Marked Increase of Trypsin(ogen) in Serum of Linitis Plastica (Gastric Cancer, Borrmann 4) Patients

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ABSTRACT

Linitis plastica, or Borrmann 4 gastric cancer, shows very poor prognosis, and the reason has not been understood. In the present study, we examined serum levels of trypsin(ogen) in 44 gastric cancer patients, including 17 early gastric cancer, 18 non-Borrmann 4 advanced gastric cancer, and 9 Borrmann 4 gastric cancer, by using the RIA gnost Trypsin kit (Hoechst Japan, Tokyo, Japan), which was expected to detect trypsin-1, trypsin-2, trypsinogen-1, and trypsinogen-2 in sera. The trypsinogen concentration was much higher in the patients with linitis plastica than in the other gross types of gastric cancer. Hypertrypsinemia was identified in ~60% of advanced gastric cancer cases. Lymph node involvement, liver metastasis, or poorly differentiated adenocarcinoma is an important factor of hypertrypsinemia. The serum trypsinogen level in linitis plastica patients was $3484.4 \pm 2319.7$ ng/ml (mean $\pm$ SD), which was significantly higher not only than that of the early gastric cancer (381.4 $\pm$ 92.1) but also the stage IV gastric cancer patients (578 $\pm$ 440.4), excluding those with linitis plastica. The elevated serum trypsinogen level in linitis plastica patients may be related to the malignant behavior of this type of cancer cell. Serum trypsinogen of linitis plastica shows significantly higher concentrations than do the other types of advanced gastric cancer. Therefore, serum concentration of trypsinogen might be a good marker of gastric cancer of linitis plastica.

INTRODUCTION

Trypsinogen, a major proteolytic enzyme precursor produced by pancreatic acinar cells, is known to also be秘密ed by cancer cells such as ovarian cancer (1), colon cancer (2), pancreatic cancer (3), and cholangiocarcinoma (4). The activated form of trypsinogen, trypsin, plays an important role in the pathophysiology of acute pancreatitis to degrade i.p. tissues, and it may be involved in the extracellular matrix degradation by invasive cancer cells (2).

Thus far, four types of trypsin have been identified: cationic trypsin (trypsin-1), anionic trypsin (trypsin-2), meso-trypsin (trypsin-3), and trypsin-4. Pancreatic juice contains cationic and anionic trypsinogen in the ratio of 2:1 and a little meso-trypsin. The cDNA for trypsinogen-4 was cloned from human brain (5).

Serum levels of anionic trypsinogen, trypsinogen-2, are elevated not only in patients with acute pancreatitis but also in patients with hepatic, biliary, or pancreatic malignancies such as cancer of the ampulla Vateri, hepatocellular carcinoma, cholangiocarcinoma, and pancreatic cancer (4).

It has been reported previously that human gastric carcinoma cell lines also secrete trypsinogen (6, 7). Gastric cancer is the leading cause of death attributable to cancer in Japan. Surgical prognosis for advanced gastric cancers is improving by excessive dissection of regional lymph nodes. However, the 5-year survival rate of advanced gastric cancer, overall, is still <50%. Moreover, the special gross-type linitis plastica, or Borrmann 4 gastric cancer, which shows a leather-bottle shape in upper gastrointestinal series, still has a very poor prognosis. Although many factors of linitis plastica, such as growth factor secretion, hormonal properties, and plasminogen activity, have been investigated, the cause of its poor prognosis is unclear. In the present study, we examined serum levels of trypsinogen, including trypsinogen-1 and trypsinogen-2, in gastric cancer patients. The concentration was much higher in the patients with linitis plastica than in the other gross types of gastric cancer.

MATERIALS AND METHODS

Samples. Serum was obtained preoperatively from 44 gastric cancer patients (Table 1), including 9 with linitis plastica, during the years 1995–1997 at Yokohama City University Hospital and was kept at $-80^\circ$C until use. Cancer gross classification and staging were according to Japanese guidelines (8).

RIA. Serum levels of trypsinogen were measured by a classic simultaneous-addition, double-antibody RIA (9), using the RIA gnost Trypsin kit (Hoechst Japan), with antihuman trypsinogen rabbit antibodies. The kits were used according to the manufacturer's instructions. Briefly, 0.1 ml of patient’s serum was incubated with 0.2 ml of antihuman trypsinogen...
Serum Trypsin(ogen) Levels in Linitis Plastica Patients

<table>
<thead>
<tr>
<th>Gross type&lt;sup&gt;a&lt;/sup&gt;</th>
<th>n</th>
<th>Stage</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>18</td>
<td>I</td>
<td>19</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>II</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>III</td>
<td>7</td>
</tr>
<tr>
<td>4 (LP)</td>
<td>9</td>
<td>IV</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td></td>
<td>44</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td></td>
<td>44</td>
</tr>
</tbody>
</table>

<sup>a</sup> Type 0, superficial, flat tumors; type 2, ulcerated type with clear margin; type 3, ulcerated type with infiltration; type 5, unclassified type. Staging refers to the classification by the Japanese Research Society for Gastric Cancer (8).

Table 1

Serum concentrations of trypsin(ogen) in preoperative gastric cancer patients. [□] groups with normal concentrations of serum trypsin(ogen); [■], hypertrypsinemia groups. For the definition of early and advanced gastric cancer, see the guidelines published by the Japanese Research Society for Gastric Cancer (8).

**RESULTS**

Serum trypsin(ogen) levels of 25 (56.8%) of 44 gastric cancer patients were within the normal range (110–460 ng/ml; Fig. 1). Fifteen cases (60%) were early gastric cancer (defined as carcinoma within mucosa or submucosa; Ref. 8), and among 17 patients of early gastric cancer, only 2 patients (13%) were over the normal upper limit.

In contrast, 17 (63%) of 27 advanced gastric cancer patients were over the upper limit (460 ng/ml) of the normal range of serum trypsin(ogen), and 9 of them were linitis plastica patients. The ratio of the patients showing hypertrypsinemia was significantly higher in the cases with lymph node metastases (P = 0.00177) or with liver metastases (P = 0.00505; Table 2). However, peritoneal dissemination was not a significant factor for hypertrypsinemia (P = 0.0898). Poorly differentiated adenocarcinoma or signet ring cell carcinoma showed hypertrypsinemia more frequently than did well or moderately differentiated adenocarcinoma.

Linitis plastica, a special gross type of advanced gastric cancer, showed a much greater frequency of hypertrypsinemia. In stage IV gastric cancer, which was defined as direct invasion to other organs, para-aortic lymph node metastases, peritoneal dissemination, or metastases to remote organs, according to Japanese guidelines, only 60% of the cancers excluding linitis plastica showed values over the upper limit of serum trypsin(ogen). However, 100% of linitis plastica patients showed high concentrations of serum trypsin(ogen).

Moreover, the averaged serum trypsin(ogen) level of linitis plastica patients was 384.1 ± 2319.7 ng/ml (mean ± SD), which was significantly higher than that of the early gastric cancer patients (384.1 ± 92.1; Fig. 2) as well as that of the stage IV gastric cancer patients as defined above (578 ± 440.4), excluding the linitis plastica patients.

**DISCUSSION**

Hypertrypsinemia is detected in ~60% of advanced gastric cancer, and this study shows that the sera of linitis plastica patients contain a high level of trypsin(ogen). Gastric cancer is a leading cause of death attributable to cancer in Japan. In particular, linitis plastica, or Borrmann 4 gastric cancer, which is a diffusely infiltrative carcinoma, shows significantly poor prognosis. In Japan, the cumulative 5-year survival rate of linitis plastica (0–13%) is far worse than that of the other types of gastric cancer (20.2–50.3%; Ref. 12). Therefore, it is urgently necessary to establish methods of early detection and effective therapy for linitis plastica.

Biological properties of the linitis plastica gastric cancer are: (a) progressive fibrosis of tumor, so-called scirrhous carcinoma; and (b) quick, wide, and deep invasion of cancer cells in the stomach wall. Cancer cells of linitis plastica make surrounding fibroblasts synthesize collagen by some paracrine factors secreted from cancer cells, such as epidermal growth factor (13) and transforming growth factors α and β (14). Mai et al. (15)
reported that linitis plastica patients showed high concentrations of serum tissue plasminogen activator, urokinase-type plasminogen activator, and their inhibitor, plasminogen activator inhibitor, which might support progressive fibrolysis before scirrhous formation (15). Urokinase-type and tissue plasminogen activators, as well as trypsinogen/trypsin, are members of matrix serine proteinases. Urokinase-type and tissue plasminogen activators activate plasminogen to plasmin, which shows proteolytic activity toward various extracellular proteins and can activate latent forms of some matrix metalloproteinases. Trypsin more strongly degrades extracellular matrix proteins and more effectively activates the latent forms of matrix metalloproteinases than plasmin.

Trypsin was considered to be specifically secreted from pancreatic acinar cells; thus, it was expected to become a more specific marker of acute pancreatitis than amylase. However, recent studies have shown that trypsinogen is expressed in various normal human tissues (16) as well as in vascular endothelial cells of cancer tissues (17). It was also reported that human pancreatic cancer cell lines (18), which had been derived from pancreatic ductal cells but not acinar cells and ovarian carcinoma (1), also secreted trypsinogen or tumor-associated trypsinogen; and they might correlate to invasion (19) of cancer carcinoma (1), also secreted trypsinogen or tumor-associated trypsinogen activator, and their inhibitor, plasminogen activator inhibitor, which might support progressive fibrolysis before scirrhous formation (15). Urokinase-type and tissue plasminogen activators, as well as trypsinogen/trypsin, are members of matrix serine proteinases. Urokinase-type and tissue plasminogen activators activate plasminogen to plasmin, which shows proteolytic activity toward various extracellular proteins and can activate latent forms of some matrix metalloproteinases. Trypsin more strongly degrades extracellular matrix proteins and more effectively activates the latent forms of matrix metalloproteinases than plasmin.

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Koshikawa et al. (6) found that some human gastric cancer cell lines, e.g., STKM-1 and MKN 28, also secreted a trypsino-
gen-like protein at high levels. More recently, it was found that trypsin is expressed in many human cancer cell lines derived from the stomach, colon, and breast in culture and that in stomach cancers the trypsin expression is higher in malignant, noncohesive types than in the cohesive type (21). In this study, a high concentration of serum trypsin(ogen) was detected in gastric cancer patients, especially in linitis plastica. The serum trypsin(ogen) concentration in linitis plastica was much higher than that in the other types of stage IV gastric cancer. Interestingly, STKM-1 (6), which highly secretes trypsin, was derived from pleural effusion of a linitis plastica patient (we could not determine whether MKN 28, which was from lymph node metastases of moderately differentiated adenocarcinoma, was derived from linitis plastica).

Serum trypsin(ogen) in linitis plastica patients is probably derived from the cancer cells. In fact, Ohta et al. (18) reported in their immunohistochemical study that this trypsin(ogen) occurred in 92% of the scirrhous type of advanced gastric cancer cases but only 25% of the intestinal type. Linitis plastica is the most advanced gross type of scirrhous-type gastric cancers.

Serum concentrations of trypsin(ogen) in linitis plastica patients all exceeded 1000 ng/ml, which is a much higher concentration than those in the patients with acute pancreatitis (11). Massive invasion of gastric cancer to the pancreas may release the pancreatic trypsin(ogen) into blood, increasing its serum level. In this study, however, five of seven linitis plastica patients did not show invasion to the pancreas. On the other hand, there were three patients who later received pancreatoduodenectomy because of direct invasion of gastric cancer to the pancreas head. These patients were not classified as having linitis plastica and did not show high levels of serum trypsin(ogen). Moreover, one linitis plastica patient, who received a curative operation, had a decrease of serum trypsin from 1800 to 360 ng/ml after the operation. He survived for 2 years and died from peritoneal recurrence. His serum trypsin level re-increased to 1200 ng/ml just before he died. For the six linitis plastica patients, noncu-

\[ \text{Table 2 Hypertrypsinemia} \]

<table>
<thead>
<tr>
<th>s-trypsin(^a)</th>
<th>n(+)</th>
<th>n(-)</th>
<th>( \chi^2 )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;460</td>
<td>15</td>
<td>2</td>
<td>9.7119</td>
<td>0.00177</td>
</tr>
<tr>
<td>110–460</td>
<td>10</td>
<td>15</td>
<td>7.858</td>
<td>0.00505</td>
</tr>
<tr>
<td>s-trypsin</td>
<td>H(+)</td>
<td>H(-)</td>
<td>2.877</td>
<td>0.0898</td>
</tr>
<tr>
<td>&gt;460</td>
<td>5</td>
<td>13</td>
<td>2.877</td>
<td>0.0898</td>
</tr>
<tr>
<td>110–460</td>
<td>0</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>s-trypsin</td>
<td>P(+)</td>
<td>P(-)</td>
<td>7.503</td>
<td>0.006</td>
</tr>
<tr>
<td>&gt;460</td>
<td>6</td>
<td>12</td>
<td>7.503</td>
<td>0.006</td>
</tr>
<tr>
<td>110–460</td>
<td>3</td>
<td>22</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) s-trypsin, serum concentration of trypsin-1/trypsinogen-1 (ng/ml). n(+), patients with lymph node metastasis; n(-), patients without lymph node metastasis; H(+), patients with liver metastasis; H(-), patients without liver metastasis; P(+), patients with peritoneal dissemination; P(-), patients without peritoneal dissemination; poor, poorly differentiated adenocarcinoma; sig, signet ring cell carcinoma; well, well-differentiated adenocarcinoma; mod, moderately differentiated adenocarcinoma.
rative simple total gastrectomy was performed to arrest continuous hemorrhage from cancer lesions. Serum trypsin(ogen) levels in three of the six cases markedly decreased, compared with preoperative values. The other three linitis plastica patients, whose serum trypsin(ogen) levels did not decrease after their operations, had massive para-aortic lymph node metastases or massive peritoneal dissemination. Thus, an adequate decrease of cancer volume by surgery resulted in a decrease of serum trypsin(ogen) levels.

Trypsin cannot be used as a sensitive marker of gastric cancer, because 88% of early gastric cancer cases did not exceed the normal range (Fig. 1). The serum level of trypsinogen-2, which changes more sensitively than trypsinogen-1 in acute pancreatitis (20) and cholangiocarcinoma (4) patients, should be measured in comparison with trypsin(ogen) as the marker of gastric cancer. The pathological meaning of the elevated serum trypsinogen concentration in linitis plastica patients is still unclear. However, it seems likely that serum trypsin(ogen) will become a good marker of linitis plastica.

ACKNOWLEDGMENTS

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REFERENCES

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