

Increased Expression of Sucrase and Intestinal-type Alkaline Phosphatase in Human Gastric Carcinomas with Progression

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The activities of sucrase, total alkaline phosphatase (total ALP) and intestinal-type alkaline phosphatase (I-ALP) were assayed in gastric carcinomas and in their surrounding mucosae from 57 patients with advanced cancers, and the localization of sucrase in 203 carcinomas, including 86 early cancers, was examined immunohistochemically using polyclonal anti-sucrase antibody. All three enzymes were active in the 57 carcinomas as well as in their surrounding mucosae, but the levels were fairly low as compared to those in normal jejunum mucosa. A considerable part of the total ALP activity in tumor specimens was assumed to be due to I-ALP itself. Increased sucrase and I-ALP were found with greater depth of invasion by undifferentiated-type carcinomas. The pattern of immunohistochemical localization of sucrase in the 203 carcinomas also clearly indicated increased expression with greater depth of invasion even in differentiated-type carcinomas.

Key words: Sucrase — Intestinal-type alkaline phosphatase — Gastric carcinomas — Depth of invasion

Generally, human gastric cancers are classified into 2 groups¹⁾: differentiated adenocarcinomas, including papillary and well-differentiated tubular adenocarcinomas, and undifferentiated adenocarcinomas, including signet-ring cell carcinomas, poorly differentiated and mucinous adenocarcinomas. We have shown²⁻⁵⁾ that human and rat gastric cancer cells of different histologic types can be classified into 2 categories: a gastric epithelial cell type, comprising pyloric gland cells and surface mucous cells, and an intestinal epithelial cell type, comprising goblet cells and intestinal absorptive cells, based on our recent results obtained by mucin histochemistry [paradoxical concanavalin A (Con A), galactose oxidase Schiff (GOS), and sialidase-GOS staining] and enzyme immunohistochemistry [pepsinogen staining].

It is well established⁶⁾ that sucrase and intestinal-type alkaline phosphatase (I-ALP) are useful functional markers for intestinal epithelial cell-type cancer cells. Sucrase, which is normally present in the small intestinal striated cell border membrane,^{7, 8)} has been found to be strongly expressed in intestinal metaplasias (considered to be precursor lesions for well-differentiated adenocarcinomas), whereas the enzyme could not be detected in normal gastric mucosae.^{9, 10)} There are at least four alkaline phosphatase isozymes (ALPs), i.e., tissue-unspecific (liver/bone/kidney), intestinal (I-), placental (P-) and germ cell or placental-like (P-like), whose gene structures were recently classified.¹¹⁻¹⁴⁾ In 1976, Miki *et al.*¹⁵⁾ initially re-

ported the co-expression of three types of ALPs: tissue unspecific, P- and I-ALPs in 23 cases of gastric carcinomas. In contrast, no ALP was detectable in normal gastric mucosae except for the I-ALP activity in intestinal metaplasias. We have reported¹⁶⁾ relatively low contents of ALPs in 13 out of 38 human primary signet-ring cell carcinomas. Other authors^{6, 17)} have also demonstrated a low level of ALP expression in various human gastric carcinomas.

A phenotypic shift from gastric to intestinal epithelial cell type occurs with progression of rat glandular stomach carcinogenesis.^{2, 3)} In order to cast light on the increase of intestinal phenotypic expression during the development of gastric cancers, the immunohistochemical localization of sucrase was examined in 203 carcinoma specimens, including 86 early cancers, and sucrase, total ALP and I-ALP activities were assayed in 57 advanced gastric carcinomas.

MATERIALS AND METHODS

A total of 203 gastric carcinoma specimens comprising 86 early and 117 advanced cancers, were obtained at surgery from patients operated on at Aichi Cancer Center Hospital. The patients (129 male and 74 female) ranged in age from 29 to 82 years (mean 63.7 years). A total of 57 of the 117 advanced cancers were resected with surrounding mucosae, washed with 0.9% NaCl solution, frozen and stored at -80°C until use. The histological classification of 203 gastric carcinoma specimens was made ac-

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ording to "The General Rules for the Gastric Cancer Study" (the 12th edition, 1993, Japanese Research Society for Gastric Cancer) taking into account the dominant features.

Enzyme assay Fifty to 150 mg samples of carcinomas and surrounding mucosae were cut into small pieces and homogenized with 2.0 to 4.0 ml of ice-cold 100 mM sodium phosphate buffer (pH 6.8) containing 1% Triton X-100. The homogenates were centrifuged at 105,000g for 30 min at 4°C, and the resulting supernatants were used for assay of sucrase, total ALP and I-ALP.

Protein content was determined by the method of Lowry *et al.*¹⁸⁾ Sucrase activity was assayed by a minor modification of the method of Carnie and Porteous.¹⁹⁾ Total ALP activity was assayed with an ALP S-test kit (Iatron Laboratories, Tokyo) with phenyl phosphate as the substrate.²⁰⁾ I-ALP activity was determined by the MICA method²¹⁾ using a monoclonal anti-I-ALP antibody that we had previously prepared.²¹⁾ The enzyme activity of each sample was determined by means of duplicate assays and expressed as units/g wet tissue. One unit of enzyme activity was defined as the amount catalyzing 1 μmol substrate/min at 37°C. All data were statistically analyzed with Student's unpaired test. Examination of the heat stability of total ALP and inhibition of enzyme activities by L-phenylalanine was performed as described previously.²²⁾

Immunohistochemistry of sucrase To investigate the intestinal phenotypic expression of cancer cells from early

lesions which were too small for biochemical analysis, the immunohistochemical localization of sucrase in 203 formalin-fixed tumors, including 86 early carcinomas, was determined by the ABC method,²³⁾ using our polyclonal anti-sucrase antibody.²⁴⁾ The χ² test for trend was applied to analyze the incidence of sucrase-positive cancers with different depths of invasion.

RESULTS

Enzyme activities Table I summarizes data on the activities of sucrase, total ALP and I-ALP determined in gastric carcinomas and surrounding mucosae from 57 patients with advanced cancers. All the enzymes were detectable in each of the specimens tested. In these 57 specimens (male 36, female 21), no marked differences in the activities of the three enzymes between males and females could be observed.

The contents of total ALP were generally comparable with those of I-ALP and therefore a considerable amount of the total ALP was assumed to be due to the expression of I-ALP itself. In order to verify these observations, the inhibitory effects of heat treatment and addition of L-phenylalanine on the total ALP activities were examined. The residual enzyme activities after heat treatments at 60°C for 10 min and 65°C for 10 min were found to be about 60% and 35% of the initial enzyme activities, respectively. In the presence of 20 mM L-phenylalanine the

Table I. Relationship between Enzyme Activity and Depth of Invasion in Gastric Carcinomas and Surrounding Mucosa

Depth of invasion ^{a)}	Effective No.	Enzyme activities (U/g tissue)					
		Sucrase		Total-ALP		I-ALP	
		carcinoma	mucosa	carcinoma	mucosa	carcinoma	mucosa
Differentiated adenocarcinoma							
mp	4	5.42±1.52 ^{b)}	5.71±2.38	1.43±0.80	1.37±1.43	0.75±0.88	0.66±0.79 ^{c)}
ss	9	3.99±1.92	6.88±4.81	1.21±1.62	2.71±3.01	1.38±1.97 ^{c)}	1.53±1.28
se	7	3.88±0.88	5.70±2.25	1.34±0.60	2.56±1.63	2.07±1.21	2.08±2.17
total	20	4.24±1.66 ^{c)}	6.22±3.67 ^{c)}	1.30±1.20 ^{c)}	2.39±2.39 ^{c)}	1.49±1.63 ^{c)}	1.55±1.67 ^{c)}
Undifferentiated adenocarcinoma							
mp	2	2.03	2.36	0.89	1.03	0.16	0.75
ss	21	2.43±1.28 ^{d)}	3.49±1.55	0.78±0.71	1.14±0.53	0.55±0.93 ^{d)}	0.77±0.98
se	14	3.45±3.95	4.01±2.16	0.93±0.75	1.05±0.49	0.99±0.96	0.56±0.41
total	37	2.83±2.68	3.63±1.81	0.84±0.71	1.10±0.50	0.70±0.95	0.69±0.81
Whole	57	3.32±2.46	4.54±2.91	1.00±0.94	1.56±1.56	0.98±1.29	0.99±1.25

a) mp, carcinoma involving the muscularis propria; ss, carcinoma invading below the subserosa; se, carcinoma exposed through the serosa or invading other organs.

b) Mean±SD.

c) Statistical significance of differences between differentiated and undifferentiated adenocarcinomas (P<0.01 in sucrase, P<0.05 in total ALP and I-ALP).

d) The t test showed a correlation between enzyme activities and depth of invasion (t test, P<0.05).

activities of total ALP decreased to about 50% of those in its absence. On the basis of these results, a large proportion of the total ALP, assumed to be composed of four isozymes, tissue-unspecific ALP, I-ALP, P-ALP and P-like ALP, was again concluded to be accounted for by I-ALP enzyme itself.

The level of sucrase in carcinomas ranged from 1.17 to 17.47 units/g tissue, with an average level of 3.32 ± 2.46 units/g tissue. In the surrounding mucosae the range was from 1.12 to 15.07 units/g tissue, and the average was 4.54 ± 2.91 units/g tissue. The total ALP in carcinomas ranged from 0.11 to 5.56 units/g tissue, with an average of 1.00 ± 0.94 . The range in surrounding mucosae was from 0.19 to 10.50 units/g tissue, and the average was 1.56 ± 1.59 units/g tissue. Similarly, the level of I-ALP in carcinomas ranged from 0.02 to 6.01 units/g tissue, with an average of 0.98 ± 1.29 . The range in the surrounding mucosa was from 0.01 to 7.22 units/g tissue, with an average of 0.99 ± 1.25 . Thus, considerable variability was noted for all three parameters. The activities of sucrase, total ALP and I-ALP were somewhat higher in the differentiated than in the undifferentiated adenocarcinomas, and in the surrounding mucosa than in the carcinoma tissues, in both cases ($P < 0.01$ for sucrase, $P < 0.05$ for total ALP and I-ALP).

In the differentiated carcinomas, a statistically significant increase of I-ALP activities was observed with

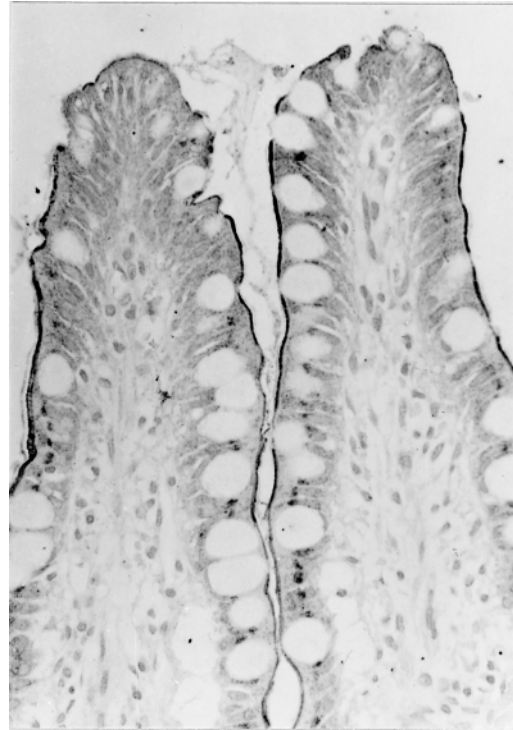


Fig. 1. Intestinal metaplasia with sucrase positivity in striated cell borders of metaplastic absorptive cells. Sucrase immunohistochemistry; magnification $\times 150$.

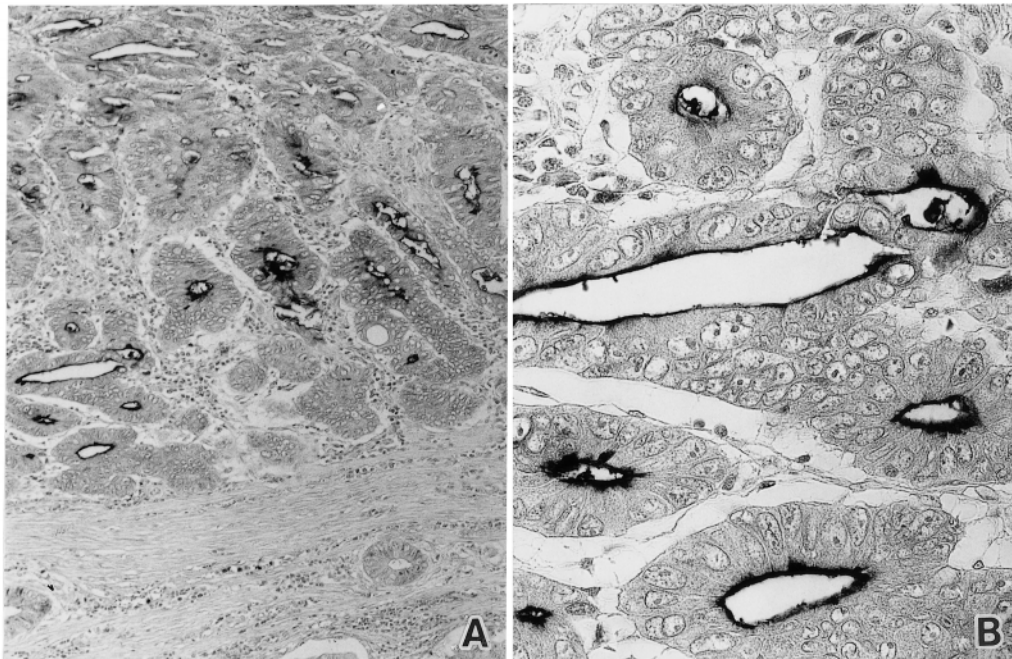


Fig. 2. Positive sucrase reaction on incomplete striated cell borders of gastric cancer cells of well differentiated adenocarcinomas. Sucrase immunohistochemistry; A, magnification $\times 30$. B, magnification $\times 150$.

greater depth of invasion ($P < 0.05$). In the undifferentiated carcinoma case, the activities of all three enzymes increased with invasion from mp to se, statistical significance being attained for sucrase ($P < 0.05$) and I-ALP ($P < 0.05$).

Immunohistochemistry of sucrase In the 57 adenocarcinomas assayed for enzyme activities, over 10% of the surrounding, gastric mucosal areas in histological sections was replaced by intestinal metaplasia in 17 of 20 differentiated and in 12 of 37 undifferentiated adenocarcinomas. Sucrase was detected immunohistochemically on the striated cell borders of the intestinal absorptive cells of intestinal metaplastic glands (Fig. 1). In the well-differentiated adenocarcinomas, a few cancer cells with incomplete striated cell borders exhibited sucrase activity (Fig. 2). In undifferentiated adenocarcinomas a positive sucrase reaction was occasionally found in the apical portions of cancer cells surrounding tiny irregular lumens (Fig. 3) or on the surfaces of intracellular microcysts. The incidence of the cases with sucrase-positive cancer cells increased in accordance with the depth of invasion both for differentiated and undifferentiated cancers. The sucrase-positive cancer cells were found to be randomly distributed within cancer tissues, including in the mucosal region. Data on the immunohistochemical sucrase positivity for the 203 cancers are summarized in Fig. 4. In the 108 differentiated adenocarcinomas, the proportion of sucrase-positive lesions in-

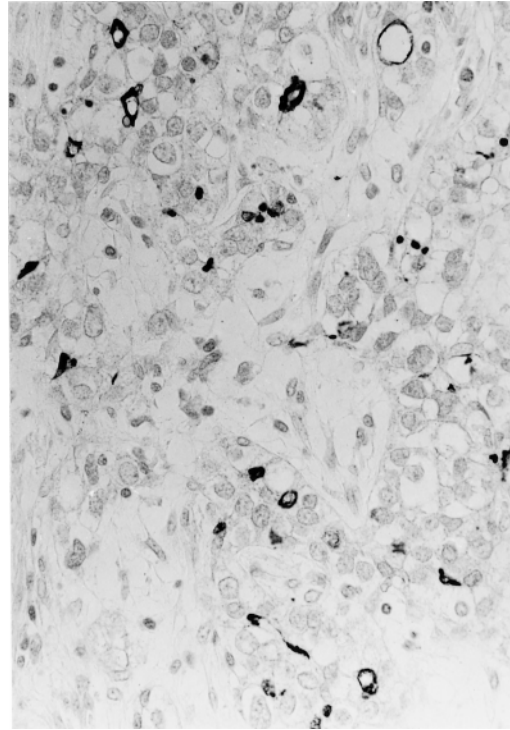


Fig. 3. Positive sucrase reaction in tiny lumens surrounded by gastric cancer cells of a poorly differentiated adenocarcinoma. Sucrase immunohistochemistry; magnification $\times 150$.

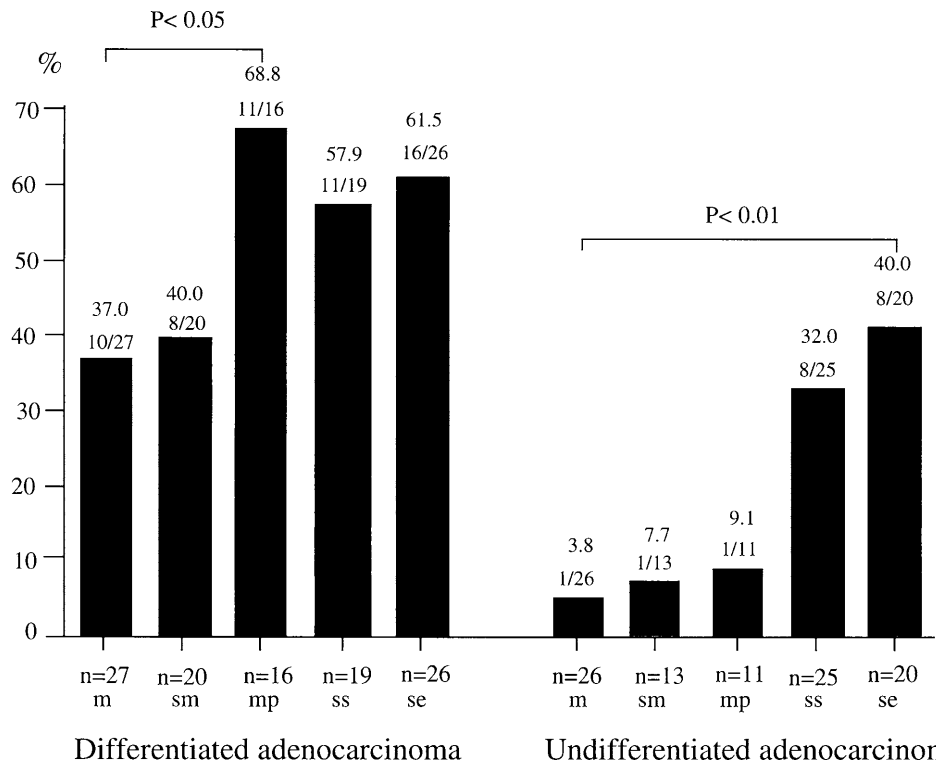


Fig. 4. Results for immunohistochemical sucrase reactivity in differentiated and undifferentiated adenocarcinomas with progression. m, carcinoma limited to the mucosa; sm, carcinoma involving the submucosa; mp, carcinoma involving the muscularis propria; ss, carcinoma invading below the subserosa; se, carcinoma exposed through the serosa or invading other organs. The increase in the incidence of sucrase-positive cancers with depth of invasion was significant ($P < 0.05$ in differentiated adenocarcinoma, $P < 0.01$ in undifferentiated adenocarcinoma) by the χ^2 test for trend.

creased significantly ($P<0.05$) with progression from 37% (stage m) to 61.5% (stage se). A similar significant increase ($P<0.01$) with progression from 3.8% (stage m) to 40% (stage se) was observed in the 95 undifferentiated adenocarcinomas studied. In young adult cases (below the age 39 years), the number of undifferentiated carcinomas was 11, and 4 of those were scirrhous carcinomas. Three of these cases (stages sm, pm, and se) were sucrase-negative and only one (stage ss) was positive, indicating a similar tendency to that found overall for undifferentiated adenocarcinomas.

DISCUSSION

The present study revealed a progressive increase in intestinal features with progression for both differentiated and undifferentiated carcinomas of the human stomach. Since tissue-unspecific ALP is more sensitive than I-ALP to heat treatment at 65°C for 10 min, while P-ALP is most stable, and the activities of I-ALP and P-ALP, but not tissue-unspecific ALP, are strongly inhibited by L-phenylalanine,^{25, 26)} our results suggest that neither tissue-unspecific ALP nor P-ALP is a main component of the total ALP (data not shown). Considering the fair comparability between values for I-ALP and total ALP, we can therefore conclude that a considerable part of the total ALP activity in gastric carcinomas is due to I-ALP itself.

The present biochemical assays demonstrated activities for sucrase, total ALP and I-ALP in all of 57 carcinomas and surrounding mucosae, in clear contrast to the lack of expression in human normal gastric mucosa, based on biochemical and immunohistochemical analyses.^{9, 10)} Intestinal metaplasia was less pronounced in mucosa surrounding undifferentiated carcinomas, while it was prominent in association with differentiated lesions. Therefore the apparent differences of enzyme activities in surrounding mucosae in the two types of cancers (Table I) can be assumed to be mainly due to the variation in intestinal metaplasia.

We have earlier argued that the phenotypic expression of carcinoma cells tends to be the same as that of the tissue of origin.³⁾ This naturally leads to the question of what is the significance of sucrase and I-ALP expression, both enzymes normally being expressed specifically in the small intestinal striated cell border membrane.

Our series^{3-6, 16, 27)} of studies of enzymes and mucins has shown that all human and rodent gastric carcinomas

consist mainly of gastric epithelial cells (pyloric gland cells and surface mucous cells), with a few intestinal epithelial cells in the early stages of neoplasia. We have proposed²⁷⁻³¹⁾ that, in humans, intestinal metaplasia and the appearance of the intestinal epithelial cell phenotype in gastric carcinomas during carcinogenesis are independent phenomena, although it had earlier been suggested⁹⁾ that the intestinal-type carcinomas might develop from intestinal metaplasia and that diffuse-type carcinomas might originate from nonmetaplastic mucosa.

The results shown in Table I demonstrate that the expression of sucrase and I-ALP as typical functional markers of intestinal-type properties was observed in both differentiated and undifferentiated adenocarcinomas, but with an increased expression at the stage of invasion in both cases. The present data indicate at least a tendency for increased expression of enzymes with progression of invasion, and there was a statistically significant relation between average values for enzyme activities and the depth of invasion ($P<0.05$ or $P<0.01$, shown in Table I). Tumor invasion depends to a certain extent on time and we can therefore consider the depth of invasion as an index of carcinoma age. Phenotypic expression of human gastric cancer cells (both differentiated and undifferentiated) has been classified into gastric epithelial cell and intestinal epithelial cell types.^{2, 16)} The higher incidences of sucrase-positive cancers (both differentiated and undifferentiated) in the deeper invasive classes thus indicates a phenotypic shift from gastric-type to intestinal-type expression with time, consistent with early cancer consisting of gastric epithelial cells. Future clarification of why intestinal phenotypic expression occurs in differentiated adenocarcinomas and surrounding gastric mucosa is necessary, because in these, sucrase and I-ALP enzymatic activities and the immunohistochemical appearance of sucrase were greater than in stage-matched undifferentiated carcinoma cases.

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