TAOS1, a Novel Marker for Advanced Esophageal Squamous Cell Carcinoma

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Abstract. Background: TAOS1 (tumor amplified and overexpressed sequence 1) was recently cloned and found to be possibly important in driving the amplification of 11q13 in oral squamous cell carcinoma (SCC). Materials and Methods: Quantitative RT-PCR was performed to determine the possible relationship between TAOS1 gene expression levels and clinicopathological features in esophageal SCC. Results: TAOS1 overexpression was observed in 7 out of 38 (18%) esophageal SCCs and CCND1 overexpression was observed in 4 out of 38 (11%), suggesting that TAOS1 was more frequently overexpressed than CCND1 in esophageal SCC. The examination of the correlation of TAOS1 overexpression with the clinicopathological features revealed a significant difference in lymph node metastasis (p=0.014) and a trend towards advanced TNM stages (p=0.074). Conclusion: The present results suggest that TAOS1 might serve as a new marker for predicting the malignancy of esophageal SCC.

Accumulating evidence indicates that a series of genetic changes in dominant oncogenes such as ΔNp63 and hst1/int 2 and tumor suppressor genes such as p53 and p16 are involved in the pathogenesis of human esophageal squamous cell carcinoma (SCC) (1-5). Plasminogen activator inhibitor-1 (PAI-1) expression was demonstrated to significantly increase along with the tumor stage and was a strong and independent prognostic factor for esophageal SCC (6). The search for novel genetic changes that might indicate the malignancy of esophageal SCC is necessary.

Abbreviations: TAOS1, tumor amplified and overexpressed sequence 1; SCC, squamous cell carcinoma; RT-PCR, reverse transcription-PCR.

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Chromosomal band 11q13 seems to be one of the most frequently amplified lesions in human cancer (7) and is associated with a poor prognosis (8). It has long been considered that CCND1 should play a role in driving 11q13 amplification because it is overexpressed in various cancers, especially esophageal SCC (9). TAOS1 (tumor amplified and overexpressed sequence 1) has recently been cloned from this chromosomal region and found to be possibly important in driving the amplification of 11q13 in oral SCC (10). This result prompted us to examine the TAOS1 expression level in esophageal SCC.

To test the hypothesis that TAOS1 may serve as a candidate marker for the malignancy of esophageal SCC, a quantitative RT-PCR was performed and the relationship between TAOS1 gene expression levels and clinicopathological features in esophageal SCC was evaluated.

Materials and Methods

Tissue specimens. The study group consisted of 38 esophageal SCC patients who had undergone surgical operations in our Gastroenterological Surgery Department at Nagoya University Graduate School of Medicine, Japan, from 1994 to 2002. All tumors and corresponding normal tissues were collected at the surgical resection and were stored at –80° C. The patients were classified into two groups for analysis according to gender, tumor location, depth of invasion, lymph node metastasis and TNM stage.

RNA preparation and reverse transcription. Total RNA was extracted from esophageal SCC and corresponding normal tissues with guanidium thiocyanate, as described previously (11). The amount of RNA was measured spectrophotometrically by absorbance at 260 nm. First-strand cDNA was generated from RNA, as described previously (12).

Quantitative reverse transcription (RT)-PCR. Quantitative RT-PCR was performed in an ABI sequence detection system 7700. Thermocycling was carried out in a final volume of 50 μL containing 2.0 μL of the cDNA sample, 1.0 μL each of the TAOS1 or CCND1 primers (forward and reverse) and 25 μL of qPCR Mastermix (including Tag DNA polymerase, reaction buffer, and deoxynucleotide triphosphate mixture) (Applied Eurogentec,
Seraing, Belgium). The TAOS1 and CCND1 primers for quantitative RT-PCR were described previously (10). The PCR amplification consisted of 45 cycles (95°C for 15 sec and 60°C for 60 sec) after an initial denaturation step (95°C for 12 min). To correct for differences in both quality and quantity between samples, ribosomal 18S was used as an internal control. TAOS1, CCND1 and ribosomal 18S mRNA variabilities were determined from triplicate samples. The quantitative error of all triplicate samples was less than 10%. An average quantity of triplicated samples was applied and the targets were obtained from the same mRNA preparations.

**TAOS1 or CCND1 expression score.** The relative amounts of TAOS1 or CCND1 in esophageal SCC (T) and the corresponding normal tissue (N) mRNA that were normalized to an internal control of ribosomal 18S mRNA were calculated. The average amounts of TAOS1 or CCND1 in all Ns were subsequently calculated in each tissue. The TAOS1 or CCND1 expression score in each tissue was defined as follows: relative amount of T / average relative amount of all Ns.

**TAOS1 or CCND1 overexpression was determined when the expression score was more than 1.5.**

**Statistical analysis.** The Fisher’s exact test was used to examine the possible association between TAOS1 or CCND1 expression and clinicopathological features. Differences between the means of analyzed variables observed were calculated by the Mann-Whitney U-test. *P*<0.05 (two-tailed) was considered significant.

**Results**

**TAOS1, CCND1 and ribosomal 18S expression levels were first analyzed in an esophageal SCC cell line using a quantitative RT-PCR.** The mRNA concentrations were determined after extensive optimization of PCR conditions, including reaction temperature and cycling times. The results of these trials provided us with a highly sensitive, specific and reproducible real-time RT-PCR for the specific detection of these mRNAs (Figure 1).

**TAOS1, CCND1 and ribosomal 18S expression levels were then examined in 38 esophageal SCCs.** The histograms of the TAOS1 and CCND1 expression scores in these tissues are provided in Figure 2. The mean TAOS1 and CCND1 expression scores were 1.76±1.88 and 1.09±1.05, respectively. Subsequently, the 38 esophageal SCCs were divided according to the TAOS1 and CCND1 expression scores (more or less than 1.5). TAOS1 overexpression was observed in 7 out of 38 (18%) esophageal SCCs and CCND1 overexpression in 4 out of 38 (11%), suggesting that TAOS1 was more frequently overexpressed than CCND1 in esophageal SCC, as reported previously for oral SCC (10).

To determine the role of TAOS1 overexpression in esophageal SCC, the correlation of TAOS1 overexpression with clinicopathological features was examined. There was no significant difference in the distribution of patients with **TAOS1 overexpression in terms of age, gender, tumor location, tumor size or depth of invasion.** However, a significant difference was observed in the incidence of lymph node metastasis (*p*=0.014) and a trend toward advanced TNM stages (*p*=0.074) (Table I). Although the possible correlation between CCND1 overexpression and clinicopathological features was examined, there were no significant differences in these parameters (data not shown). These results suggest that TAOS1 might be a useful marker for advanced esophageal SCC.

**Discussion**

The 11q13 region is frequently amplified in various cancers and CCND1 in 11q13 has long been considered a target of the amplification. CCND1 is one of the cyclins that induces the cell cycle progression through the G1/S checkpoint. Several reports have indicated that CCND1 overexpression was significantly associated with poor prognosis in SCCs and several other types of cancer (9, 13-15).

Recently, Huang et al. reported a novel candidate oncogene, TAOS1, which was cloned from chromosome 11q13 (10). TAOS1 is located approximately 12-kb distal to the CCND1 gene. TAOS1 spans 2,494 bp and consists of 5 exons, the functions of which are currently unknown. Using quantitative RT-PCR, TAOS1 was examined and found to be more frequently overexpressed in oral SCCs compared to CCND1. This result implied that TAOS1 overexpression might also be associated with the malignancy of cancers, especially SCCs, just like CCND1.

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**Table I. Clinicopathological features and TAOS1 expression scores in esophageal SCC.**

<table>
<thead>
<tr>
<th>Clinicopathological features</th>
<th>Variable</th>
<th>No. of cases</th>
<th>TAOS1 expression score</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt;1.5 &amp; 1.5≥</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>51 to 77</td>
<td>38</td>
<td>64±7</td>
<td>0.62¹</td>
</tr>
<tr>
<td>Gender</td>
<td>male</td>
<td>32</td>
<td>6</td>
<td>&gt;0.99²</td>
</tr>
<tr>
<td>Tumor location</td>
<td>upper half</td>
<td>12</td>
<td>0</td>
<td>0.074²</td>
</tr>
<tr>
<td>Tumor size (mm)</td>
<td>17 to 140</td>
<td>38</td>
<td>47±14</td>
<td>0.96¹</td>
</tr>
<tr>
<td>Depth of tumor</td>
<td>≤mt³</td>
<td>14</td>
<td>1</td>
<td>0.22²</td>
</tr>
<tr>
<td>invasion</td>
<td>mt&lt;</td>
<td>24</td>
<td>6</td>
<td>0.014²</td>
</tr>
<tr>
<td>Lymph node</td>
<td>–</td>
<td>16</td>
<td>6</td>
<td>0.074²</td>
</tr>
<tr>
<td>TNM stage</td>
<td>I, II</td>
<td>12</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>III, IV</td>
<td>26</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

¹Mann-Whitney U-test.  
²Fisher’s exact test.  
³Muscular tunic.
To our knowledge, the present study has demonstrated for the first time that \textit{TAOS1} overexpression was significantly associated with lymph node metastasis and showed a trend towards advanced TNM stages. The results suggest that \textit{TAOS1} might serve as a new marker predicting the malignancy of esophageal SCC.

This study also provides a solid basis for additional research into the molecular mechanism of \textit{TAOS1} expression in esophageal SCCs. Since esophageal SCC is one of the most aggressive of all forms of cancer, changing the overall survival rate using only information regarding \textit{TAOS1} expression levels might not be feasible. However, \textit{TAOS1} expression may well serve as a valuable marker for determining the extent of resection in esophageal SCC.

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**References**


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