in the precancerous and cancerous lesions of the rat stomach (Tatematsu et al.,1983). Rather than intestinal metaplasia, they considered the changes of pepsinogen isozymes in the pyloric glands and the pyloric metaplasia of the fundic mucosa to indicate the preneoplastic change (Tatematsu et al.,1987, 1989). Furihata et al. (1984) described the prevalence of intestinal type cells not only in intestinal but also in diffuse type human gastric cancers. Our study also demonstrated that the intestinal phenotype of tumor cells was present in all types of carcinoma without a specific association with any one. As for the relationship between the intestinal metaplasia and intestinal property of the tumor cells, 7 out of 19 cases with intestinal expression lacked background intestinal metaplasia of the non-neoplastic mucosa, and only about half (12/23) of the tumors with intestinal metaplasia expressed intestinal property. These results are in accordance with the conclusion by Tatematsu et al. that the two phenomena are independent. However, overall incidence of the intestinal metaplasia in these cancer-bearing gastric mucosa was high (70%), with a higher incidence in the well-differentiated (80%) group than in the poorly differentiated (50%) group. This fact suggests that the differentiation of the tumor cells (not phenotypic expression) might be influenced by a preexisting intestinal metaplastic change.

Fiocca et al. (1987) suggested some correla-

tion between the pattern of diffuse, glandular, or mucoid growth and the predominant cell type of the tumor. Such a tendency was not demonstrated in the present study. They described that foveolar cells were prominent in intramucosal signet ring cell carcinomas, but four out of 8 tumors in this study were predominantly of the intestinal type, and 2 were of the pyloric gland type, with the remaining 2 being surface mucous cell types. This pattern was more unique than in the other types, since neither intestinal nor pyloric gland type cells were found to be major components in well- or poorly differentiated adenocarcinomas. Tatematsu et al. (1986) observed high incidence of intestinal type cells in human signet ring cell carcinomas transplanted into nude mice. They described that 19 of 40 consisted entirely of gastric type cells, but did not discriminate them into either pyloric gland or surface mucous cell type. Foveolar cell phenotype was reported to be prominent in intramucosal signet ring cell carcinomas (Fiocca et al.,1987). On the contrary, in our study, 7 out of 8 signet ring cell carcinomas had intestinal properties, with accompanying intestinal metaplasia in 6. Thus, signet ring cell carcinomas seemed to be more related to the presence of intestinal metaplasia than the other types. Moreover, class Ⅲ mucin, which was focally present in only 7 out of 25 well- and poorly differentiated carcinomas, was common in signet ring cell carcinomas. It is of interest that the signet ring cell carcinomas may have a distinct histogenesis, case by case, in spite of their indistinguishable cytology on routine histologic examination.

The histogenesis of gastric cancers and role of intestinal metaplasia in it still remain unclear. Recently new techniques, including a modified method of staining with labeled peanut agglutinin, the glactose oxidase-Schiff (GOS) reaction, and the sialidase-GOS reaction, have been developed for a more detailed discrimination of various cells of the alimentary tract (Tatematsu et al.,1986, 1989, 1990). Paradoxical Con A staining and other methods for mucin histochemistry would be very useful in the investigation of human gastric carcinogenesis.

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