MACROSCOPIC DEMONSTRATION OF THE DISTRIBUTION OF INTESTINAL METAPLASIA BY LEUCINE AMINOPEPTIDASE ACTIVITY

We know of the common association of intestinal metaplasia with well-differentiated adenocarcinoma of the stomach. Furthermore, some gastric cancer of animals induced by chemical substances has been reported to be associated with intestinal metaplasia. In order to examine the relation of gastric cancer to intestinal metaplasia, we found it expedient to supplement histological analysis by adapting Burstone's histochemical method for the demonstration of leucine aminopeptidase (aminopeptidase (cytosol), EC 3.4.11.1) in the resected specimen of a stomach. Leucine aminopeptidase is specific to intestinal metaplasia and normal mucosa of the small intestine. A similar approach, but using alkaline phosphatase (EC 3.1.3.1) was first reported by Graham and improved by Stemmermann. Sugimura used disaccharidases for the same purpose. Histochemical method by Nachlas et al. was also adaptable for the same approach for less diffusion, but its technique is comparatively complicated.

The freshly resected stomach was opened along the greater curvature. The mucosa was spread out on a cork-board and fixed in 10% Formalin which had been buffered with 2% calcium acetate (pH 7.1) for 12 hr in a refrigerator. The stomach was inserted into a plastic bag, filled with a substrate prepared according to the following formula: For each specimen, 20 mg L-leucyl-2-naphthylamide (Sigma Chemical Co., St. Louis, U.S.A.) was dissolved in 400 ml of distilled water, 100 ml of 0.2M Tris buffer (pH 7.2) was added, followed with 300 mg of Fast Garnet GBC (Sigma). The plastic bag was incubated at 24° for 15 min. Intestinalized mucosal surface was stained red. (Positive area by Nachlas' method is stained blue.) The duodenal cuff contributed as a positive control. Preliminary examinations revealed that areas showing positive reaction for leucine aminopeptidase coincided with zones of intestinal metaplasia examined histologically. Immediately after the staining using leucine aminopeptidase, it was possible to test for alkaline phosphatase activity as well. We adopted Stemmermann's method for the demonstration of area of intestinal metaplasia with alkaline phosphatase activity, but substituted Red Violet LB salt for Fast Blue RR salt (Sigma) in his formula. By this modified Stemmermann's method, duodenal mucosa and intestinal metaplasia were stained blue. If intestinal metaplasia had the activity of both enzymes, it was stained dark violet. If intestinal metaplasia had only the activity of leucine aminopeptidase, it remained red. Areas demonstrated by leucine aminopeptidase activity were always more extensive than areas demonstrated by alkaline phosphatase activity. The former usually encompassed the latter.

This method is quite simple to apply and never interferes with histological examination. Further details of the present study will be published in the near future.

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References


**EXPLANATION OF PLATES**

Photo 1. The red areas show the presence of intestinal metaplasia and normal duodenal mucosa demonstrated by leucine aminopeptidase activity.

Photo 2. The same specimen is stained by alkaline phosphatase activity. The dark violet areas mean the presence of intestinal metaplasia and normal duodenal mucosa showing positive reaction to leucine aminopeptidase and alkaline phosphatase. The areas remaining red mean the presence of intestinal metaplasia with only leucine aminopeptidase activity.

*National Kyushyu Cancer Center*

595 Notame, Minami-ku

*Fukuoka 815*

Kunihiro Nakahara (中原國廣)
Hirotugu Tomoda (友田博次)
Motonosuke Furusawa (古澤元之助)
Muneaki Abe (安部宗顕)
Akio Horie (堀江昭夫)