Human placental form of glutathione S-transferase (GST-π) was detected in human colonic carcinomas and adenomas by peroxidase anti-peroxidase method using antibody raised against GST-π. Of 60 carcinomas, including differentiated adenocarcinomas and undifferentiated carcinomas, 88% were positive for GST-π staining, and 47% of 23 adenomas were also positive. In the normal colonic mucosa, GST-π was not detectable or was only weakly stained in the basal parts of the absorptive cells or in the cytoplasm of the cells containing little mucin. These results indicate that GST-π is a possible new marker for immunohistochemical detection of human colonic carcinoma and some adenomas.

Key words: Glutathione S-transferase — Placental isoenzyme — Tumor marker — Human colonic carcinoma

Many kinds of tumor markers have been reported as being helpful for diagnosis and follow-up of tumors. Recently, the placental form (GST-P or GST 7-7) of glutathione S-transferase (GST), one of the multifunctional detoxifying enzyme families, has been reported as a new marker enzyme for (pre)neoplastic lesions arising during chemical carcinogenesis in rat liver and hamster pancreas. The human placental form of GST (GST-π) is immunologically related to rat GST-P and, assuming that interspecies similarities exist in neoplastic development, might be expected to be a useful marker for (pre)neoplastic lesions in human organs. Therefore, to examine this possibility, we tried to detect GST-π expressed in colonic carcinomas and adenomas by application of the peroxidase anti-peroxidase (PAP) method using anti-GST-π antibody. GST-π was purified from the terminal placenta by S-hexylglutathione column chromatography followed by chromatofocusing, and anti-GST-π antibody was prepared in a rabbit as previously described. Formalin-fixed and paraffin-embedded tissue blocks from 60 carcinomas and 23 adenomas of the colon obtained surgically or at biopsy were examined. Paraffin sections (5 μm) were treated with anti-GST-π antibody optimally diluted (600-fold), then with swine anti-rabbit IgG antibody (Dako Co., Ltd., Denmark) followed by rabbit peroxidase-anti-peroxidase soluble complex (Dako Co., Ltd.). Finally, they were treated with DAB solution to visualize the location of GST-π, and counterstained with hematoxylin. Tissue which was mostly stained dark (or thick) brown was regarded as being strongly positive for GST-π staining, while tissue stained light (or thin) brown was regarded as being weakly positive. However, as the intensity of staining was variable depending upon the staining and other conditions, the total response, including strongly and weakly positive GST-π staining, was evaluated (i.e., staining was evaluated simply as positive or negative). Negative control staining was done by replacing the GST-π immune serum with pre-immune rabbit serum and further by absorption of the immune serum with the purified GST-π. As tissue controls, normal portions of the colonic mucosa were examined.
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The results are summarized in Table I. Of 60 carcinomas, including 44 adenocarcinomas and 16 undifferentiated carcinomas, 62% were strongly and 26% weakly positive for GST-π staining, giving a total of 88% positive and 12% negative (not stained) cases. Of 23 adenomas, 4% were strongly and 43% weakly positive: a total of 47%. In both adenocarcinoma and undifferentiated carcinoma, GST-π was stained throughout the tissue, diffusely in the cytoplasm and rather more strongly in the luminal surfaces of the cells (Fig. 1). In adenomas, GST-π was stained mainly in the basal parts of the cellular cytoplasm or diffusely in the cytoplasm of the cells containing little mucin (Fig. 2). In the normal colonic mucosa (Fig. 3), GST-π was not detectable, or was only weakly stained in the basal parts of the absorptive cells or diffusely stained in the cytoplasm of cells containing little mucin. GST-π was not detectable in cells containing much mucin. These findings indicate that GST-π is a possible marker useful for immunohistochemical detection of human colonic carcinoma and some adenomas.

With regard to the relationship to differentiation, 73% of well-differentiated adenocarcinomas (n=15) were positive for GST-π staining, while 93% of the moderately dif-

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Table I. GST-π Staining in Human Colonic Carcinomas and Adenomas

<table>
<thead>
<tr>
<th></th>
<th>Strongly positive (%)</th>
<th>Weakly positive (%)</th>
<th>Positive (total) (%)</th>
<th>Negative (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcinoma (60)</td>
<td>62 (37)</td>
<td>26 (16)</td>
<td>88 (53)</td>
<td>12 (7)</td>
</tr>
<tr>
<td>Adenocarcinoma (44)</td>
<td>61 (27)</td>
<td>25 (11)</td>
<td>86 (38)</td>
<td>14 (6)</td>
</tr>
<tr>
<td>well-differentiated (15)</td>
<td>60 (9)</td>
<td>13 (2)</td>
<td>73 (11)</td>
<td>27 (4)</td>
</tr>
<tr>
<td>moderately differentiated (14)</td>
<td>57 (8)</td>
<td>36 (5)</td>
<td>93 (13)</td>
<td>7 (1)</td>
</tr>
<tr>
<td>poorly differentiated (15)</td>
<td>67 (10)</td>
<td>27 (4)</td>
<td>94 (14)</td>
<td>6 (1)</td>
</tr>
<tr>
<td>Undifferentiated carcinoma (16)</td>
<td>63 (10)</td>
<td>31 (5)</td>
<td>94 (15)</td>
<td>6 (1)</td>
</tr>
<tr>
<td>Adenoma (23)</td>
<td>4 (1)</td>
<td>43 (10)</td>
<td>47 (11)</td>
<td>53 (12)</td>
</tr>
<tr>
<td>Normal mucosa (56)</td>
<td>0 (0)</td>
<td>18 (10)</td>
<td>18 (10)</td>
<td>82 (46)</td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate the numbers of samples examined.

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Fig. 1. A well-differentiated adenocarcinoma of the colon. PAP staining using anti-GST-π antibody and counterstaining with hematoxylin (×33). GST-π is diffusely stained in the carcinoma cells.
ferentiated (n=14) and 94% of the poorly differentiated (n=15) cases gave positive results. Similarly, 94% of undifferentiated carcinomas (n=16) were positive. These results suggest a tendency for GST-π to increase with increased carcinoma dedifferentiation. In rat chemical hepatocarcinogenesis, GST-P appears to be a more specific marker for preneoplastic lesions than for poorly differentiated hepatomas, but, at least in human colon, the opposite seems to be the case for GST-π expression. Thus, adenomas, which are thought to be premalignant, are less likely to be positive than the carcinomas, the marker becoming most useful with tumor progression. Indeed, the focal carcinoma por-

Fig. 2. An adenoma of the colon. PAP staining using anti-GST-π antibody and counterstaining with hematoxylin (×33). GST-π is stained mainly in the basal parts of the cytoplasm of the cells.

Fig. 3. Normal colonic mucosa (×33). The PAP staining procedure was the same as that for Figs. 1 and 2, but there is an almost complete absence of reaction product.
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In adenomas were stained more strongly than were the surrounding adenomatous portions (data not shown).

We have reported that the content of GST-π, determined immunochemically, was significantly increased in hepatic tumors, irrespective of whether they were primary hepatomas or metastatic hepatic tumors originating from the stomach and colon. However, this is the first report of the application of anti-GST-π antibody for immunohistochemical detection of cancerous alteration in human tissues. Similar investigations on the potential of GST-π as a marker for (pre)neoplastic lesions in other human organs such as the stomach, pancreas and uterine cervix are being carried out using our anti-GST-π antibody by several groups in Japan.

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