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Expression of Cytoplasmic Galectin-3 as a Prognostic Marker in Tongue Carcinoma

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Abstract

Galectin-3 is a member of the β-galactoside-binding mammalian lectin family with affinity to ABH group epitopes, cell surface and extracellular poly saccharide glycans. It has been shown to be involved in differentiation, morphogenesis, tumor progression, and metastasis. Here we questioned the possible involvement of galectin-3 in the neoplastic progression of the tongue epithelium and evaluated its prognostic value in tongue cancer patients. Galectin-3 expression was analyzed by the immunohistochemical method in 77 tongue specimens (54 squamous cell carcinomas and 23 specimens of distinct normal mucosa). Levels of nuclear expression of galectin-3 markedly decreased during the progression from normal to cancerous states (P < 0.0001), while cytoplasmic expression increased (P < 0.0001). Enhanced expression of galectin-3 in the cytoplasm was associated with a reduced disease-free survival of tongue cancer patients. Multivariate analysis identified enhanced expression of cytoplasmic galectin-3 as an independent predictor of disease recurrence (P = 0.0120). These results suggest that the observed translocation of galectin-3 from the nucleus to the cytoplasm during neoplastic progression may serve as a prognostic factor for tongue cancer patients.

Introduction

HNSCC is among the most morbid of human malignancies. The global incidence of this carcinoma is more than 500,000 cases annually and is increasing gradually, most probably due to the prolongation of the average life span and increment of cigarette smoking and alcohol consumption (1). Despite intensive efforts in primary prevention, screening, and therapy, the long-term survival rate has not improved substantially for the last two decades. Standard treatment consists of deforming surgical procedures coupled with radiotherapy; recurrence of primary diseases, together with the development of second primary tumors, is the major cause of treatment failures (2). Thus, it is of clinical significance to establish whether a specific gene product is involved in the pathogenesis of HNSCC and in disease progression because a better prognostic stratification of patients should lead to a more effective therapy.

Galectin-3 is a member of a growing family of carbohydrate-binding proteins that share affinity for β-galactoside-containing glycoconjugates and a conserved sequence of the carbohydrate-binding site (3). Galectin-3 is a M, ~30,000 protein that consists of three distinct structural motifs: (a) a short NH₂ terminus of 12 amino acids; (b) a repetitive collagen-like sequence rich in glycine, tyrosine, and proline; and (c) a COOH-terminal half domain with a globular structure encompassing the carbohydrate-binding site (3). A wide range of glycoconjugates, including IgE (4), laminin (5, 6), lysosome-associated membrane proteins (7), Mac-2-binding protein (7–9), carci noembryonic antigen (10), and mucin (11), have been shown to be ligands for galectin-3, reflecting the multifunctionality of this molecule. In fact, a series of in vitro experiments have indicated that galectin-3 is involved in multiple biological phenomena, including cell growth (12, 13), inflammation (14), apoptosis (15, 16), metastasis (8, 17), and neoplastic transformation (18).

Conflicting data have been reported regarding the expression of galectin-3 in different malignancies. Galectin-3 is up-regulated in cancers of the thyroid, liver, stomach, and central nervous system (19–23). In contrast, galectin-3 was found to be down-regulated in carcinomas of the ovary, uterus, and breast, compared with normal counterparts (24–26). In colon carcinomas, some have reported increased expression of galectin-3 (27, 28), whereas others have reported decreased expression of galectin-3 (29, 30).

The present study was designed to determine whether galectin-3 expression is involved in the pathogenesis of tongue cancer, one of the most common HNSCCs, and, to this end, we have retrospectively examined the expression of galectin-3 in a spectrum of surgically excised tongue lesions with immunohis-
to chemotherapy and assessed the prognostic value of the results with patient outcome.

Materials and Methods

Tissue Specimens. The specimens used for immunohistochemical analysis were obtained from 54 tongue cancer patients who had undergone curative initial treatments without any prior therapy at Osaka University Medical School Hospital and Osaka Teishin Hospital (Osaka, Japan) between 1986 and 1993. Twenty-three cases of normal mucosa adjacent to the proximal resection margins of the glossectomy specimens were also examined. The International Union against Cancer TNM classification (31) was used to categorize the cancer patients (37 males and 17 females; median age, 53 years; age range, 27–83 years). Histologically, 46, 5, and 3 cases were well-, moderately, and poorly differentiated squamous cell carcinomas, respectively. Tumor sizes were T1 (18 cases), T2 (25 cases), T3 (9 cases), and T4 (2 cases). Regional metastases were found in 10 patients with no distant metastases at the time of initial treatment. Accordingly, there were 17 stage I patients, 25 stage II patients, 7 stage III patients, and 5 stage IV patients. All of the patients were treated with standard therapy based on the clinical stage. Briefly, patients at stages I and II underwent surgery alone, whereas those at stages III and IV underwent combination therapy of surgery and successive radiation. The patients were followed-up for 54.1 ± 27.8 months (mean ± SD).

Immunohistochemistry. Immunohistochemistry was performed using a modification of the avidin-biotin peroxidase complex technique. Briefly, 4-μm-thick tissue sections were deparaffinized in xylene, rehydrated in ethanol, incubated with freshly prepared 3% hydrogen peroxide in methanol for 10 min to inhibit endogenous peroxidase activity, and washed with PBS (pH 7.4). Normal rabbit serum (5%) was applied to block nonspecific binding sites and removed by blotting. The sections were incubated at room temperature for 2 h with 2-fold diluted hybridoma supernatant containing rat anti-galectin-3 antibody produced by TIB166 hybridoma (American Type Culture Collection, Manassas, VA), washed with PBS, and then incubated at room temperature for 30 min with biotinylated secondary antibody from a VECTASTAIN Elite ABC kit (Vector Laboratories, Burlingame, CA). After washing with PBS, the sections were incubated with avidin-biotin complex reagent, (Vector Laboratories) for 30 min, followed by washing with PBS. Subsequently, the sections were incubated with diaminobenzidine (Abbott Laboratories, Abbott Park, IL) for 1 min to visualize the bound antibody by colored peroxidase reaction product, washed with tap water, counterstained with hematoxylin, washed with tap water and PBS, dehydrated, and mounted. Controls receiving either no primary antibody or a nonspecific rat IgG exhibited no background staining.

The percentage of epithelial cells positive for either nuclear or cytoplasmic galectin-3 was evaluated on a ×100 field (×10 objective and ×10 ocular), and the average percentage of five fields was determined. Sections were examined by two independent observers who were blinded to the clinical outcome and clinicopathological features of the patients (interobserver reliability, r = 0.95).

Statistical Analysis. Comparison of either nuclear or cytoplasmic galectin-3 expression between different states of tongue squamous epithelium was carried out using the Mann-Whitney analysis. The Mann-Whitney procedure was used for comparison of either nuclear or cytoplasmic galectin-3 expression between groups defined by clinicopathological parameters. Overall survival duration was measured from the date of surgery to the date of the last follow-up or death, whereas disease-free survival duration was measured from the date of surgery to the date of recurrence. Overall survival and disease-free survival curves were calculated using the method of Kaplan and Meier, and the differences were analyzed by using the log-rank test. Multivariate analysis for factors related to disease recurrence was performed by using the Cox proportional hazard model. Statistical significance was set at P < 0.05. StatView software (Abacus Concepts Inc., Berkeley, CA) was used for all statistical analyses.

Results

Galectin-3 Expression in Tongue Carcinoma. Fifty-four tongue carcinomas of known stage were examined for galectin-3 expression. Initially, we verified the specificity of the rat anti-galectin-3 monoclonal antibody and found by immunoblotting that it detects a single band of M, 31,000 of the total proteins extracted from tongue tissues (data not shown).

It was reported previously that in carcinomas, a shift from nuclear to cytoplasmic expression of galectin-3 may occur (29). Thus, we evaluated galectin-3 expression in both cellular compartments. In the normal squamous mucosa of the tongue, staining was distinct, with constant topological distribution. Galectin-3 was scattered in the nucleus of epithelial cells of the basal and parabasal layers, whereas cytoplasmic galectin-3 was observed in the cells of the superficial and parasureal layers (Fig. 1A). Fig. 2 illustrates the frequency of galectin-3 localization in the cells, and the proportion of epithelial cells positive for nuclear staining varied from 5–40% (21.5 ± 9.6%, mean ± SD); similarly, cytoplasmic expression of galectin-3 ranged between 10% and 41% (21.9 ± 8.7%, mean ± SD).

Squamous cell carcinomas showed a subcellular distribution of galectin-3, which was strikingly different from that of normal mucosa (Fig. 1, B and C). Thirty-five of 54 tongue carcinomas (64.8%) were negative for nuclear galectin-3 staining (Fig. 2), and tumor cells expressing nuclear galectin-3 were limited in number even in the positive cases. On average, nuclear galectin-3 was observed in 4.3 ± 6.9% (mean ± SD) of tumor cells, which was significantly less frequent compared with normal mucosa (P < 0.0001), whereas cytoplasmic galectin-3 was detected in the majority of tumor cells (84.9 ± 12.2%; mean ± SD; Fig. 2), representing a significant increase compared with normal mucosa (P < 0.0001). Tumor cells negative for cytoplasmic galectin-3 were generally located in superficial areas rather than infiltrating areas of the tumors. Intratumor heterogeneity in staining intensity of cytoplasmic galectin-3 was not significant.

Collectively, in tongue epithelial cells, expression of nuclear galectin-3 decreased during the progression from normal to cancerous states, whereas that of cytoplasmic galectin-3 increased during the progression from normal to cancerous states.
Prognostic Significance of Cytoplasmic Galectin-3 Expression. Based on an average of 84.9% of tumor cells expressing cytoplasmic galectin-3, patients were divided into two subgroups using the mean value as a cutoff point to evaluate the possible prognostic value of cytoplasmic galectin-3 staining. The group with high galectin-3 expression group was defined as ≥85% of tumor cells showing cytoplasmic galectin-3 expression, and the group with low galectin-3 expression was defined as <85% of tumor cells showing cytoplasmic galectin-3 expression. As summarized in Table 1, clinicopathological parameters, including age, sex, tumor size, nodal status, tumor stage, and tumor differentiation, were well balanced between the groups. Follow-up information was available for the patients included in the study. Fig. 3 depicts the Kaplan-Meier plot of overall and disease-free survival curves stratified by galectin-3 status. The 5-year overall survival rates of the groups expressing high and low levels of galectin-3 were 83.9% and 95.7%, respectively. There was a trend toward a less favorable survival rate in the group expressing high levels of galectin-3, which was not significant (P = 0.1675; Fig. 3A), whereas a significant difference in the disease-free survival rate was noted in favor of the patients with low expression of galectin-3 (P = 0.0210; Fig. 3B). The 5-year disease-free survival rates of the groups expressing high and low levels of galectin-3 were 40.8% and 73.9%, respectively. To evaluate the independent predictive value of galectin-3 expression for disease recurrence, multivariate analysis with the Cox proportional hazard model was carried out. The risk ratio of disease recurrence was 3.514 among patients who belonged to the group with high galectin-3 expression versus those who belonged to the group with low galectin-3 expression (P = 0.0120; Table 2). Next, we questioned whether nuclear galectin-3 expression correlates with the clinical outcome of tongue cancer patients. The survival rate among patients lacking cell staining for nuclear galectin-3 versus those showing nuclear staining was compared. The 5-year overall and disease-free survival rates for the group without nuclear galectin-3 staining were 86.4% and 59.1%, respectively, whereas those for the group with nuclear galectin-3 staining were 90.6% and 51.2%, respectively. These results imply no statistically significant difference in both overall survival and disease-free survival between the two groups (P = 0.6140 and 0.8392, respectively).

Discussion

In the present study, we analyzed galectin-3 expression as well as its subcellular distribution to examine the possible involvement of galectin-3 in neoplastic progression of the tongue squamous epithelium. We demonstrated that the expression of cytoplasmic galectin-3 is up-regulated during the progression from normal to cancerous states and established an intrinsic relevance of cytoplasmic galectin-3 expression to the neoplastic progression of the tongue. This result is in agreement with previous reports on the expression pattern of galectin-3 in cancers of the thyroid, liver, stomach, central nervous system, and colon (19–23, 27, 28). In contrast, reports have shown down-regulation of cytoplasmic galectin-3 in malignancies of the ovary, uterus, and breast (24–26). It remains unclear why galectin-3 expression is regulated differently among organs during neoplastic progression. Nevertheless, it was recently reported that the serum levels of galectin-3 are significantly elevated in cancer patients, including patients with breast cancer, gastrointestinal cancer, lung cancer, ovarian cancer, melanoma,

Fig. 1 Galectin-3 staining in tongue lesions. A, normal squamous epithelium; B, well-differentiated squamous cell carcinoma; C, poorly differentiated squamous cell carcinoma. A modification of the avidin-biotin peroxidase complex technique with a hematoxylin counterstain was used to visualize galectin-3. Note that a keratin pearl is unstained, whereas tumor islands are darkly stained. Original magnification, ×100.
and non-Hodgkin’s lymphoma, as compared with normal individuals. Moreover, galectin-3 concentrations in sera from patients with metastatic disease were higher than those in sera from patients with localized tumors (32).

The dissociated expression of cytoplasmic and nuclear galectin-3 during neoplastic progression of the tongue epithelium suggests different biological roles for galectin-3, depending on its subcellular localization. In normal squamous epithelium, cells positive for nuclear or cytoplasmic galectin-3 are distributed in the basal and parabasal layers or superficial and para superficial layers, respectively. Nuclear and cytoplasmic galectin-3 is likely to be linked with proliferation and differentiation, respectively, in normal epithelium. This assumption may be supported by previous findings such as: (a) mitogenic stimulation of quiescent fibroblasts results in a prompt increase of nuclear galectin-3 expression (13, 33); and (b) nuclear galectin-3 is involved in ribonuclear complexes (34) and identified as a factor in pre-mRNA splicing (35). In normal colonic mucosa, cytoplasmic galectin-3 is predominantly observed in the upper areas of the crypt and in the surface epithelium, i.e., terminally differentiated cells (30), and in normal squamous epithelium of the head and neck, distribution of cytoplasmic galectin-3 is confined to the superficial and intermediate layers (36).

To evaluate the prognostic value of cytoplasmic expression of galectin-3 in tongue cancer patients, we divided them into two subgroups, i.e., groups with high and low galectin-3 expression, and compared the clinical outcome between the two groups. The group with high galectin-3 expression showed statistically significant poor disease-free survival compared with the group with low galectin-3 expression, whereas there was no significant difference in overall survival between the two groups. Multivariate regression analysis revealed that the level of galectin-3 expression was an independent variable in predicting disease recurrence because the risk ratio of disease recurrence was 3.514 among patients who belonged to the high

Table 1  Clinicopathological features of the study population according to cytoplasmic galectin-3 expression

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Low galectin-3 (n = 23)</th>
<th>High galectin-3 (n = 31)</th>
<th>P value</th>
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<tbody>
<tr>
<td>Age (yrs)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>8 (34.8)</td>
<td>7 (22.6)</td>
<td>0.3222</td>
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<tr>
<td>&lt;60</td>
<td>15 (65.2)</td>
<td>24 (77.4)</td>
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<tr>
<td>Gender</td>
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<td>Male</td>
<td>15 (65.2)</td>
<td>22 (71.0)</td>
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<tr>
<td>Female</td>
<td>8 (34.8)</td>
<td>9 (29.0)</td>
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<tr>
<td>Tumor size</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>T1 + T2</td>
<td>21 (91.3)</td>
<td>22 (71.0)</td>
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<tr>
<td>T3 + T4</td>
<td>2 (8.7)</td>
<td>9 (29.0)</td>
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<td>Nodal status</td>
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<td></td>
</tr>
<tr>
<td>N0</td>
<td>20 (87.0)</td>
<td>24 (77.4)</td>
<td>0.3723</td>
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<tr>
<td>N1 + N2 + N3</td>
<td>3 (13.0)</td>
<td>7 (22.6)</td>
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<tr>
<td>Tumor stage</td>
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<tr>
<td>I + II</td>
<td>20 (87.0)</td>
<td>22 (71.0)</td>
<td>0.1623</td>
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<tr>
<td>III + IV</td>
<td>3 (13.0)</td>
<td>9 (29.0)</td>
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<td>Histological differentiation</td>
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<tr>
<td>Well</td>
<td>19 (82.6)</td>
<td>27 (87.1)</td>
<td>0.6462</td>
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<tr>
<td>Moderate/poor</td>
<td>4 (17.4)</td>
<td>4 (12.9)</td>
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* Analyzed by χ² test.
Table 2  Multivariate Cox proportional hazard analysis of prognostic factors for disease recurrence

<table>
<thead>
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<th>Variable</th>
<th>Hazard ratio</th>
<th>95% CI*</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>Cytoplasmic galectin-3 status</td>
<td>3.514</td>
<td>1.318–9.368</td>
<td>0.0120</td>
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<tr>
<td>(high vs. low)</td>
<td></td>
<td></td>
<td></td>
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<td>Age (≥60 vs. &lt;60)</td>
<td>1.628</td>
<td>0.615–4.305</td>
<td>0.3262</td>
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<td>Gender (male vs. female)</td>
<td>2.144</td>
<td>0.818–5.623</td>
<td>0.1208</td>
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<td>Tumor size (T4 vs. T1 + T2)</td>
<td>2.570</td>
<td>0.537–12.295</td>
<td>0.2372</td>
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<tr>
<td>Nodal status (N1 vs. N2)</td>
<td>5.175</td>
<td>0.870–30.763</td>
<td>0.0707</td>
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<tr>
<td>Differentiation (moderate/poor vs. well)</td>
<td>2.766</td>
<td>0.931–8.219</td>
<td>0.0671</td>
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</table>

* CI, confidence interval.

gallactin-3 expression group versus those who belonged to the low galectin-3 expression group (P = 0.0120). It is likely that overexpression of galectin-3 could serve as a marker to identify a distinctive group of tongue cancer patients who potentially harbor a high risk of death caused by disease recurrence and thus may benefit from more aggressive treatment. A future study with a longer follow-up period and/or a larger patient population will corroborate whether galectin-3 expression could serve as a prognostic marker for the clinical outcome of tongue cancer patients. While this work was under review, it was reported in a subset of prostate cancer patients that the exclusion of galectin-3 from the nucleus is associated with disease progression (38), similar to the data shown here.

In conclusion, cytoplasmic galectin-3 expression increased during the progression from normal to cancerous states, whereas nuclear galectin-3 expression decreased during the progression from normal to cancerous states. Enhanced expression of cytoplasmic galectin-3 serves as a predictor of disease recurrence in patients with tongue carcinomas.

Acknowledgments

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References

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