Tumor Suppressor Gene Maspin Induces Apoptosis in Stomach and Colon Cancer

Atsushi Ishino, Nobuyuki Ohike, Koichi Nagasaki*, Takeyoshi Kitayama, Kaoru Katou and Toshio Morohoshi

Abstract: The tumor suppressor gene maspin serves to inhibit cancer infiltration/metastasis, and maspin's involvement in apoptosis-inducing action has been noted. In the current research, we studied the relationship between maspin expression and apoptosis using clinical specimens of stomach and colon cancer. Subjects were 39 cases of stomach cancer (20 cases of early cancer, 19 cases of advanced cancer) and 36 cases of colon cancer (16 cases of early cancer, 20 cases of advanced cancer) in which the cancer was surgically resected. We performed immunohistochemical staining of maspin and ss-DNA for apoptotic cell extraction. Based on the degree of maspin expression, we classified subjects into a high expression and low expression group, calculated the number of ss-DNA-positive cells for both, and compared the proportion of these cells. In early stomach cancer, the proportion of ss-DNA-positive cells was significantly higher in the group with high maspin expression (high expression group vs. low expression group 0.014 vs. 0.005; P = 0.022). In advanced cancer, a significant difference was not found (0.013 vs. 0.005; P = 0.09). However, the proportion of such cells with advanced stomach cancer tended to be high in high expression groups. With colon cancer, the proportion of ss-DNA-positive cells with both early and advanced cancer was significantly higher in the group with high maspin expression (early cancer 0.009 vs. 0.002, P = 0.021; advanced cancer 0.012 vs. 0.003, P = 0.006). Results suggested that the expression of maspin in both stomach and colon cancer may induce apoptosis regardless of the extent of the tumor. Future study of molecular therapies targeting maspin is anticipated for cancer that is difficult to cure surgically.

Key words: maspin, apoptosis, stomach cancer, colon cancer, tumor suppressor gene

Introduction

In recent years, opportunities for the early detection and resection of stomach and colon cancers have increased, and the prognosis for these conditions has improved markedly. The 5-year survival rates after standard surgery for both stage IB or lower stomach cancer and stage I or lower colon cancer exceed 85% and are extremely favorable1,2. With stage IV,
in contrast, the 5-year survival rates for stomach and colon cancer decline markedly to 10–20% \(^1,2\). With the increase in early cancer, limited surgery such as endoscopic mucosal resection or functional preservation has garnered attention, but the current reality is that highly advanced cancer that is determined to be unresectable at diagnosis is not remarkably decreasing \(^1,2\), so improvement of prognosis in advanced cancer is desired. The mainstays of treatment for these cases of unresectable cancer are palliative surgery and chemotherapy, but gastrointestinal cancer traditionally has a low sensitivity to anticancer agents and there is a strong impression that chemotherapy is adjunctive treatment. Thus, new anticancer agents should be developed and new treatment strategies like molecular-targeting therapies should be examined.

The tumor suppressor gene maspin is a type of serine protease inhibitor and serves to inhibit cancer infiltration and metastasis and neovascularization \(^3-5\). In both stomach and colon cancer, maspin has been reported to be closely involved in tumor progression and neovascularization \(^6-13\). The current authors reported a high expression of maspin in previous cases of stomach and colon cancer and, with regard to cases expressing maspin, a significantly lower proportion of vascular invasion in cases of early cancer \(^14,15\). Recently, the apoptosis-inducing action of maspin on tumor cells has garnered attention \(^16-18\). The mechanism of maspin expression in cancer cells has already been elucidated \(^19\), and clinical applications using this apoptosis-inducing action are anticipated. Nevertheless, the relationship between maspin and apoptosis in stomach and colon cancer has not been examined previously.

The aim of the current research is to examine the possibility that maspin expression induces apoptosis in stomach and colon cancer.

**Materials and Methods**

Subjects were 39 cases of stomach cancer and 36 cases of colon cancer in which the cancer was surgically resected at Showa University Hospital between May 2004 and April 2005. For cases of stomach cancer, the male-to-female ratio was 1.5 : 1 and the mean age was 65.6 years of age; for cases of colon cancer, the male-to-female ratio was 1.4 : 1 and mean age was 68.3 years of age. The histological type of either cancer was limited to adenocarcinoma. Thin sections of 3 \(\mu m\) were made from formalin-fixed paraffin-embedded blocks including the maximum cut surface of the tumors for use in immunostaining. Maspin expression was examined immunohistochemically and apoptotic cell extraction was examined by immunostaining using ss-DNA polyclonal antibody. Anti-human maspin monoclonal antibody (PharMingen International, San Diego, CA, U.S.A.; diluted 1 : 75) was used for immunohistochemistry, which was performed in accordance with the EnVision ChemMate method (for details, see \(^21\)). Briefly, for antigen retrieval, sections were pretreated by microwaving for 10 min in 10 mM citrate buffer. Sections were incubated with anti-human maspin monoclonal antibody for 30 min and then incubated with HRP-conjugated dextran polymer reagent (Dako Japan, Kyoto, Japan) for 30 min. 3'3'-Diaminobenzidine was used as the chromogen. ss-DNA (Dako Japan; diluted 1 : 400) staining was performed in a similar manner.

In accordance with the paper by Ohike et al\(^{14}\), maspin expression is considered to be a high level when tumor cells that were moderately to strongly positive accounted for 1 / 3 of the tumor as a whole (Fig. 1). For assessment of ss-DNA staining, 3 locations in ss-DNA-
Maspin Induces Apoptosis in Cancer

Fig. 1. Diffuse cytoplasmic and nuclear staining of maspin is demonstrated in the colonic cancer.

Fig. 2. Two ss-DNA-positive cells (arrowhead) are observed in the same area of Fig. 1.

Table 1. Frequency of high expression of maspin

<table>
<thead>
<tr>
<th></th>
<th>Early cancer</th>
<th>Advanced cancer</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric cancer</td>
<td>17 / 20 (85%)</td>
<td>17 / 19 (89%)</td>
<td>NS</td>
</tr>
<tr>
<td>Colonic cancer</td>
<td>10 / 16 (63%)</td>
<td>14 / 20 (70%)</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS: not significant

stained samples (containing 100 or more tumor cells) were selected at random at 800 × magnification and imaged using Win ROOF image processing software (Mitani Co., Tokyo, Japan) (Fig. 2). ss-DNA-positive cells were extracted and the number of positive cells per 100 cells was calculated (as %).

Based on the preceding assessments, maspin expression and the extent of the tumor were compared and the relationship between maspin expression and ss-DNA-positive cells was studied. The Student’s t-test was used for statistical analysis with p < 0.05 indicating a significant difference.

Results

In stomach cancer, high maspin expression was seen in 87% of the specimens overall; expression was high for both early cancer and advanced cancer at 85% and 89%, respectively. In colon cancer, high maspin expression was seen in 67% of the specimens overall; expression was high for both early cancer and advanced cancer at 63% and 70%, respectively. A significant difference in maspin expression and the extent of the tumor was not found for either stomach or colon cancer (Table 1).

For ss-DNA staining, all of the cases with low maspin expression (3 cases of early stomach cancer and 2 of advanced cancer; 6 cases of early colon cancer and 6 of advanced
Table 2. Frequency of expression of ss-DNA in gastric cancers

<table>
<thead>
<tr>
<th>Maspin expression</th>
<th>n</th>
<th>High 10</th>
<th>Low 5</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>total</td>
<td>15</td>
<td>0.0136</td>
<td>0.0056</td>
<td>0.0019</td>
</tr>
<tr>
<td>early</td>
<td>8</td>
<td>0.0142</td>
<td>0.0057</td>
<td>0.0221</td>
</tr>
<tr>
<td>advanced</td>
<td>7</td>
<td>0.0130</td>
<td>0.0054</td>
<td>0.0933</td>
</tr>
</tbody>
</table>

Table 3. Frequency of expression of ss-DNA in colonic cancers

<table>
<thead>
<tr>
<th>Maspin expression</th>
<th>n</th>
<th>High 10</th>
<th>Low 12</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>total</td>
<td>22</td>
<td>0.0112</td>
<td>0.0032</td>
<td>0.0001</td>
</tr>
<tr>
<td>early</td>
<td>11</td>
<td>0.0099</td>
<td>0.0029</td>
<td>0.0217</td>
</tr>
<tr>
<td>advanced</td>
<td>11</td>
<td>0.0124</td>
<td>0.0036</td>
<td>0.0065</td>
</tr>
</tbody>
</table>

cancer) and 20 cases with high expression (in order of lower file numbers, 5 cases of early stomach cancer, 5 of advanced cancer, 5 of early colon cancer, and 5 of advanced cancer) were selected and staining performed.

With stomach cancer, the proportion of ss-DNA-positive cells was significantly higher overall in the high expression group than in the group with low maspin expression (1.36% vs. 0.56%; P = 0.0019). In early cancer, the proportion was significantly higher in the high expression group than in the group with low maspin expression (1.42% vs. 0.57%; P = 0.0221). In advanced cancer, a significant difference between the groups with high and low maspin expression was not found (1.3% vs. 0.54%; P = 0.0933), but ss-DNA-positive cells tended to be more prevalent in the group with high maspin expression (Table 2).

With colon cancer, the proportion of ss-DNA-positive cells was significantly higher overall in the high expression group than in the group with low maspin expression (1.12% vs. 0.32%; P = 0.0001). In early cancer, the proportion was significantly higher in the high expression group than in the group with low maspin expression (0.99% vs. 0.29%; P = 0.021). In advanced cancer, the proportion was also significantly higher in the high expression group than in the group with low maspin expression (1.24% vs. 0.36%; P = 0.006) (Table 3).

**Discussion**

The current study found a positive correlation between the expression of maspin and apoptosis of tumor cells for both stomach and colon cancer. This thus suggests that maspin expression may induce apoptosis in the two types of cancer. Moreover, this correlation was unaffected by differences in the extent of early and advanced cancer. The lack of a significant difference for advanced stomach cancer alone is believed to be because there were only 2 cases with low maspin expression. Latha et al reported that apoptosis-inducing action by maspin in cancer cells is through an intrinsic pathway. Specifically, maspin transported to mitochondria enhances the permeability of the mitochondrial outer mem-
brane, promotes the release of cytochrome c from the intermembrane space, and induces apoptosis by activating caspase protease. In this event, the expression of Bel-2 in the mitochondrial outer membrane decreases. Furthermore, maspin's involvement in apoptosis induction has also been demonstrated because, as a consequence of mutations in the RSL region of maspin, transport of maspin to mitochondria does not occur. The current research is the first to demonstrate a positive relationship between maspin and apoptosis using clinical cancer specimens.

Many previous studies of maspin expression using immunohistochemical techniques in clinical specimens of stomach and colon cancer reported that a decrease in maspin expression is correlated with a poor prognosis and incidence of poor prognostic factors. However, Terashima et al reported that enhancement of maspin expression is positively correlated with a positive rate of lymph node metastasis. The current authors previously examined the relationship between maspin expression and clinical/pathological factors for both stomach and colon cancer but reported that it may not necessarily serve as a prognostic marker. With regard to this conflicting result, the current authors discussed the fact that maspin displays immunohistochemically heterogeneous expression in advanced cancer with an increased tumor burden and particularly with adenocarcinoma and that this heterogeneity invites confusion when studying the correlation to clinical/pathological prognostic factors. In any event, these phenomena do not negate the cancer-suppressing action of maspin; rather, the current results are significant in that they suggest that the cancer-suppressing action of maspin is by means of apoptosis.

The factors responsible for determining cell characteristics such as cancer growth and infiltration/metastasis have been clarified by recent cancer research, and new therapies are being developed with these factors as molecular targets. Clinical use primarily of therapeutic agents targeting the EGFR family has begun and is being studied further. In addition, angiogenesis factors and apoptosis-related factors occupy a vital position as targets for these molecular-targeting therapies. The current research has demonstrated induction of apoptosis by maspin in stomach and colon cancer regardless of the extent of cancer. Apoptosis induction by maspin is an important potential target for molecular-targeting therapies.

Reference

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[Received June 12, 2007: Accepted July 11, 2007]