Association of Matrilysin Expression with Recurrence and Poor Prognosis in Human Esophageal Squamous Cell Carcinoma

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Abstract

Matrix metalloproteinase-7 (matrilysin) has been implicated in tumor invasion and metastasis as well as tumor initiation and growth. In this study, we analyzed an association between immunohistochemically detected matrilysin expression at the invasive front in esophageal squamous cell carcinomas and clinicopathological characteristics and determined whether matrilysin predicts recurrence and/or survival. Matrilysin expression at the invasive front was detected in 49% of 100 carcinoma tissues and was associated with the depth of invasion ($P < 0.0001$), advanced tumor stage ($P = 0.0159$), recurrences ($P = 0.0002$), and overall survival differences when compared to those with a matrilysin-negative one ($P < 0.0001$). Matrilysin remained a significant predictive value for disease-free and overall survival in multivariate analysis, including conventional clinicopathological factors ($P = 0.0007$ and 0.0004, respectively). Our results suggest that matrilysin may play a key role in the progression of esophageal carcinoma and that its detection may be useful for the prediction of recurrence and poor prognosis and, possibly, for selecting patients for anti-matrix metalloproteinase therapy.

Introduction

It has become clear that cancers develop and progress through the accumulation of various genetic alterations. Esophageal carcinoma is one of the most aggressive malignant tumors, and its prognosis is still worse than that of other digestive tract cancers. Five-year overall survival after potentially curative surgical resection is still very low, due to the high rate of local and distant recurrences (1). Although conventional pathological staging has served as the standard measure of prognosis of patients with esophageal carcinoma, it does not always define the individual risk of recurrence after surgical resection. In other words, poor prognosis of these patients is not necessarily predicted by the TNM classification alone. Recent advances in the molecular genetics of esophageal carcinoma have stimulated attempts to evaluate the prognostic significance of specific genetic alterations in this tumor (2, 3). Identification of a prognostic marker that is both sensitive and specific, and a potency to start an activation cascade of MMPs (4), such as a minimum MMP structure, wide spectrum of substrate specificity, and a potency to start an activation cascade of MMPs (4, 5). In addition to the overexpression in a variety of cancer tissues (4–8), in vitro and animal data suggest that matrilysin may play a key role in tumor invasion (7, 9–11) and metastasis (12) as well as tumor initiation and growth (13–15). Its advantage as a possible biological marker of tumor behavior is a production by cancer cells themselves. We have reported that matrilysin expression at the invasive front correlates with the progression of gastric cancers (16). Matrilysin expression at the invasive front was also frequently observed in a small number of esophageal SCC tissues that we examined (16). Another advantage is susceptibility to direct therapeutic intervention. Administration of a synthetic MMP inhibitor batimastat to Min mice reportedly suppresses tumor multiplicity by nearly 50%, which is similar to that observed in matrilysin-deficient Min mice (14, 17). Indeed, clinical trials of several synthetic MMP inhibitors are currently underway (18). Inhibition of matrilysin by an antisense expression vector or antisense oligonucleotides has been demonstrated to suppress the in vitro invasive potential and in vivo metastatic potential of tumor cells (10, 19, 20).

On the basis of these findings, we analyzed immunohistochemically matrilysin expression in 100 primary esophageal SCC tissues to determine whether matrilysin expression at the invasive front correlates with clinicopathological characteristics, disease recurrence, and survival.

Patients and Methods

Patients and Tissue Samples. Paraffin-embedded tumor specimens from 100 Japanese patients with esophageal SCC were used for immunohistochemical analysis. All patients had undergone surgical resection at Keiyukai Sapporo Hospital. The clinicopathological characteristics were evaluated according to the guidelines of the Union Internationale Contre le Cancer.

Immunohistochemistry. The 5-mm sections were dewaxed in xylene, rehydrated in alcohol, and then heated to 105°C in an autoclave for 10 min. The endogenous peroxidase activity was suppressed by a solution of 3% hydrogen peroxide in methanol for 5 min. After being rinsed twice in PBS, the sections were treated for 18 h with an antihuman matrilysin monoclonal antibody (141-7B2; 10 μg/ml, Fuji Chemical, Toyama, Japan) diluted in PBS (21). After washing three times in PBS, the sections were treated with biotinylated goat antimouse immunoglobulin (DAKO, Glostrup, Denmark) for 10 min, followed by horseradish peroxidase-avidin complex, diluted as recommended by the manufacturer (DAKO) for 10 min. The slides were then washed in PBS and developed in 0.05 M Tris-HCl (pH 7.5) containing 0.6 mg/ml 3,3′-diaminobenzidine at room temperature. The sections were counterstained in Mayer’s hematoxylin and mounted. Immunostaining signals at the invasive front were scored in two sections each by two independent observers. The scores were calculated as the number of stained cells divided by the total number of carcinoma cells, as described previously (22). Cases were considered positive when >30% of carcinoma cells at the invasive front were stained with the antibody.

Statistical Analysis. Matrilysin expression was assessed for associations with clinicopathological parameters using following statistical tests: Student’s
Results

Fig. 1 shows representative results of immunohistochemistry for matrilysin in esophageal SCC tissues. The cytoplasm and cell membrane of carcinoma cells were stained for matrilysin, but stromal cells other than some monocytes were not stained. There was no detectable immunoreactivity with the control AI-206 antibody (data not shown). Sections with immunostaining signals in >30% of carcinoma cells at the invasive front were judged as being positive for matrilysin, which was thus observed in 49% of 100 cases. The relationship between the expression of matrilysin and clinicopathological characteristics is listed in Table 1. The expression of matrilysin was associated with the depth of invasion \((P < 0.0001)\), advanced tumor stage \((P = 0.0159)\), recurrences \((P = 0.0002)\), and recurrences within the first postoperative year \((P = 0.002)\). On the other hand, there were no significant relationships between matrilysin expression and age, sex, lymph node, or distant metastasis (Table 1 and data not shown).

Patients with matrilysin-positive carcinoma had significantly shorter disease-free \((P < 0.0001)\) and overall \((P < 0.0001)\) survival times than did those with matrilysin-negative carcinoma (Fig. 2). In the univariate analysis, significant prognostic variables for predicting both disease-free and overall survival were matrilysin expression, depth of invasion, lymph node metastasis, distant metastasis, and TNM stage (Table 2). In the multivariate analysis of these variables, only matrilysin remained a significant prognostic variable for predicting disease-free and overall survival (Table 2).

Discussion

In this study, matrilysin expression in carcinoma cells at the invasive front was immunohistochemically seen in 49% of patients with esophageal SCC and was associated with the depth of invasion and advanced tumor stage. Thus, matrilysin at the invasive front is likely...
to contribute to the more invasive phenotype of carcinoma cells, resulting in the progression of esophageal SCC.

The implication of matrilysin expression at the invasive front was further substantiated by its correlation with disease recurrence and shorter disease-free and overall survival time. Moreover, only matrilysin provided a significant predictive value for disease-free and overall survival in the multivariate analysis, suggesting that matrilysin expression could be a powerful predictor of recurrence and poor prognosis, with a significance equaling or surpassing other conventional clinicopathological factors.

Identification of matrilysin as a molecular marker that correlates with disease recurrence and poor prognosis would provide new insights into disease management by making it possible to define a high risk of recurrence, thus providing a more accurate estimation of prognosis of patients with esophageal SCC. Consequently, early postoperative screening and/or intense postoperative therapy should be performed on patients with matrilysin-positive carcinoma. Immunohistochemical analysis is a technique that is feasible in daily clinical practice, and therefore, analysis of matrilysin expression could be an important routine part of the management of patients with esophageal SCC.

Nevertheless, the effect of conventional therapy may be limited for patients with high malignant potential such as those with matrilysin-positive carcinoma. Indeed, several attempts have been made to try to develop a novel therapeutic approach. In this regard, it is noteworthy that matrilysin could be a potential target for therapeutic intervention. The use of synthetic broad-spectrum MMP inhibitors is one of possible strategies (18). Considering our results, matrilysin could be a primary target of such inhibitors in esophageal SCC. Although considerable optimization is necessary in many aspects, the use of matrilysin-specific antisense oligonucleotides may be another possibility in the future (10, 19, 20). Thus, the immunohistochemical analysis of matrilysin in esophageal SCC tissues could also form the basis of a new therapeutic strategy via broad and/or selective MMP inhibitors.

The diagnostic strategy shown in this study and the advances in therapeutic approaches would improve the prognosis of patients with esophageal SCC.

### Table 2

**Univariate and multivariate analyses of disease-free and overall survival**

<table>
<thead>
<tr>
<th></th>
<th>Disease-free survival</th>
<th>Overall survival</th>
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<tbody>
<tr>
<td></td>
<td>Univariate</td>
<td>Multivariate</td>
</tr>
<tr>
<td></td>
<td>RR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Matrilysin expression</td>
<td>4.1 (2.1–8.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Depth of invasion</td>
<td>4.8 (1.7–13.5)</td>
<td>0.0030</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td>4.2 (1.8–10.1)</td>
<td>0.0012</td>
</tr>
<tr>
<td>Distant metastasis</td>
<td>2.6 (1.3–5.3)</td>
<td>0.0070</td>
</tr>
<tr>
<td>TNM stage</td>
<td>4.0 (1.9–8.4)</td>
<td>NS</td>
</tr>
</tbody>
</table>

*RR, relative risk; CI, confidence interval; NS, not significant.*

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**References**


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