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Precancerous Changes in the Stomach[1]

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Summary

Intestinal metaplasia is often associated with human gastric carcinoma. Intestinalization seems to be a typical example of abnormal differentiation and is possibly a precancerous state. For investigation of intestinal metaplasia, a method for visualizing disaccharidases using Tes-Tape was developed; this method was applied to many specimens of stomach surgically removed for the treatment of gastric carcinoma.

More than 130 specimens of human stomach were investigated. Intestinalization was classified into types I and II intestinal metaplasia. In type I intestinal metaplasia, sucrase, maltase, trehalase, alkaline phosphatase, goblet cells, and Paneth cells were present; while the type II intestinal metaplasia, sucrase and maltase were present but alkaline phosphatase and trehalase were absent. In type II, goblet cells were present but not Paneth cells.

The histochemical technique for sucrase was newly devised. Some of the villi with goblet cells in the area of intestinalization in the stomach were not stained by sucrase activity, although most of the villi were stained. The presence of a third type of metaplasia was suggested.

Purified sucrases obtained from the intestine and one case of type I intestinal metaplasia showed blood group reactivity due to the oligosaccharide side chain. However, purified sucrases obtained from two cases of type II intestinal metaplasia were negative in blood group reactivity.

A close relation between distribution of α-fetoprotein and carcinoembryonic antigen in gastric carcinoma and that in surrounding intestinal metaplasia is discussed.

Introduction

Stomach carcinoma is still very common in some countries in the world. Epidemiological and experimental data indicate that there is a latent period between carcinogenic impact and the development of cancer. The studies on this latent period, which may be called a "precancerous stage," are crucially important to understand the carcinogenic process and to conquer this ugly human disease.

Many investigations on various biochemical parameters have been carried out in an attempt to find critical differences between normal and cancerous cells. It has been generally accepted that cancerous cells lose specific enzymes and other markers that are found in the original tissue cells and acquire new markers such as AFP,[3] CEA's, and isozymes, which are not found in the original normal tissues.

Intestinal metaplasia, often associated with gastric carcinoma (2, 6, 13, 15), is more frequently found in the Japanese people than in other nationalities (5). It has been considered to be a possible precancerous state. This is supported also by the fact that striated cell borders or enzymes characteristic of intestinal epithelium are sometimes found in carcinoma cells. Intestinal metaplasia is found 4 times more frequently in specimens from patients with stomach cancer than in those from patients with gastric ulcer.

We devised a method for the visualization of intestinalization by detecting disaccharidases in specimens of human stomach removed surgically from patients with gastric carcinoma and ulcer, in order to investigate the relationship between intestinal metaplasia and gastric carcinoma (9, 16). Disaccharidases, including sucrase, maltase, and trehalase, were selected as marker enzymes and were detected by Tes-Tape and histochemical techniques using natural disaccharides as substrates. We previously reported the presence of intestinal metaplasia type I (complete type of intestinal metaplasia) and of intestinal metaplasia type II (incomplete type of intestinal metaplasia) (17).

The intestinal disaccharidases are located on the external surface of the enterocyte membrane. Kelly and Alpers (10) reported that human intestinal maltase and sucrase were glycoproteins and that blood group reactivity was associated with an oligosaccharide side chain covalently linked to the enzyme. We found that there was blood group reactivity in sucrase purified from intestine but not in that from some cases of intestinal metaplasia of the stomach.

Materials and Methods

Specimens. Specimens were obtained by gastrectomy of gastric carcinoma patients at the National Cancer Center Hospital, Tokyo.

Detection of Disaccharidases with Tes-Tape. A method of detecting disaccharidases with Tes-Tape has been developed (9, 16). The stomach specimens were rushed into the laboratory from the operating room. The surfaces of the mucosa were gently and carefully washed with cold 0.9%

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[3] The abbreviations used are: AFP, α-fetoprotein; CEA, carcinoembryonic antigen.

NaCl solution. The stomach was spread out on a thick glass plate. A solution of 5% sucrose, maltose, or trehalose in 10 mM sodium phosphate buffer was sprayed on the surface of the mucosa and was incubated at 37° for 5 min. Then the entire surface of the stomach was covered with many pieces of Tes-Tape. Glucose was enzymatically cleaved from disaccharides in areas with disaccharidases and the Tes-Tape turned green.

Sucrase Preparation. Sucrase of intestine or intestinal metaplasia of the stomach was purified by the method of Cogoli *et al.* (1) using papain solubilization, gel filtration on BioGel P-300, and chromatography on DEAE-cellulose. Intestine was the jejunum part which was removed for the treatment of colon tumor. All the specimens were from patients with blood types B and A; the area of intestinal metaplasia of the stomach was located by the Tes-Tape method.

Blood Group Antigenicity. Blood group testing was performed by the hemagglutination inhibition test using a microtitration system (8). The end-point titer was determined by the maximum dilution that showed half-inhibition of complete agglutination of RBC in the wells.

Histochemical Procedure. The chromogenic reagent was prepared by adding to a 0.1 M phosphate buffer (pH 6.8) the following reagents: phenazine methosulfate, 0.05 mg/ml; nitro blue tetrazolium, 0.25 mg/ml; and glucose oxidase, 0.15 mg/ml. The sucrose solution was 10 mg/ml in distilled water. The 15-μm-thick sections prepared in cryostat were preincubated in 0.9% NaCl solution on the glass slide for 60 min at 37° to exhaust the endogenous substrate. Excess NaCl solution was removed with a filter paper. Substrate solution:chromogenic reagent (1:9) was dropped on the section, a coverslip was placed on it, and it was incubated for 3 hr at 37°. This length of time usually gave a suitable blue stain. The section was washed carefully in 15% ethanol and embedded in glycerol with a coverslide (7).

Results

Disaccharidases as Markers of Intestinalization. Fig. 1 shows the gross appearance of a resected stomach from a 63-year-old man with stomach carcinoma. The stomach carcinoma was located in the antrum and diagnosed histologically as a tubular adenocarcinoma. As shown in Fig. 2, a strong green color with Tes-Tape was obtained in the antrum except for the cancerous portion by using sucrose as a substrate. Spotted green color reactions were obtained in the corpus area. The cancerous area usually gave a negative reaction, although the surrounding noncancerous area gave a positive reaction for sucrase. The area of intestinalization visualized by this Tes-Tape technique agreed well with the area of intestinalization that was shown by histological examination of 5-mm strips parallel to the lesser curvature (9).

The adoption of this Tes-Tape method made it possible to analyze many specimens of resected stomach efficiently and rapidly.

Presence of 2 Types of Intestinalization. More than 130 specimens of human stomach were examined. Some specimens were subjected also to the test for alkaline phosphatase and histological examination after this rapid disaccharidase test. From the data obtained on many specimens we could classify intestinalization into 2 types, *i.e.*, type I and type II intestinal metaplasia. In type I intestinal metaplasia, sucrase, maltase, trehalase, alkaline phosphatase, goblet cells, and Paneth cells were present; while in type II intestinal metaplasia, sucrase, maltase, and goblet cells were present but alkaline phosphatase, trehalase, and Paneth cells were absent. Type I may be a complete form of intestinalization, whereas type II may be an incomplete form. It is also possible to speculate that type II is a degenerated form that is derived from type I intestinalization. However, statistical analysis indicated that no preferential development of cancer occurred in either type I or type II intestinalization.

Intestinalization and Stomach Cancer. Ninety-six of 117 cases of stomach carcinoma were found in the area showing either type I or type II intestinalization. Fifty-five cases of type I and 41 cases of type II were found.

Tumors that arose from areas of intestinalization were mainly intestinal adenocarcinomas. Only 18% of 117 cases of stomach cancer arose from areas where intestinalization was not observed. Most of these carcinomas were diffuse-type adenocarcinoma or signet ring cell adenocarcinoma.

Histochemical Demonstration of Sucrase in Intestinal Metaplasia. Histochemical examination of sucrase was carried out in order to investigate the presence of disaccharidase markers. As shown in Fig. 3, in most villi, in areas of intestinalization with positive Tes-Tape test, the reaction for sucrase was definitely positive. However, some villi with areas of intestinal metaplasia showed a negative reaction for sucrase although goblet cells were demonstrated histologically. A typical case is shown in Fig. 3. Sucrase reaction was observed usually on the top of the villi but not in the crypt, just as in the case of normal intestine. The absence of sucrase in some villi even on the top portion means that intestinalization at the histological level could be classified into more than 3 types, as will be discussed later.

Blood Group Activity of Purified Sucrase from Intestinal Metaplasia. Sucrase from human intestine was purified to give a single-band homogeneity on polyacrylamide gel electrophoresis (Fig. 4). Duplicate samples were applied to 5% polyacrylamide gels in Tris-glycine buffer (pH 9.4) and separated at 2 ma/gel for 150 min. One set of gels was stained with Coomassie blue, and the other was cut into many slices with a gel slicer. These slices were extracted individually. The fractions were assayed both for sucrase activity and blood group determinant activity. Similarly, sucrase from type I intestinal metaplasia (complete form) was purified to give a single-band homogeneity on polyacrylamide gel electrophoresis. Each purified sucrase preparation from intestine and type I metaplasia was found to have blood group activity, as determined by hemagglutination inhibition (Chart 1, *a* and *b*). It is clear that sucrase activity migrated to Fractions 9 to 10, which is in agreement with the position of the Coomassie blue-positive staining. In addition, blood group activity was also found in Fractions 9 to 10 (Chart 1, *a* and *b*). However, purified sucrase from type II intestinal metaplasia (incomplete type) lacked blood group reactivity (Chart 1*c*) in this preliminary experiment.

Blood group activity that was found in the top portion of

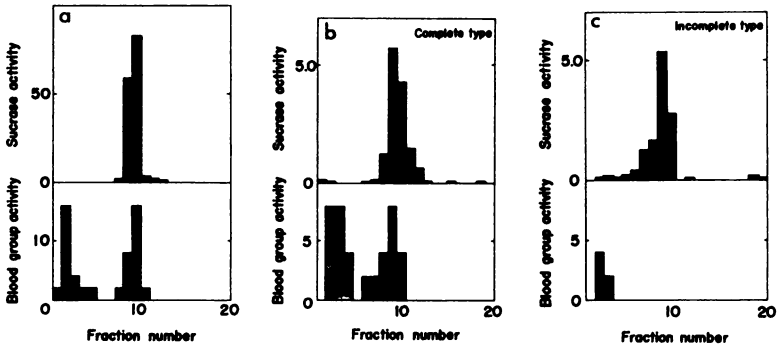

Chart 1. Polyacrylamide gel electrophoresis of *(a)* purified intestinal sucrase, *(b)* purified sucrase from type I intestinal metaplasia, and *(c)* purified sucrase from type II intestinal metaplasia. All sucrases were purified from specimen of patients with blood type Group B. About 7 to 27 μg of sucrase were applied on 5% Tris-glycine polyacrylamide gels and separated at 2 ma/gel for 150 min. The gel was cut with a gel slicer and the slices were eluted. The resulting gel fractions were assayed for sucrase activity and blood group activity. Sucrase activity is expressed as absorbance at 530 nm. Blood group activity is expressed as titer of dilutions of the purified sucrase solution.

the polyacrylamide gel may have been derived from the carbohydrate moiety adsorbed during the preparation of purified sucrase. Naturally, reactions showing blood group activity in the top portion of the gel did not show sucrase activity nor was the gel stained by Coomassie blue.

Discussion

We could classify intestinal metaplasia into at least 2 types from the data of Tes-Tape test on many specimens of resected stomach. Type I (complete type) intestinalization showed positive reaction of sucrase, maltase, trehalase, and alkaline phosphatase, and it contained goblet and Paneth cells. In type II (incomplete type) intestinalization, trehalase and alkaline phosphatase activity were negative but sucrase and maltase were positive. These areas contained goblet but no Paneth cells.

Sometimes both type I and type II intestinalization were found in a single stomach. Among the specimens with intestinalization, one-third showed type I intestinalization, one-third showed type II intestinalization, and one-third showed both types. The frequencies of cases of cancer originating from types I and II were not significantly different.

By using histochemical techniques we demonstrated a new type of intestinal metaplasia, type III. Some villi with intestinalization with goblet cells but without sucrase activity existed, at least as microscopic foci.

Both type I and type II intestinalization showed definite sucrase activity. Purified sucrase from type I intestinalization was found to have blood group activity, but purified sucrase from type II intestinalization lacked blood group activity. Denk *et al.* (3) have reported that some stomach carcinomas showed blood group determinants while others did not.

Disaccharidase activity was demonstrated to be located in the cell membrane of the intestinalized area. It is also frequently mentioned that the cell membrane is altered during the carcinogenic processes. The appearance of disaccharidases as a possible precancerous change and their disappearance during malignant conversion suggest that abnormal differentiation associated with cellular surface changes is related to malignant transformation.

Kitaoka *et al.* (11) indicated that AFP was found in the tumor tissues of about 30% of well-differentiated stomach adenocarcinomas. A high content of AFP was demonstrated to be present also in areas with intestinal metaplasia surrounding cancer. However, AFP was not detected or its content was very low in poorly differentiated and in anaplastic cancers. The content of AFP in intestinal metaplasia surrounding these types of gastric carcinoma was also low (11, 12).

In well-differentiated stomach adenocarcinoma, CEA was sometimes present on the surface of the tumor cells, but in a smaller amount than in colonic cancers. CEA was detected on the luminal surface of the cells in intestinal metaplastic areas but was absent in anaplastic stomach cancers (4). There is a good correlation between gastric carcinoma and surrounding intestinal metaplasia based on the intensity of CEA staining by fluorescence antibody (14).

Epidemiological analyses have shown that Japanese emigrants and their descendents have stomach carcinoma and also intestinal metaplasia less frequently than do the residents of Japan (5).

All the above findings support the idea that intestinalization is closely related to the development of stomach cancer. Further investigation on molecular biology of intestinalization is desirable.

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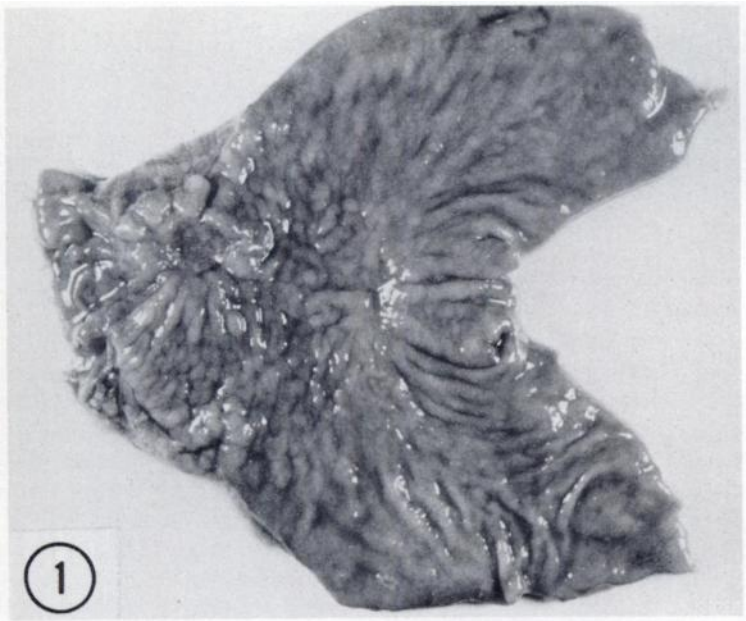

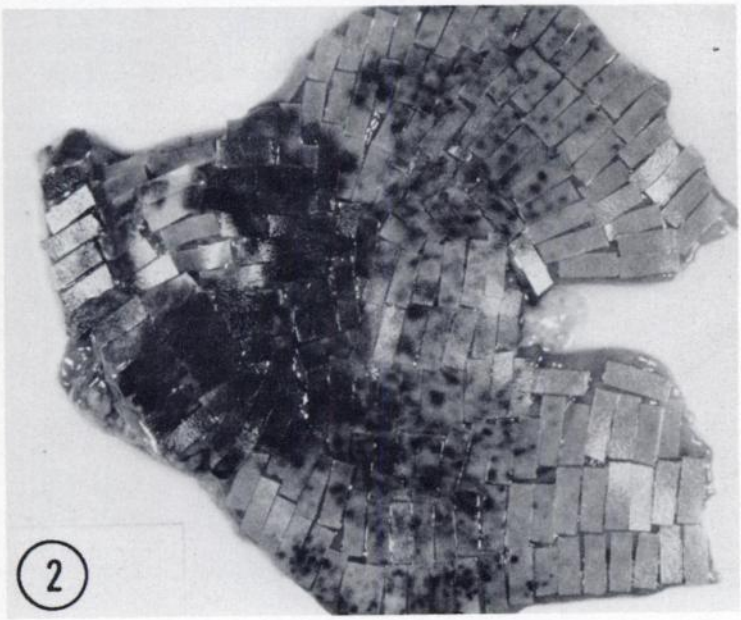

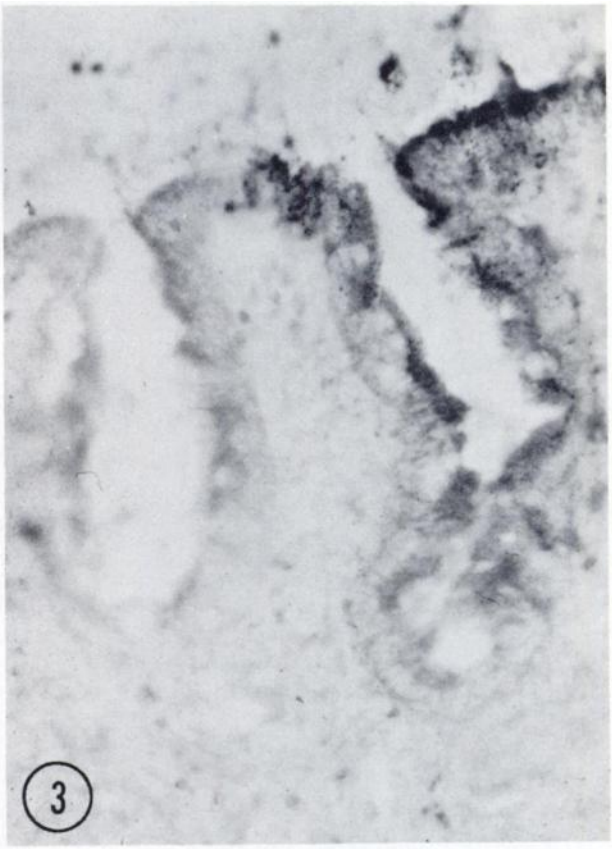

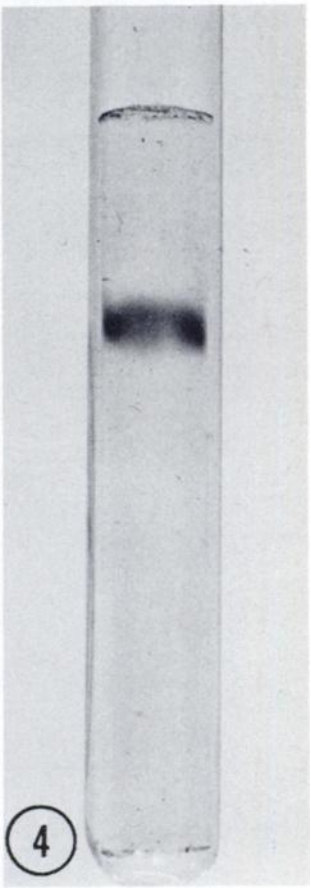

Fig. 1. Gross appearance of the stomach of a 63-year-old man with carcinoma of the antrum.

Fig. 2. Tes-Tape test on the same specimen as in Fig. 1. Intestinal metaplasia was shown with positive sucrase reaction. The area of carcinoma showed no sucrase activity.

Fig. 3. Histochemical demonstration of sucrase activity in villi of intestinal metaplasia. Specimen from a 63-year-old man with gastric carcinoma.

Fig. 4. Polyacrylamide gel electrophoresis of purified sucrase from human intestine. Seven μg of protein were applied to 5% acrylamide gel and subjected to electrophoresis for 150 min at 2 ma/gel. Gel was stained with Coomassie blue.