Expression and clinical significance of SPARC in clinical stage II tongue squamous cell carcinoma

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**Background and Objective:** Secreted protein acidic and rich in cysteine (SPARC) is expressed widely in malignant tumors. It is related to prognosis and biological behaviors of tumors. This study was to detect the expression of SPARC in stage II tongue squamous cell carcinoma and analyze its relationship with prognosis. **Methods:** Tongue carcinoma samples (T2N0M0) were obtained from 55 patients treated at Sun Yat-sen University Cancer Center from January 1992 to December 2003. Twenty-five squamous epithelium samples with tongue inflammation nearby were taken as control. Immunohistochemistry was used to detect the expression of SPARC. Its relationships with survival, occult lymph node metastasis and recurrence were analyzed. **Results:** The positive rate of SPARC was 49.1% in tongue cancer tissues and 0 in normal tissues (p < 0.001). The accumulative 5-year survival rate was significantly lower in SPARC-positive patients than in SPARC-negative patients (30.0% vs. 85.3%, p = 0.005). The positive rate of SPARC was significantly higher in tissues with occult lymph node metastasis than in those without metastasis (86.7% vs. 35.0%, p = 0.001), and higher in tissues with recurrence than in those without recurrence (100% vs. 31.5%, p < 0.001). The expression of SPARC was positively correlated to occult lymph node metastasis (r = 0.46, p < 0.001) and recurrence (r = 0.595, p < 0.001). **Conclusion:** SPARC is highly expressed in stage II tongue squamous cell carcinoma, and positively correlated to survival, occult lymph node metastasis and recurrence.

Recurrent and occult lymph node metastasis are two major causes of treatment failure in stage II tongue squamous cell carcinoma. Looking for biological indices that can be used to predict prognosis will help to improve follow-up and conduct early treatment.

**Materials and Methods**

**Clinical data.** Tongue carcinoma samples were collected from 55 patients treated at Sun Yat-sen University Cancer Center from January 1992 to December 2003. All patients underwent no preoperative radiotherapy or chemotherapy but received hemiglossectomy and cervical lymph node clearance. According to the 2002 UICC/AJCC staging system for oral cancer, all cases were postoperatively staged as stage II disease (pT2N0M0). Twenty-five samples of peri-inflammation tongue tissue were taken as controls. All samples were pathologically confirmed. All patients were followed up for 15–110 months, with a median of 73.5 months.

**Major reagents and instruments.** SP-9000 Histostain Plus Kits was purchased from Zymed Co., USA; mouse anti-human SPARC monoclonal antibody (SC-74295), preserved at 4°C, was purchased from Santa Cruz Co., USA; goat anti-mouse or goat anti-rabbit IgG antibodies was purchased from Santa Cruz Co., USA; normal goat serum, normal saline injection, PBS solution and DAB reagents, preserved at 4°C, were purchased from Beijing Zhongshan Biotechnology Co., Ltd., Beijing.

**Immunohistochemical detection of SPARC expression.** After all samples were fixed in 10% neutral buffered formalin and paraffin-embedded, slices of 4 µm thickness were cut, and subjected to routine dewaxing, hydration and antigen retrieval in 0.1 mmol/L PBS solution for 15 min. The remaining steps were conducted according to the instructions provided with the kit. Each batch of slides contained positive and negative controls. Negative controls were run...
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by replacing the primary antibody with PBS, while known esophageal squamous cell carcinoma tissues were used as positive controls. Immunohistochemical staining results were evaluated independently by two pathologists according to the criteria established by Infante et al. Ten high-power visual fields were chosen. Negative results were considered when the staining extent was less than 10% or the intensity was lighter than pale yellow, while positive results were considered when the staining extent was more than 10% or the intensity was deeper than pale yellow (yellowish-brown or dark brown).

Statistical analysis. Statistical analysis was performed using the SPSS13.0 software package. The differences in the expression of SPARC among groups were compared using the Chi-square test and Fisher's exact test. The cumulative survival rate was calculated using the Kaplan-Meier method. Intergroup differences were compared using the log-rank test. Correlation analysis was conducted using the Spearman rank correlation. A p value of < 0.05 was considered significant.

Results

The positive rates of SPARC were 49.1% (27/55) in tongue squamous cell carcinoma tissues and 0 in peri-inflammation tongue epithelial tissues (p < 0.001). The positive signals for SPARC were mainly localized on the membrane and in the cytoplasm of epithelial cells (Fig. 1A and B). The positive rates of SPARC in stromal cells were 94.6% (53/55) in tongue squamous cell carcinoma tissues and 96.0% (24/25) in peri-inflammation tongue tissues (p = 0.937). SPARC was unspecifically expressed in multiple types of cells, mainly including fibrocytes, endothelial cells and striated muscle cells (Fig. 1C).

The accumulative 5-year survival rate was significantly lower in SPARC-positive patients than in SPARC-negative patients (30.0% vs. 85.3%, p = 0.005, Fig. 2). The correlations of SPARC expression to sex, age, differentiation degree, occult lymph node metastasis and recurrence were shown in Table 1. SPARC expression was positively correlated to differentiation degree (r = 0.397, p = 0.003), occult lymph node metastasis (r = 0.460, p < 0.001), and recurrence (r = 0.595, p < 0.001).

Table 1 The correlation of SPARC expression to clinico-pathologic features of stage II tongue carcinoma

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cases</th>
<th>SPARC expression [cases (%)]</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>30</td>
<td>18 (60.0)</td>
<td>0.076</td>
</tr>
<tr>
<td>Female</td>
<td>25</td>
<td>9 (36.0)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤45</td>
<td>13</td>
<td>3 (23.1)</td>
<td>0.733</td>
</tr>
<tr>
<td>&gt;45</td>
<td>42</td>
<td>14 (33.3)</td>
<td></td>
</tr>
<tr>
<td>Differentiation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>49</td>
<td>21 (42.9)</td>
<td>0.011</td>
</tr>
<tr>
<td>Moderate/poor</td>
<td>6</td>
<td>6 (100)</td>
<td></td>
</tr>
<tr>
<td>Occult lymph node metastasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>15</td>
<td>13 (86.7)</td>
<td>0.001</td>
</tr>
<tr>
<td>No</td>
<td>40</td>
<td>14 (35.0)</td>
<td></td>
</tr>
<tr>
<td>Recurrence</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Yes</td>
<td>14</td>
<td>14 (100)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No</td>
<td>41</td>
<td>13 (31.7)</td>
<td></td>
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</tbody>
</table>

Figure 1. The expression of SPARC in tongue tissues (SP×400). (A) No expression of SPARC is detected in adjacent epithelium in tongue inflammation tissue. (B) SPARC is expressed (in brown) on membrane and in cytoplasm of tongue carcinoma cells. (C) SPARC is expressed (in light yellow) in fibroblast, endothelia, striated muscle cells, and so on, in adjacent stroma of tongue inflammation tissue.

Figure 2. Overall survival curves of tongue carcinoma patients with or without SPARC expression.
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Discussion

SPARC gene family is a subfamily of Ca²⁺-binding matrix protein family and is involved in the regulation of cell-cell and cell-stroma interactions. Different from other extracellular matrix components, such as collagens, fibronectins, laminins and integrins, SPARC has anti-adhesion effect, which may be due to its direct interfering effects on cell adhesion molecules (matrix components). SPARC is widely expressed in normal human tissues, including the bone, kidney, hematopoietic tissue and nervous tissue. In the context of environmental changes, such as inflammation and wound healing, the expression of SPARC shows dynamic changes. In addition to regulating cell proliferation and differentiation and altering the micro-environment for cell growth, SPARC is closely associated with the development and progression of tumors.¹

Studies showed that SPARC is expressed at different levels in malignant melanoma,² non-small cell lung cancer,³ breast cancer,⁴ prostate cancer,⁵ renal cell carcinoma,⁶ liver cancer,⁷ gastric carcinoma,⁸ esophageal squamous cell carcinoma,⁹ and so on, with positive rates ranging from 45% to 95%. The Cancer Genome Anatomy Project established by the National Cancer Institute used serial analysis of gene expression (SAGE) to analyze the expression of SPARC, and found that SPARC expression was extremely unbalanced in normal and tumor tissues;¹¹ SPARC was expressed highly in astrocytoma, hemangioma, breast cancer and pancreatic cancer, but lowly in malignant melanoma; studies on breast cancer indicated that SPARC expression was correlated to the degree of malignancy and invasiveness of the cancer;¹² studies on colon cancer showed that the expression level of SPARC in tumor epithelial cells was two times as high as that in normal colonic epithelial cells.¹³ Our study proved that SPARC was highly expressed in tongue squamous cell carcinoma tissues, which is consistent with the results obtained by Duan et al.¹⁴ However, compared with other types of tumors, the positive rate of SPARC in stage II tongue squamous cell carcinoma is not high, which may due to its lower expression levels in early-stage carcinoma. No SPARC expression was found in normal tongue squamous epithelial tissues, suggesting that SPARC may be related with tumorigenesis. SPARC was more highly expressed in poorly differentiated tongue squamous cell carcinomas or those with lymph node metastases, suggesting that the expression level of SPARC is correlated to the degree of malignancy of tongue squamous cell carcinoma.

There are great differences in the expression level of SPARC in different tumor tissues. According to the relationship between the expression level of SPARC and prognosis, tumors are classified into three types: (i) for most tumors, such as malignant melanoma,³ breast cancer,⁵ liver cancer,⁶ gastric carcinoma,⁸ esophageal cancer,¹⁰ and so on, higher expression level of SPARC in tumor tissues suggests stronger invasiveness and poorer prognosis; (ii) for few tumors, such as ovarian cancer¹⁵ and neuroblastoma,¹⁶ the expression level of SPARC in tumor tissues is negatively correlated to tumor prognosis; (iii) for pancreatic cancer¹ and non-small cell lung cancer,⁴ higher expression level of SPARC in pericancerous stromal cells suggests poorer prognosis. The expression of SPARC in squamous cell carcinoma and adenocarcinoma are not consistent. The detailed mechanism for this difference is still unclear and may be associated with its specific expression in tumors.¹ Our results indicated that the expression pattern of SPARC in stage II tongue squamous cell carcinoma should be classified as the first type as described above. Though SPARC was highly expressed in tumor and normal stromas, its expression locations and cell types expressing SPARC were not specific. Furthermore, the expression of SPARC has no obvious correlation to prognosis. The possibility that the high-level expression of SPARC in peri-inflammation stroma is caused by other factors such as inflammation and overstaining could not be ruled out.

Although recurrence and lymph node metastases are independent factors affecting the prognosis of early stage tongue carcinoma, it is difficult to predict when recurrence and lymph node metastases will develop.¹⁷,¹⁸ In this study, re-staging of all postoperative stage II tongue squamous cell carcinoma samples and postoperative dissection of cervical lymph nodes revealed no metastasis, and the surgical margins of the tongue were all negative. However, some patients still developed recurrence and cervical lymph node metastases (mainly located at the ipsilateral level II), mostly within two years. After re-treatment, the prognosis of the patients developing recurrence and lymph node metastases was still poor. This result indicates that regular clinical re-examination is inadequate for detecting recurrence and lymph node metastasis. It is necessary to find some indices that can be used to predict the biological behavior of tumors, which can help to preliminarily predict tumor prognosis postoperatively or even preoperatively. In this study, we found that the positive rate of SPARC was over 85% in recurrent tongue carcinoma tissues or tissues with lymph node metastases, and the sensitivity was satisfactory. Correlation analysis indicated that SPARC expression was correlated to recurrence and occult lymph metastases. Thus, SPARC may be used as a maker for predicting the prognosis of stage II tongue squamous cell carcinoma. This result is consistent with that reported by Kato et al.¹⁹ Therefore, for SPARC-positive patients, more thorough cervical lymph node clearance and intensive postoperative follow-up are needed. However, since the number of recurrent and metastatic samples is not so large, we cannot exclude the possibility that relatively high positive rate of SPARC obtained in this study is due to insufficient sample size.

References

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