Abstract

Objective
1. Study the correlation of tumor susceptibility gene 101 (TSG101) expression with tumor differentiation in squamous cell carcinomas of head and neck.
2. Understand the relationship between the expressions of TSG101 and the epidermal differentiation markers including SP1 and transglutaminase I.

Methods

There were 138 paraffin-embedded specimens of squamous cell carcinomas of head and neck in different differentiation statuses between 1988 and 2006 included in the study. Immunohistochemical study was employed for the evaluation of TSG101, SP1, and transglutaminase I expressions. In situ hybridization for the detection of TSG101 mRNA was also performed. One-way ANOVA analysis was used to evaluate TSG101 expressions between tumors of different differentiation. Spearman's correlation method was used to evaluate the correlation between the expressions of SP1 and transglutaminase I expression.

Conclusion

Our results have provided evidences to support the role of TSG101 on tumor differentiation in squamous cell carcinomas of head and neck.

Introduction

Human TSG101 was known to be associated with tumor susceptibility. Some previous studies revealed that TSG101 has multiple biological functions, including regulations of transcription, protein degradation, protein trafficking, cell survival and proliferation and epithelial cell differentiation. Notably, our previous study demonstrated that in carcinomas, TSG101 expression in the carcinomatous parts was higher than in the sarcomatous parts by immunohistochemistry (IHC) study despite the tissue origin. Thus, in addition to the role of tumorgenesis previously described, TSG101 may have a causal link with tumor susceptibility.

Immunohistochemical study and scoring

Tumor differentiation

Table 1. Results of IHC study on 138 HNSCC cases

<table>
<thead>
<tr>
<th>Tumor differentiation</th>
<th>TSG101**</th>
<th>SP1**</th>
<th>Transglutaminase I**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well differentiated</td>
<td>0 23 50 0</td>
<td>27 46 0</td>
<td>25 48 0</td>
</tr>
<tr>
<td>Moderately differentiated</td>
<td>0 52 32 24 30 1 0</td>
<td>43 12 0</td>
<td></td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>6 4 0 6 0 6 4 0</td>
<td></td>
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</tbody>
</table>

IHC versus ISH for TSG101 expression on a representative case of HNSCC.

A) IHC with TSG101 Ab. B) ISH with TSG101 antisense probe. C) ISH with TSG101 sense probe as negative control.

Selection of the specimens and preparation of the sections

Representative paraffin-embedded tissue blocks of 138 HNSCC between 1988 and 2006 were retrieved and sectioned for IHC and ISH.

Immunohistochemical study and scoring

The TSG101 antibodies (rabbit polyclonal antisera #654, 1:100 dilution) was used for IHC as previously reported [Liu et al., Oncogene 2002]. SP1 (H-225) and transglutaminase I (H-87) polyclonal antibodies were purchased from Santa Cruz Biotechnology. The intensity of IHC was scored as − (no staining), 1+ (weak staining), 2+ (moderate staining), and 3+ (strong staining).

Preparation of TSG101 riboprobes by in vitro transcription

The plasmid pBluescript SK(-) that contained TSG101 cDNA encoding amino acid residues 1-291 was used for in vitro transcription to generate both anti-sense and sense riboprobes.

In situ hybridization

ISH was performed with heat-denatured antisense TSG101 riboprobe at 55°C overnight. Immunodetection was performed with rabbit HRP-anti-DIG antibody (1:100 dilution). The DAKO GenPoint kit was used to amplify the signal. Hybridization with sense probe was performed simultaneously as the negative control.

Results

Clinical data

There were 131 males and 7 females in the HNSCC study cohort (mean age, 56.2 ± 10.6 years; range, 35 to 95 years). The tumors were located in oral cavity (31 cases), tongue (39 cases), tonsil (28 cases), hypopharynx (19 cases), and larynx (21 cases), respectively.

IHC and ISH analysis

In IHC study, TSG101 was strongly expressed over the parabasal, intermediate and cornified layers but only weakly expressed in the basal layer of the squamous epithelium. In the HNSCC cases, TSG101 expression was readily detected in the tumor cells, but was weakly expressed in the poorly differentiated tumors. The expression of TSG101 was significantly correlated with tumor differentiation (p < 0.001) (Table 1). When the tumor differentiation was poorer, the expression of TSG101 was weaker (Figure 1). None of gender, age or tumor location was significantly correlated with TSG101 expression. TSG101 mRNA distribution was consistent with its protein expression by ISH study (Figure 2). Similar to that of TSG101, the expression of transglutaminase I was mainly in the parabasal, intermediate and cornified layers of the squamous epithelium. However, it showed a distinct membranous pattern. In the HNSCC cases, the expressions of both SP1 and transglutaminase I were also significantly correlated with tumor differentiation (p < 0.001) (Table 1) (Figure 1). Furthermore, the expression of TSG101 was significantly correlated with that of SP1 (r = 0.856, p = 0.012) and transglutaminase I (r = 0.914, p = 0.007), respectively among the HNSCC cases.

Conclusions

1. TSG101 could be regarded as a differentiation marker in squamous cell carcinomas.
2. TSG101 might play a role in the regulation of squamous epithelial differentiation through modulation of SP1 gene expression and possibly interact with transglutaminase I.
3. Further study is essential to investigate the direct role of TSG101 in squamous cell differentiation by organic cell culture in vitro.

TSG101 expression in head and neck squamous cell carcinomas

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