PTCH Gene Expression as a Potential Marker for Esophageal Squamous Cell Carcinoma

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Abstract. Background: The PTCH expression level in esophageal squamous cell carcinoma (SCC) was examined. Materials and Methods: To test whether PTCH can serve as a candidate marker for esophageal SCC, a quantitative reverse transcription (RT) -PCR for the PTCH gene was performed and the possible relationship between PTCH gene expression levels and clinicopathological findings in esophageal SCC was evaluated. Results: A low PTCH expression score was observed in 18 out of 29 (62%) esophageal SCCs and it was significantly correlated with a poor prognosis in esophageal SCC patients (p=0.0073). Conclusion: These results suggest that PTCH might serve as a new parameter for the prediction of prognosis in esophageal SCC.

Esophageal squamous cell carcinoma (SCC) is a most aggressive cancer. Accumulating evidence indicates that a series of genetic changes in dominant oncogenes, such as cyclic D1 and hst1/int 2, and in tumor suppressor genes, such as p53 and p16, are involved in the pathogenesis of human esophageal SCC (1-4).

Recently, it was proven that the activation of the Hedgehog (Hh)-signaling pathway results in the initiation and propagation of gastrointestinal and pancreatic cancer (5,6). Hh signaling was originally identified as an essential pathway for cell differentiation and organ formation during the embryogenesis of Drosophila (7). PTCH, a transmembrane receptor of Hh ligands, is a key modulator of signaling in the Hh pathway, and blocks the activation of Smo, another transmembrane protein. As a result, the transcriptional activation of Hh target genes is repressed. In this manner, PTCH acts as a tumor suppressor in such tumors. Since PTCH gene expression is expected to be repressed in some tumors, its expression level in esophageal SCC was examined.

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

Materials and Methods

Patients and tissue specimens. The study group consisted of 29 esophageal SCC patients who underwent surgical operations at the Department of Gastroenterological Surgery of the Nagoya University Graduate School of Medicine, Japan.

All tumors and corresponding normal tissues were collected at surgical resection and stored at –80°C. The tumors were graded according to their tumor-node-metastasis (TNM) stage as follows: 1 was stage 0 disease; 8 stage II; 12 stage III; and 8 were stage IV disease.

RNA preparation and reverse transcription. Total RNA was extracted from esophageal SCC and corresponding normal tissues with guanidium thiocyanate, as described previously (8). The amount of RNA was measured spectrophotometrically by absorbance at 260 nm. First-strand cDNA was generated from RNA, as previously described (9).

Quantitative reverse transcription (RT)-PCR. Quantitative RT-PCR was performed with the ABI sequence detection system 7000 using the SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA). Thermocycling was carried out at a final volume of 50 μL containing 2.0 μL of the cDNA sample, 1.0 μL each of the PTCH primers (forward and reverse) and 25 μL of Mix SYBR Green I / enzyme (including Tag DNA polymerase, reaction buffer and deoxynucleotide triphosphate mixture). The PTCH primers for quantitative RT-PCR have been described previously (5). PCR amplification consisted of 40 cycles (95°C for 10 sec, 65°C for 15 sec and 75°C for 30 sec) after an initial denaturation step (95°C
for 10 min). To correct for differences in both the quality and quantity among samples, GAPDH was used as an internal control. The GAPDH primers were purchased from Applied Biosystems. PTCH and GAPDH mRNA variability was determined from triplicate samples. The difference in the quantity of triplicate samples was less than 10%.

PTCH expression score. The relative amounts of PTCH in esophageal SCC (T) and corresponding normal tissue (N) mRNA that were normalized to an internal control (GAPDH mRNA) were calculated. The PTCH expression score in each tissue was defined as follows: relative amount of T / average relative amount of all Ns.

We considered that a high or low PTCH expression score was more or less than 1.0, respectively.

Statistical analysis. The Fisher’s exact test was used to examine the possible association between PTCH expression and clinicopathological features. The differences between the means of analyzed variables observed were calculated by Student’s t-test. The survival rates were calculated by the Kaplan-Meier method for analysis of censored data.

Results

PTCH expression levels were first analyzed in 29 esophageal SCC samples using quantitative RT-PCR. The distribution of the PTCH expression scores is provided in Table I. Subsequently, the 29 esophageal SCCs were divided according to the score assigned (more or less than 1.0). Low PTCH expression was observed in 18 out of 29 (62%) esophageal SCCs, while high PTCH expression was found in 11 out of 29 (38%), suggesting that the PTCH expression levels were ubiquitously distributed in esophageal SCCs.

In order to determine the role of PTCH expression in esophageal SCC, the correlation of PTCH expression scores with the clinicopathological features was examined. There were no significant differences in the distribution of patients according to PTCH expression in terms of age, sex, tumor size, or lymph node metastasis. However, we found that a low PTCH expression showed a trend toward advanced TNM stages (p=0.065) (Table II).

The cumulative survival of the patient groups was examined according to the PTCH expression scores. Interestingly, the low-score group showed significantly worse survival rates than the high-score group (Figure 1, p=0.0073). To confirm the prognostic significance of other factors, additional clinicopathological variables that might affect survival were further analyzed. Analysis by the Kaplan-Meier method revealed that lymph node metastasis (p=0.0067) and TNM stage (p=0.0397) were significantly correlated with survival (Table III). Taken together, the PTCH expression score as well as lymph node metastasis and TNM stage provided a useful marker to estimate the prognosis in esophageal SCC patients.

Discussion

Hh signaling is activated through the binding of 3 mammalian Hh (sonic Hh, Indian Hh and desert Hh) to a membrane receptor, PTCH. This ligand-receptor interaction inhibits PTCH function and subsequently de-represses Smo.
leading to the activation of the Hh signaling pathway (10, 11). Therefore, PTCH may be involved in this pathway that is so important for tumorigenesis in common with the Wnt signaling pathway (12).

In this study, we demonstrated, for the first time, that a low PTCH expression showed a trend toward advanced TNM stages and was significantly correlated with the survival of esophageal SCC patients. These results suggest that PTCH might act as a tumor suppressor gene and could serve as a new parameter for the prediction of prognosis in esophageal SCC. Recently, Berman et al. demonstrated that no wild-type PTCH gene expression was ever detected in murine medulloblastoma, indicating a lack of the functional PTCH gene product that should result in Hh pathway activation (13). Aboulkassim et al. showed that PTCH gene expression was frequently observed to be significantly decreased in papillary bladder tumors with LOH in the 9q22 region, suggesting that this gene is a putative suppressor, and that the haploinsufficiency of this gene may be an important event in the development of this tumor (14). These reports support the idea that the inactivation of the PTCH gene might be an important factor in the tumorigenesis of some tissues.

On the other hand, PTCH gene expression was paradoxically found to be increased in primary gastric and pancreatic cancers (5), suggesting that, in these cancers, the transcription of PTCH might be induced by an activated Hh pathway, but that Smo was insensitive to the regulation of PTCH, or that the PTCH gene was inactivated by mutation. These results suggest that PTCH might present various expression levels depending on the tissue types and the gastroenterological tissues.

This study provides a solid basis for additional studies on the molecular mechanism of PTCH expression in esophageal SCCs. Since esophageal SCC is a most aggressive cancer, the overall survival rate may not be improved using information regarding PTCH expression levels alone. However, PTCH expression could prove useful as a marker for predicting the outcome of resection-treated esophageal SCC patients.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Variable</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph node metastasis</td>
<td>+ / –</td>
<td>0.0067</td>
</tr>
<tr>
<td>TNM stage</td>
<td>0, I, II, III / IV</td>
<td>0.0397</td>
</tr>
<tr>
<td>PTCH expression score</td>
<td>&lt;1.0 / 1.0 ≤</td>
<td>0.0073</td>
</tr>
</tbody>
</table>

Figure 1. The cumulative survival of patient groups according to PTCH expression scores (more or less than 1.0). The low PTCH expression-score group showed significantly worse survival rates than the high PTCH expression-score group (p=0.0073).
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References


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