Serum Levels of Transforming Growth Factor α in Gastrointestinal Cancer Patients

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Abstract

Transforming growth factor α (TGFα), a polypeptide growth-stimulating factor, has been implicated to play a role in the progression of gastrointestinal (GI) cancer. It has been suggested that TGFα expression in tumors or TGFα in the biological fluids of cancer patients may have tumor marker value. The serum levels of TGFα in GI cancer patients have not been reported. In this study, the serum TGFα levels of 100 GI cancer patients, as well as 74 healthy individuals, were determined by a TGFα-specific RIA kit. All of the cancer patient sera and as well as 74 healthy individuals had detectable levels of TGFα. The TGFα concentrations in GI cancer patients ranged from 119 to 760 pg/ml, with a mean value of 269 ± 102 pg/ml. Fifty normal individuals had detectable levels of TGFα, and their levels ranged from 120 to 207 pg/ml, with a mean value of 147 ± 18 pg/ml. Differences in serum TGFα concentration between cancer patients and healthy individuals were found to be statistically significant, as evaluated by Mann-Whitney and Student's t tests. Serum TGFα levels were found to be significantly elevated in all disease stages of gastric, pancreas, colon, and rectal cancers, and only in the late stages of esophageal cancer. Serum carinoembryonic antigen levels were significantly elevated only in the late stages of these diseases. The potential of serum TGFα as a tumor marker for GI malignancy, therefore, warrants further investigation.

Introduction

TGFα is a 50-amino acid single-chain polypeptide growth factor with a molecular weight of 56,000 (1-5). Its precursor form is a 160-amino acid membrane-bound protein that is proteolytically cleaved to release the mature form (3, 4). TGFα has 40% amino acid homology to EGF (6) and mediates its proliferative effect through the cell-surface EGF receptor (1, 7-9).

TGFα plays a role in normal cellular physiology as a growth-stimulating factor, especially with cells of epithelial origin (5, 10, 11). It may also play an important role in embryogenesis (12-17), angiogenesis (18), and wound healing (19-21).

Since the discovery of TGFα in 1978 (22), the results of several studies have suggested that TGFα may also be involved in cancer progression. In general, tumor cells secrete high levels of TGFα (6, 23-25), as do cells transformed by oncogenes or retroviruses (6, 22, 26, 27). The constitutive expression of TGFα in normal cells induces a transformed phenotype (28-32). Transgenic mice expressing high levels of TGFα tend to develop liver and mammary tumors in addition to hyperplasia and dysplasia in other organs (33-36). TGFα may mediate its action through the cell surface EGF receptor via an autocrine loop (30-31, 37-43). Thus, abnormal expression of TGFα and its receptor, abnormal cellular responses to TGFα, or both may be involved in cellular transformation and in the maintenance of the transformed phenotype.

Normal and preneoplastic GI tissues express TGFα (10, 11, 44-49). GI cancer tissues express TGFα transcripts (10, 46-50), and human GI carcinoma cells express and secrete TGFα and respond to it in an autocrine or paracrine manner (23-25, 43, 51-55). Elevated TGFα levels (determined by immunoassays, biological assays, or EGF receptor competition assays) have been detected in the urine, ascites, and pleural effusions of patients with GI cancers (56-60). It has therefore been suggested that the TGFα levels in the effusions of GI cancer patients may have diagnostic or prognostic value (59). With the advent of more sensitive and specific immunoassays, it is possible to detect TGFα in human plasma (61-64). Since the levels of serum TGFα in GI cancer patients were not reported previously, we quantitated the TGFα levels in the sera of 100 GI cancer patients and 74 healthy individuals. The serum immunoreactive TGFα levels in the cancer patients were significantly higher than those of normal individuals. Serum CEA levels were also determined. No correlation was found between serum CEA and TGFα levels. Therefore, the in vivo role of TGFα in human GI malignancy and the potential of serum TGFα as a marker for GI cancer warrants further investigation.

Materials and Methods

Sera were obtained from 100 GI cancer patients registered at M. D. Anderson Cancer Center (56 men and 44 women). Their ages ranged from 26 to 78, with a mean age of 58. Their GI cancer types are summarized in Table 1. Medical records for the patients were reviewed up to the time of serum sampling, their tumors were staged by TNM staging,
Serum TGFα in Gastrointestinal Cancer

The distribution of serum immunoreactive TGFα levels in GI cancer patients and normal individuals is shown in Fig. 1. The mean TGFα levels are shown in Table 1. All 100 GI cancer patients had detectable TGFα levels. The TGFα concentrations in GI cancer patients ranged from 119 to 760 pg/ml with a mean of 269 pg/ml. TGFα levels in esophageal cancer patients ranged from 119 to 760 pg/ml with a mean of 269 pg/ml. TGFα levels in gastric cancer patients ranged from 179 to 375 pg/ml with a mean of 231 pg/ml. TGFα levels in pancreas cancer patients ranged from 185 to 480 pg/ml with a mean of 255 pg/ml. TGFα levels in colon cancer patients ranged from 175 to 540 pg/ml with a mean of 288 pg/ml. TGFα levels in rectal cancer patients ranged from 200 to 610 pg/ml with a mean of 279 pg/ml. Of the 74 normal sera used in this study, 24 had TGFα levels below the threshold of detectability of the assay (100 pg/ml). The remaining 50 normal sera had TGFα concentrations in the range of 120 to 207 pg/ml, with a mean of 147 pg/ml (Fig. 1). ANOVA showed that the difference in serum TGFα levels in cancer patients and normal individuals was statistically significant by either Student’s t test or the Mann-

Table 1 GI cancers and TGFα levels

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Number of patients</th>
<th>Mean TGFα ± SD (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>74</td>
<td>147 ± 18</td>
</tr>
<tr>
<td>Total GI</td>
<td>100</td>
<td>269 ± 102</td>
</tr>
<tr>
<td>Esophageal</td>
<td>14</td>
<td>260 ± 166</td>
</tr>
<tr>
<td>Gastric</td>
<td>11</td>
<td>231 ± 53</td>
</tr>
<tr>
<td>Pancreas</td>
<td>22</td>
<td>255 ± 64</td>
</tr>
<tr>
<td>Colon</td>
<td>36</td>
<td>288 ± 95</td>
</tr>
<tr>
<td>Rectal</td>
<td>17</td>
<td>279 ± 111</td>
</tr>
</tbody>
</table>

Table 2 Number of GI patients per disease and treatment histories

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Esophagus</th>
<th>Gastric</th>
<th>Pancreas</th>
<th>Colon</th>
<th>Rectum</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>2</td>
<td>2</td>
<td>16</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Ongoing</td>
<td>9</td>
<td>6</td>
<td>3</td>
<td>21</td>
<td>10</td>
</tr>
<tr>
<td>Post-treatment</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>11</td>
<td>22</td>
<td>36</td>
<td>17</td>
</tr>
</tbody>
</table>

Fig. 1. Distribution of serum TGFα levels in normal healthy individuals and GI cancer patients. Points, serum TGFα concentration of one individual; bs,bs,bs,bs,bs, mean TGFα concentration within a group. TGFα levels were determined in a total of 74 normal sera. Twenty-four normal sera had TGFα levels below the threshold of detection (100 pg/ml) of the assay. The TGFα levels of the other 50 normal sera are shown.

Results

The distribution of serum immunoreactive TGFα levels in GI cancer patients and normal individuals is shown in Fig. 1. The mean TGFα levels are shown in Table 1. All 100 GI cancer patients had detectable TGFα levels. The TGFα concentrations in the GI cancer patients ranged from 119 to 760 pg/ml with a mean of 269 pg/ml. TGFα levels in esophageal cancer patients ranged from 119 to 760 pg/ml with a mean of 269 pg/ml. TGFα levels in gastric cancer patients ranged from 119 to 760 pg/ml with a mean of 269 pg/ml. TGFα levels in pancreas cancer patients ranged from 179 to 375 pg/ml with a mean of 231 pg/ml. TGFα levels in pancreas cancer patients ranged from 185 to 480 pg/ml with a mean of 255 pg/ml. TGFα levels in colon cancer patients ranged from 175 to 540 pg/ml with a mean of 288 pg/ml. TGFα levels in rectal cancer patients ranged from 200 to 610 pg/ml with a mean of 279 pg/ml. Of the 74 normal sera used in this study, 24 had TGFα levels below the threshold of detectibility of the assay (100 pg/ml). The remaining 50 normal sera had TGFα concentrations in the range of 120 to 207 pg/ml, with a mean of 147 pg/ml (Fig. 1). ANOVA showed that the difference in serum TGFα levels in cancer patients and normal individuals was statistically significant by either Student’s t test or the Mann-

Discussion

Serum tumor markers can be operationally defined as serum molecules whose levels can be used in the diagnosis or clinical management of malignant diseases. The utility of CEA, the best characterized tumor marker, and the more recently recognized tumor markers such as CA 19-9 in managing GI cancer are constantly being debated (63–69).
There remains a great need for identification of new tumor markers.

In this study 100 GI cancer patients had serum immunoreactive TGFα levels significantly higher than those of 74 normal healthy individuals. All the GI cancer patients, but only 67% of the normal individuals, had detectable levels of serum TGFα. The cancer patients represented a cross-section of the GI cancer patients at M.D. Anderson Cancer Center with esophageal, gastric, pancreas, colon, and rectal cancers (see Table 1). Most of the patients were receiving some form of treatment at the time of serum sampling (see Table 2); fewer patients were in the follow-up phase of treatment. Finally, the fewest patients were untreated. The effect of treatment on serum immunoreactive TGFα levels is unknown. However, elevated TGFα levels were observed in most patients regardless of disease stage or treatment history, except for patients with early stage esophageal cancer. TGFα was elevated to the same degree in untreated patients, in patients receiving treatment, and in patients in the follow-up phase. However, TGFα levels did not correlate with site of metastatic disease in stage IV patients.

Of the 14 esophageal cancer patients, 12 had adenocarcinoma and 2 had squamous cell carcinoma. In these patients, disease grade was not correlated with TGFα level, although the seven patients with poorly differentiated adenocarcinomas tended to have slightly higher TGFα values. The two patients with squamous cell carcinoma had low levels of TGFα (119 and 133 pg/ml). Of the 11 gastric cancer patients, all had adenocarcinoma. Similarly, TGFα was elevated in all grades but was higher in patients with poorly differentiated tumors. Of the 22 pancreas cancer patients, 16 were untreated. Disease grade had no impact on their TGFα levels. Most of the colon and rectal cancer patients had stage IV disease and were receiving treatment at the time of serum sampling. The disease grade of their adenocarcinomas also was not correlated with TGFα levels.

CEA has been somewhat useful in managing GI malignancy; the highest CEA sensitivities and specificities have been for extensive colon and rectal cancer, especially in follow-up for recurrent disease (65, 66). In our study CEA tended to be higher in stage IV disease. In these patients high CEA levels correlated with liver metastasis in stage IV colon and rectal cancers and with the presence of unresectable tumors in stage III and IV pancreas cancer. CEA levels were not correlated with TGFα levels.

We determined the sensitivities and specificities of TGFα and CEA using cutoff levels of 176 pg/ml for TGFα and 2.5 ng/ml for CEA (see Table 4). These cutoff levels were determined as the 97.5th percentile of normal TGFα values using a standard laboratory cutoff value for CEA. TGFα was highly sensitive for all stages of GI malignancy except for early esophageal cancer. As mentioned previously, CEA sensitivity was highest for stage IV colon and rectal cancer.

In athymic mice, TGFα expression in human cancer cells has been shown to promote and maintain the transformed properties of these cells via an autocrine mechanism (31). Administering anti-TGFα antibody to athymic mice carrying human tumor xenografts suppresses tumor growth (70). Thus, elevated serum TGFα levels may play a role in disease progression in cancer patients.

The exact source of the excess serum immunoreactive
TGFA in cancer patients is not known. GI cancer cells produce and secrete TGFA in vitro (23–25, 43, 51–55). Immunohistochemical staining of sections of human tumors has shown that these tumors express TGFA (10, 46–50). Thus, the tumors themselves may be contributing to the increase in TGFA. Our study also showed that TGFA was a normal constituent of human serum, lending support to the hypothesis that TGFA has a role as a growth factor in normal cellular physiology. TGFA is also thought to play a role in the inflammatory response (71, 72) and wound healing (19–21). Thus, physiological responses to the presence of tumors, to treatment, or to both may also contribute to increased serum TGFA levels in these patients.

How serum TGFA levels can be used to manage GI malignancy has yet to be determined. Further investigation, including controlled longitudinal studies with serial measurement of TGFA in individual patients in the pretreatment, treatment and follow-up phases, will provide valuable information.

References


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