Original Article
SKA1 expression in oral squamous cell carcinoma and its relationship to P53 and clinicopathologic features

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Abstract: In this study, 57 paraffin-embedded tissue specimens from patients with oral squamous cell carcinoma (OSCC) were collected and analyzed. Spindle and kinetochore-associated complex subunit 1 (SKA1) and P53 protein expression in selected samples was detected by immunohistochemistry. The positive expression rate of SKA1 and P53 was significantly higher in oral squamous cell carcinoma tissues than in normal controls. The expression of SKA1 protein was significantly associated with tumor-node-metastasis (TNM) stage, and p53 expression was significantly correlated with pathologic differentiation grade in oral squamous cell carcinoma tissues. There was a significant correlation between SKA1 and p53 protein expression in oral squamous cell carcinoma tissues. Our results indicate that the SKA1 gene might be involved in the development of oral squamous cell carcinoma and might predict its prognosis. SKA1 is expected to be a new molecular target for oral squamous cell carcinoma.

Keywords: Spindle and kinetochore-associated complex subunit 1, P53, oral squamous cell carcinoma, prognostic

Introduction

It has been reported that 90% of oral cancers histologically originate from squamous cells, and this is defined as oral squamous cell carcinoma (OSCC) [1]. In worldwide reports, cancers of all regions of the oral cavity and pharynx are grouped and collectively represent the sixth most common cancer in the world [2]. Despite research progress and enhanced novel therapies, survival has not improved significantly in recent decades. There are no specific biomarkers for OSCC, representing a continuing challenge for biomedical science [3].

The cell division cycle is a highly ordered process that is tightly regulated by intracellular intrinsic cell cycle checkpoints to ensure faithful replication of cells [4]. Checkpoint defects may cause genetic mutations and chromosomal structural abnormalities, leading to cell proliferation and tumorigenesis [5].

Spindle and kinetochore-associated complex subunit 1 (SKA1), a microtubule-binding protein, is localized to the spindle microtubule and outer kinetochore interface during mitosis [6-11]. SKA1 mediates the binding of the SKA complex (SKA1, 2 and 3) to the microtubule, which is essential for stabilizing kinetochore-spindle microtubule attachment during mitosis. Our previous study found that depletion of SKA1 in CAL-27 cells led to G2/M phase cell cycle arrest and apoptosis [12]. As one of the most frequently studied genes, research has confirmed that p53 plays an important role in the cell cycle and apoptosis of tumor cells [13-16]. P53 is also a common tumor biomarker in squamous cell carcinoma (SCC) [17]. Therefore, we postulated that there may be a correlation between SKA1 and P53 related to their influence on the cell cycle and apoptosis. In the present study, we investigated the expression and correlation of SKA1 and P53 protein in oral squamous cell carcinoma to further clarify the roles of SKA1.

To the best of our knowledge, the correlations between SKA1 expression and the clinicopathologic features of patients with OSCC, as well as the correlation between SKA1 and p53, have not been well reported. The present study aimed to investigate the expression of SKA1 and p53 in OSCC and in adjacent oral epitheli-
SKA1 expression in OSCC and its relationship with P53

Materials and methods

Patients and specimens

The protocol of the study was approved by the Institutional Ethics Committee of the Liaocheng People’s Hospital Affiliated to Taishan Medical University (Liaocheng, China). All 57 paraffin-embedded tissue specimens analyzed were selected from patients with complete clinical data in the Department of Pathology, Liaocheng People’s Hospital (Liaocheng Clinical School of Taishan Medical University) of China, between May 2004 and June 2013. The criteria for study enrollment were as follows: patients (19 females, 38 males) with primary oral squamous cell carcinoma (OSCC) who underwent resection without preoperative chemotherapy, hormone therapy, radiotherapy, or other anticancer treatment. All cases were assessed by pathologic examination after surgery and confirmed to be squamous cell carcinoma (cancer) with a clear presence of lymph node metastasis and nerve invasion. Two independent observers reviewed all of the original hematoxylin and eosin-stained sections and chose the most representative slide from each case to perform immunohistochemical staining. According to the seventh edition of the AJCC (American Joint Committee on Cancer) Cancer Staging Manual (tumor-node-metastasis: TNM), 33 patients (57.9%) had stage I or II disease, and 24 patients (42.1%) had stage III or IV disease. Three histologic grades were established according to the International Classification of Tumors of the WHO: well differentiated tumors, moderately differentiated tumors and poorly or undifferentiated tumors. Other clinical and pathologic features of the enrolled patients are summarized in Table 1.

Immunohistochemical staining

The sections were placed in 3% H₂O₂ in distilled water for 15 min to block endogenous peroxidase activity after being dewaxed in xylene and hydrated in a graded ethanol series. Then, the sections were subjected to antigen retrieval by heating the slides in an autoclave at 120°C for 3 min in 0.1 M citric acid buffer (pH 6.0). Following antigen retrieval, the sections were blocked with BSA and then probed with rabbit anti-SKA1 (Sigma-Aldrich, 1:800) and mouse anti-p53 (Maixin, 1:300) at 4°C overnight. After rinsing with PBS, the sections were incubated with biotinylated goat anti-rabbit immunoglobulins and goat anti-mouse immunoglobulins at 37°C for 30 min and visualized using peroxidase-conjugated streptavidin and diaminobenzidine (DAB), followed by counterstaining with Mayer’s hematoxylin. The negative controls were obtained by using PBS instead of primary antibodies.

Evaluation of immunohistochemical staining

The results were evaluated independently by two pathologists blinded to all clinical data. Immunohistochemical staining for SKA1 and p53 was evaluated according to intensity and proportion. Immunopositivity of SKA1 and p53 was scored according to the percentage of positive cells in four distinct categories: 0 for 0-10%, 1 for 11-30%, 2 for 31-70%, and 3 for 71-100%. The staining intensity was then scored as 0 for bright yellow staining, 1 for yellow staining, 2 for brown-yellow staining, and 3 for red-brown staining. Both scores were multiplied together, resulting in a final score: 0, negative (-); 1, weakly positive (-~+); 2-4, positive (+); and 6-9, strong positive (++).

Statistical methods

Correlations were separately evaluated between SKA1 and p53 expression and several clinicopathologic variables according to the Pearson chi-square test and Fisher’s exact test. McNemar’s test was used to evaluate the relationship between SKA1 and p53 expression. A P value <0.05 was considered significant.

Results

SKA1 expression and its correlations with clinicopathologic variables

Positive expression of SKA1 was mainly observed in the cytoplasm and nucleus. A small number of SKA1-positive cells had reactivity present in the cytoplasm. Immunostaining results suggested significantly higher SKA1 expression in OSCC tissues than in adjacent normal tissues (Figure 1). Among 57 tumor samples, 46 (80.7%) showed high SKA1 expression, which was significantly higher than the
SKA1 expression in OSCC and its relationship with P53

The positive expression of p53 protein was located in the nuclei of the tumor cells, demonstrated by a brown color (Figure 2). The rate of positive p53 expression in OSCC (68.4%) was significantly higher than that in normal tissue adjacent to the carcinoma (9.5%, P<0.001). Correlations between p53 and clinicopathologic variables are listed in Table 1. P53 expression in OSCC was significantly associated with pathologic differentiation grade (P=0.001), and its expression in OSCC was not significantly associated with tumor size, TNM stage, age, sex, history of alcohol or tobacco use, lymph node involvement, or nerve invasion (P>0.05).

Correlation between SKA1 and p53 protein expression

As shown in Table 2, of 46 tumor tissues with positive SKA1 expression, 8 were negative and 38 were positive for p53 expression. By contrast, of 11 tumor tissues with negative SKA1 expression, 10 were negative and 1 was positive for p53 expression. McNemar’s test reflected that there was a significant correlation between SKA1 and p53 expression in OSCC (P<0.05). The expression of SKA1 and p53 showed similar trends in patients grouped by sex, age, tumor size and TNM stage.

<table>
<thead>
<tr>
<th>Factors</th>
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<th>P</th>
<th>P53</th>
<th>P</th>
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<tr>
<td></td>
<td></td>
<td>+ (n)</td>
<td>- (n)</td>
<td></td>
<td>+ (n)</td>
</tr>
<tr>
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<td>31</td>
<td>7</td>
<td></td>
<td>27</td>
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</tr>
<tr>
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</tr>
<tr>
<td>&gt;60</td>
<td>33</td>
<td>26</td>
<td>7</td>
<td></td>
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<td>21</td>
<td>7</td>
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SKA1 expression in OSCC and its relationship with P53

Discussion

To date, there are no specific biomarkers for oral cancer. To predict long-term prognosis and define individual treatment modalities for patients with OSCC, extensive studies have focused on the identification of useful biologic and molecular markers in the diagnosis and treatment of OSCC [12, 18, 19]. There is no study demonstrating the altered expression of SKA1 in oral cancer.

Spindle and kinetochore-associated complex subunit 1 (SKA1), a newly discovered gene associated with mitosis [20], has been found to silence the spindle checkpoint [6]. SKA1 is a subtype of the SKA complex that causes spindle microtubules to attach firmly to the kinetochore in mitosis [6, 21, 22]. Overexpression of SKA1 has been found in the malignant progression of several human cancers, such as hepatocellular carcinoma, gastric cancer, prostate cancer, bladder cancer, glioblastoma, and non-small-cell lung cancer [23-28], indicating that SKA1 may be associated with the occurrence and development of oral cancer.

The present study shows that SKA1 is overexpressed in OSCC and is associated with the clinicopathologic features of OSCC. In OSCC, the prognosis largely depends on factors such as smoking, alcohol consumption, medical comorbidity, and in particular, tumor stage [29]. Our study of primary OSCC samples found that patients with higher SKA1 expression were typically in the advanced stage, suggesting that SKA1 may be a new immunohistochemical prognostic marker for human OSCC. The results of our study were consistent with those of previous research [30]. Therefore,

Table 2. Relationship between SKA1 and p53 expression in oral squamous cell carcinoma (OSCC)

<table>
<thead>
<tr>
<th></th>
<th>P53</th>
<th>Total (n)</th>
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<tbody>
<tr>
<td></td>
<td>+ (n)</td>
<td>- (n)</td>
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<tr>
<td>SKA1</td>
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</tr>
<tr>
<td></td>
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<tr>
<td>Total (n)</td>
<td>39</td>
<td>18</td>
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SKA1 expression may provide a theoretical basis for diagnosis and staging and help to predict the prognosis of oral cancer. In addition, our study found that SKA1 expression was not significantly related to alcohol and tobacco use.

Among the genes related to oral cancer, p53 has been one of the most frequently studied. The corresponding relationship between P53 expression in OSCC and clinicopathologic data remains controversial [31-33]. More than 60% of our OSCC samples were p53 positive which is consistent with the results described in the literature [34]. Our results also clearly showed that p53 expression is significantly correlated with the differentiation grade of OSCC but not with tumor stage or lymph node metastasis. Patients with high p53 expression had poorly differentiated tumors, indicating that p53 may be considered as a marker for the grade of human OSCC.

SKA1 expression correlates with abnormal cell cycle distribution in a variety of tumor cells [23, 26, 28]. Research has also confirmed that p53 plays an important role in tumor cell cycle and apoptosis [13-16]. We found that the protein expression of SKA1 and p53 showed the similar trends in patients grouped by sex, age, tumor size, and TMN stage. There was a significant correlation between SKA1 and p53 expression in OSCC, suggesting that the carcinogenic potential of SKA1 may be related to p53 molecular pathways.

In conclusion, the present study indicates that the SKA1 gene might be involved in the development of OSCC. In future studies, we will increase the sample size and verify whether there is a synergistic carcinogenic mechanism between SKA1 and p53 by molecular biology experiments. With further research, SKA1 may become a molecular target in oral squamous cell carcinoma.

Disclosure of conflict of interest

None.

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