Kimihide Yoshida · Wataru Nakamura · Kazuyuki Hirano
Hirofumi Yuasa · Tetsuya Tsukamoto · Masae Tatematsu

Expression of sucrase and intestinal-type alkaline phosphatase in colorectal carcinomas in rats treated with methylazoxymethanol acetate

Received: 22 July 1998 / Accepted: 1 September 1998

Abstract In this study the small-intestine phenotype in rat colonic tumors was investigated in terms of sucrase and intestinal-type alkaline phosphatase (I-ALP) activity. F344 rats were given intraperitoneal injections of methylazoxymethanol acetate at a dose level of 25 mg/kg body weight once a week for 8 weeks and were killed 40 weeks after the first injection. Sucrase and I-ALP activities in proximal and distal colon adenocarcinomas were significantly higher than those in the normal colon epithelium. In the jejunum, by contrast, normal tissue had significantly higher levels than tumors. Immunohistochemical staining of I-ALP was also strong in striated cell borders of colon adenocarcinoma cells. These data suggest that, whereas absorptive cells of the small intestine lose their own traits with tumor development, colonocytes acquire phenotypic features of the small intestine. Intestinal enzymes associated with the striated-cell border, such as sucrase and I-ALP, may be useful markers for malignant phenotypic expression in colonocytes.

Key words Small-intestine phenotype · Sucrase · Intestinal-type alkaline phosphatase · Colon cancer · Rat

Abbreviation I-ALP intestinal-type alkaline phosphatase

Introduction

Intestinal metaplasia has been thought to be a putative preneoplastic lesion in the stomach on the basis of epidemiological, histopathological and biological findings (Morson 1955; Nakamura et al. 1968; Kawachi et al. 1976). However, we have previously shown that adenocarcinomas induced experimentally in rats are mainly composed of tumor cells of the gastric epithelial cell type and the phenotypic expression changes from the gastric epithelial cell type to the small-intestinal epithelial cell type with tumor progression. The same phenomenon is also observed in human gastric cancers and we have therefore proposed that small-intestinalization of normal epithelium and gastric cancer cells occurs independently with time (Tatematsu et al. 1983, 1984, 1992; Yamachika et al. 1997a; Nakamura et al. 1998).

We also have found, using mAb 91.9H, which is specific for colonic sulfomucins and mAb TKH-2 for sialosyl TN antigen, that the mucous phenotype shifts from the colonic to the small-intestinal striated-cell border membrane, which catalyzes the hydrolysis of dietary sucrase and some of the products of starch digestion (Lloyd and Ward 1987). In the adult colon, sucrase appears in the early gestation period and disappears totally at term (Lacroix et al. 1984).

There are four different forms of alkaline phosphatase (ALP): intestinal (I-ALP), tissue-unspecific (liver/bone/kidney), placentat and germ cell or placental-like ALP, the gene structures of which have recently been elucidated (Weiss et al. 1988; Henthorn et al. 1988;
Knoll et al. 1988; Millan and Manes 1988). I-ALP is believed to be bound to the microvillus surface of mature small-intestinal absorptive cells by a phosphatidylinositol-glycan anchor (Low 1987). However, it also persists, if only in small amounts, in the adult colon (Lacroix et al. 1984). Sucrase and I-ALP are useful functional markers for intestinal epithelial-cell-type cancer cells (Furihata et al. 1984), and can therefore be employed to assess the small-intestine phenotype in rat colonic tumors.

Materials and methods

Chemicals and animals

Methylazoxymethanol acetate was obtained from Sigma Chemical Co. Ltd. (St. Louis, USA). Monoclonal anti-(rat intestinal type alkaline phosphatase) antibody, and polyclonal anti-(rat alkaline phosphatase) antibodies were prepared as described previously (Hayashi et al. 1991).

F344 rats were purchased from Charles River Japan Inc., Kanagawa, and used at 7 weeks of age for this study. Animals were housed on hardwood chips in an air-conditioned room with a 12-h light/dark cycle. They were given continuous access to commercial rat chow (Oriental MF, Oriental Yeast Co., Tokyo) and water. Twenty-three rats were given intraperitoneal injections of methylazoxymethanol acetate at a dose level of 25 mg/kg body weight once a week for 8 weeks, and 15 rats in the control group were given physiological saline in the same way.

Twenty-three rats were given intraperitoneal injections of methylazoxymethanol acetate at a dose level of 25 mg/kg body weight once a week for 8 weeks, and 15 rats in the control group were given physiological saline in the same way. All specimens of normal and tumor tissues were fixed in 10% phosphate-buffered formalin and embedded in paraffin. Tissue sections were counterstained with hematoxylin and eosin for microscopic examination. Localization of I-ALP in each specimen was examined by immunohistochemical staining, using polyclonal anti-(rat I-ALP) antibody diluted 1:200. We have previously described the immunohistochemical method in detail (Yuasa et al. 1994).

Enzyme assays

 Samples of 4–60 mg normal and tumor tissues were homogenized with 2.0 ml ice-cold 100 mM sodium phosphate buffer, pH 6.8, containing 1% Triton X-100 in a glass homogenizer. The homogenate was centrifuged at 105 000 g at 4°C for 30 min. The resulting supernatant was employed for assays of sucrase and I-ALP.

The protein content of the extract was determined by the method of Lowry et al. (1951). Sucrase activity was assayed by a minor modification of the method of Carnie and Porteous (1962). I-ALP activity was determined by the monoclonal immunocatalytic assay method (Hayashi et al. 1991) using monoclonal anti-(rat I-ALP) antibody.

One unit of enzyme activity was defined as the amount catalyzing the conversion of 1 μmol substrate/min at 37°C. The total enzyme activity of each specimen was expressed as units per gram of wet tissue. All data were statistically analyzed by Student’s unpaired t-test; a P value less than 0.05 was considered statistically significant.

Histological and immunohistochemical examination

All specimens of normal and tumor tissues were fixed in 10% phosphate-buffered formalin and embedded in paraffin. Tissue sections were counterstained with hematoxylin and eosin for microscopic examination. Localization of I-ALP in each specimen was examined by immunohistochemical staining, using polyclonal anti-(rat I-ALP) antibody diluted 1:200. We have previously described the immunohistochemical method in detail (Yuasa et al. 1994).

Results

Tumor incidence

The entire rat colon from the cecum to the rectum was equally divided into two parts, defined as proximal and distal.

In control rats, no tumors were found in any part of the intestine. In methylazoxymethanol-acetate-treated rats, adenocarcinomas developed in the duodenum, jejunum and colon (Table 1). Most tumors were fungating and sessile.

Enzyme activity

Data for sucrase and I-ALP activities of normal jejunum and colon, and adenocarcinomas of the jejunum and colon are summarized in Figs. 1, 2.

In normal tissues, sucrase activity was highest in the jejunum, the value being significantly different from those for the proximal and distal colon (P < 0.0001, P < 0.0005 respectively). Sucrase activity in jejunum adenocarcinomas was significantly (P < 0.05) lower than that of normal jejunum. In contrast, in colon adenocarcinomas (proximal and distal) a significant (P < 0.005, P < 0.05) increase of sucrase activity in comparison with that of the normal epithelium was observed. The differences between sucrase activities of adenocarcinomas of the jejunum, proximal colon and distal colon were not statistically significant.

In normal tissues, I-ALP activity was similarly higher in the jejunum than in either the proximal or distal colon (P < 0.0001). Significantly lower activity was found in jejunum adenocarcinomas than in the normal jejunum (P < 0.05) while the values for proximal and distal colon adenocarcinomas were higher (P < 0.0001 and P < 0.0005) than those for the normal counterparts. The differences in I-ALP activities of adenocarcinomas of the jejunum, proximal colon and distal colon were not statistically significant.

Localization of I-ALP in normal tissues and adenocarcinomas

Immunohistochemically, I-ALP expression was found to be strong on the striated cell borders of normal small-

Table 1 Subsite distribution of adenocarcinomas of the intestine (n = 23)

<table>
<thead>
<tr>
<th>Site</th>
<th>No. of animals with tumors (%)</th>
<th>No. of adenocarcinomas (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distal colon</td>
<td>18 (78.3)</td>
<td>40 (40.8)</td>
</tr>
<tr>
<td>Proximal colon</td>
<td>15 (65.2)</td>
<td>22 (22.5)</td>
</tr>
<tr>
<td>Cecum</td>
<td>2 (8.6)</td>
<td>2 (2.0)</td>
</tr>
<tr>
<td>Jejunum</td>
<td>7 (30.4)</td>
<td>7 (7.1)</td>
</tr>
<tr>
<td>Duodenum</td>
<td>18 (78.3)</td>
<td>27 (27.6)</td>
</tr>
<tr>
<td>Total</td>
<td>22 (95.6)</td>
<td>98 (100)</td>
</tr>
</tbody>
</table>