

Alkaline Phosphatase Isoenzymes in Intestinal Metaplasia and Carcinoma of the Stomach¹

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Summary

The enzymological and immunological properties of alkaline phosphatase (ALP) isoenzymes in intestinal metaplasia and gastric carcinoma tissues were compared with those of purified human intestinal, placental, and hepatic ALP's to speculate the gene expression of gastric cancer cells. The results were as follows.

Intestinal metaplasia was found in 21 of 23 cases (91%) of gastric cancer, whereas normal gastric mucosa had no ALP activity. ALP extracted from the mucosa of intestinal metaplasia was identical with intestinal ALP as to enzymological and immunological properties. ALP obtained from gastric carcinoma tissues of 23 patients was separated into 3 bands (ALPa, ALPb, and ALPc) by polyacrylamide-gel disc electrophoresis. Ten cases showed ALPa only, 4 cases showed ALPa and ALPb, 5 cases showed ALPa and ALPc, and the other 4 cases showed ALPa, ALPb, and ALPc, respectively. It was concluded that ALPa was similar in its enzymological and immunological properties to hepatic-type ALP, ALPb was similar to placental-type ALP, and ALPc was similar to intestinal-type ALP. ALPb probably originates from the cancer cell itself. As for the other enzymes, ALPa and ALPc, further investigation should be made to determine whether they come from the cancer cell itself or not.

Introduction

Gastric carcinoma frequently accompanies intestinal metaplasia in the surrounding gastric mucosa (6). Some cases of gastric carcinoma may arise from the intestinal-metaplastic mucosa, possibly a precancerous condition. Recently, a close relationship has been shown between intestinal metaplasia and a certain type of gastric carcinoma, that is, well-differentiated adenocarcinoma. ALP⁴ is available as a marker enzyme of intestinal metaplasia, and its isoenzyme analysis has been of considerable value in defining the origin of ALP (1, 10).

This paper speculates the gene expression of gastric can-

cer cells from the enzymological and immunological properties of ALP isoenzymes in intestinal metaplasia and gastric carcinoma tissues.

Materials and Methods

Purification of Enzyme. Subjects were 23 patients with gastric carcinoma. Scraped gastric mucosa and carcinoma tissues of gastrectomy specimens were homogenized and their ALP's were extracted with *n*-butyl alcohol by a modification of the method of Morton (7). The aqueous extract was used for analysis. Purification of human intestinal, placental, and hepatic ALP's was performed as reported previously (8, 9).

Enzyme Assay. ALP activity was determined by a modification of the method of Kind and King (4). Heat stability at 65° and inhibition by L-phenylalanine and imidazole were also studied. Protein concentration was determined by the method of Lowry *et al* (5). The ALP activity was expressed as King-Armstrong units/ μ g protein.

Electrophoresis. Disc electrophoresis was performed using 5% polyacrylamide-gel at 4° in a 0.1 M Tris-borate buffer system (pH 9.5). Samples were run for 1 hr, at a constant current (3 mA/tube). After the run, the gel was stained with a mixture composed of 60 ml Tris-HCl buffer (pH 9.7), 25 mg α -naphthyl-phosphate, 25 mg fast blue BB salt (Sigma Chemical Co., St. Louis, Mo.), and 30 mg MgCl₂.

Neuraminidase Treatment. The neuraminidase solution (1 mg/ml) was prepared by dissolving crystalline neuraminidase (Boehringer Mannheim, Mannheim, Germany) in 100 mM acetate buffer (pH 5.5) containing 150 mM NaCl and 10 mM MgCl₂.

The samples were treated with equal volumes of neuraminidase solution at 37° for 20 hr and then subjected to polyacrylamide-gel disc electrophoresis.

Immunological Method. Anti-human placental ALP antiserum was prepared as previously reported (3). Anti-human hepatic and intestinal ALP antisera were also prepared in the same manner. Samples were incubated with optimally diluted respective antiserum at 37° for 30 min and then subjected to polyacrylamide-gel disc electrophoresis. Radioimmunoassay of intestinal ALP was performed by the double-antibody technique (3).

Results and Discussion

Properties of Metaplastic Gastric Mucosa ALP. The intestinal-metaplastic mucosa ALP was identical with human

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⁴ The abbreviations used are: ALP, alkaline phosphatase; ALPa, band of alkaline phosphatase isoenzymes similar in enzymological and immunological properties to hepatic alkaline phosphatase; ALPb, band of alkaline phosphatase isoenzymes similar to placental alkaline phosphatase; ALPc, band of alkaline phosphatase isoenzymes similar to intestinal ALP.

purified intestinal ALP as to its enzymological and immunological properties, such as electrophoretic mobility, sensitivities to L-phenylalanine and imidazole, heat stability at 65°, K_m value, sensitivity to neuraminidase, ALP staining by diazo technique, double-immunodiffusion technique, radioimmunoassay in the intestinal ALP assay system, and immunofluorescent staining.

Properties of Gastric Carcinoma Tissue ALP's. Eleven of 23 cases of gastric carcinoma were of a well-differentiated type, and the other 12 cases were of a poorly differentiated type. In 21 cases (91%), surrounding intestinal metaplasia was noted to various extents. It appeared more intensively in well-differentiated than in poorly differentiated gastric cancer.

Chart 1, *left*, shows the ALP zymograms of gastric carcinoma tissues by polyacrylamide-gel disc electrophoresis. One to 3 broad bands were observed by this method. The electrophoretic mobilities of these bands differed slightly from case to case.

Chart 1, *right*, shows the zymograms after neuraminidase

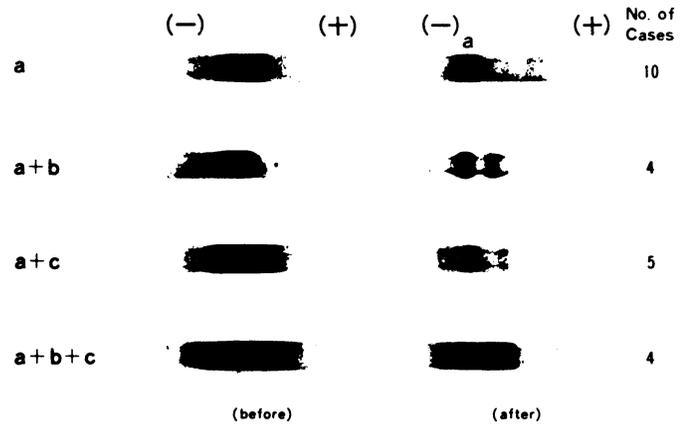


Chart 1. Neuraminidase sensitivities of gastric carcinoma tissue ALP's (polyacrylamide-gel disc electrophoresis). a, band of neuraminidase-treated human hepatic ALP; b, band of neuraminidase-treated human placental ALP; c, band of human intestinal ALP.

treatment. After neuraminidase treatment, the ALP bands were separated into 3 sharp bands (ALPa, ALPb, and ALPc). ALPa occurred in all cases, but ALPb and ALPc were found in 8 and 9 cases, respectively. Coexistence of ALPb and ALPc was seen in 4 cases.

Electrophoretic mobilities of ALPa and ALPb were similar to those of neuraminidase-treated human hepatic ALP and placental ALP, respectively. Electrophoretic mobility of ALPc was similar to that of human intestinal ALP. ALPc did not change its electrophoretic mobility by neuraminidase treatment, as did intestinal ALP.

The immunological reactions of gastric carcinoma tissue ALP's with anti-human hepatic, placental, and intestinal ALP antisera by polyacrylamide-gel disc electrophoresis showed that the ALPa band disappeared after incubation with anti-human hepatic ALP antiserum, but ALPb and ALPc bands remained unchanged. After incubation with anti-human placental or anti-human intestinal ALP antiserum, ALPb and ALPc bands disappeared, but ALPa remained. This result demonstrates that immunological cross-reaction exists between ALPa and human hepatic ALP, and between ALPb, ALPc, and human placental and intestinal ALP's, respectively.

Chart 2a shows dose-response curves of gastric carcinoma tissue ALP's in the human intestinal ALP radioimmunoassay system using double-antibody technique. ALPa and ALPb showed no response against anti-human intestinal ALP antiserum by this method. ALPc showed a similar dose-response curve to that of human intestinal ALP. ALPb, which is considered to be placental-type ALP, did not react in the intestinal ALP radioimmunoassay system.

Chart 2b shows heat stability of gastric carcinoma tissue ALP's at 65° for 20 min. ALPa and ALPc were completely inactivated by heat treatment. Only ALPb was heat stable. Placental ALP is also heat stable.

The inhibition curves of ALPa by L-phenylalanine and imidazole were similar to those of purified human hepatic ALP. However, ALPb and ALPc were imidazole insensitive

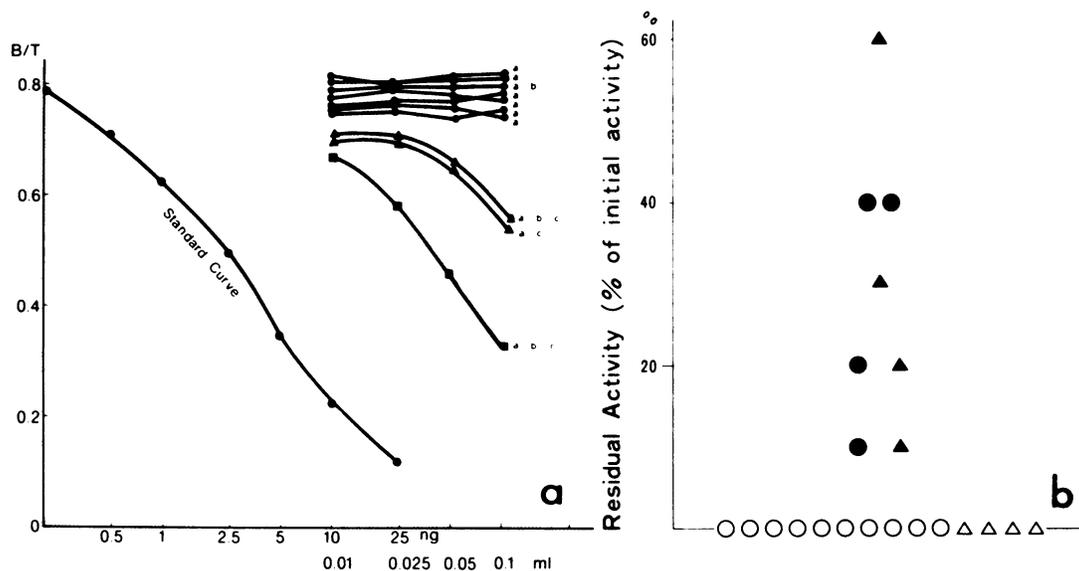


Chart 2. a, dose-response curves of gastric carcinoma tissue ALP's by radioimmunoassay system of intestinal ALP; b, heat stability of gastric carcinoma tissue ALP's (65°, 20 min). ○, a; △, a + c; ●, a + b; ▲, a + b + c.

and L-phenylalanine sensitive, as are placental and intestinal ALP's. Therefore, the enzymological and immunological properties of ALPa were similar to those of hepatic ALP, properties of ALPb were similar to those of placental ALP, and properties of ALPc were similar to those of intestinal ALP, respectively.

ALPb was seen in almost equal incidence both in well-differentiated and poorly differentiated carcinoma. No correlation was found between the occurrence of ALPb and the grade of differentiation. Fishman *et al.* (2) found the placental-type ALP in the tissue of various cancers. ALPb (placental-type ALP) appeared in gastric carcinoma tissues of 8 of 23 cases (35%) and may originate from carcinoma cells of the stomach. This may suggest an abnormality in differentiation during the growth of gastric cancer cells.

ALPc, which is of intestinal origin, appeared in the mucosa of intestinal metaplasia. In the course of chronic gastritis, an alteration of the gene expression may have occurred. ALPc was seen in increasing frequency when intestinal metaplasia of the surrounding mucosa became marked. Therefore, ALPc might come from intestinal metaplasia tissue mixed with carcinoma cells examined. As for the occurrence of ALPc, as well as ALPa, further study should be done to determine their originating cells.

Furthermore, the presence of Warnock's variant ALP (11) is also being investigated on gastric carcinoma tissues, and ALP's found in meconium and in the FL cell line have been investigated to clarify their molecular differences of ALP's.

Acknowledgments

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