Galectin-1 expression in oral squamous cell carcinoma: An immunohistochemical study

Vaibhavi Salunkhe¹, Aarti Mahajan¹, Nilima Prakash¹, Pradeep GL¹, Rekha Patil¹, Sajda Khan Gajdhar²

¹Department of Oral and Maxillofacial Pathology, MGV’s K.B.H. Dental College and Hospital, Nashik, Maharashtra, India, ²Department of Oral Pathology and Microbiology, Ibn Sina National College for Medical Studies, Jeddah, Kingdom of Saudi Arabia

Abstract

Context: Oral squamous cell carcinoma (OSCC) of the head and neck are a heterogeneous group of neoplasms with an increasing rate of mortality and morbidity. OSCCs are characterized by a high degree of local invasiveness and metastasis to cervical lymph nodes but show a lower rate of distant metastasis. Galectin-1 (Gal-1), a β-galactoside-binding lectin, is known to regulate tumor cell growth, angiogenesis, mediate cell-cell or cell-extracellular matrix adhesion and promote cancer cell migration.

Aims: This study aims to evaluate the Gal-1 expression in different clinical stages and histological grades of OSCC.

Settings and Design: Forty histopathologically diagnosed cases of OSCC, including 16 cases of well-differentiated, 18 moderately differentiated and 6 poorly differentiated carcinomas, were included in the study group.

Materials and Methods: The samples were subjected to staining using primary mouse monoclonal antibodies against Gal-1 and visualized using polymer-HRP detection system.

Statistical Analysis: The nonparametric Mann–Whitney U-test and Kruskal–Wallis ANOVA test were used for the statistical analysis.

Results: Gal-1 expression was higher in advanced stages of OSCC, and the results were statistically significant. Immunoexpression of Gal-1 increased with advancing histological grades of OSCC with statistically significant results.

Conclusion: Gal-1 plays an important role in invasion, metastasis and as a prognostic marker.

Keywords: Galectin-1, immunohistochemistry, invasion, metastasis, oral squamous cell carcinoma

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is emerging as a global health problem with 300,000 new cases of oral and oropharyngeal cancers being diagnosed over 5 years.¹ OSCC is the sixth and fifteenth common cancer in men and women, respectively. It involves approximately 94% of all oral malignancies.² The highest prevalence and incidence of OSCC is found in the Indian subcontinent.³ One of the major reasons for the significant mortality related to oral cancer is that 60% of patients present with an advanced...
The metastatic spread of tumors continues to be the main barrier to the successful treatment of malignant tumors. About two-thirds of OSCCs are already of substantial size and have clinically detectable metastases to cervical lymph nodes at the time of diagnosis. OSCCs which have metastasized to the regional lymph nodes are more aggressive and have a less favorable prognosis.

Tumor invasion and the process of metastasis is a characteristic of malignant neoplasms. Galectin-1 (Gal-1) is one of the most important lectins participating in malignant tumor development and metastasis. The mechanism by which Gal-1 contributes to cancer progression and metastasis is as follows: it regulates tumor cell growth, triggers the death of infiltrating T cells, suppresses T-cell-derived pro-inflammatory cytokine secretion, mediates cell-cell or cell-extracellular matrix adhesion, is involved in tumor angiogenesis and promotes cancer cell migration. There is evidence that Gal-1 expression increases as cancer cells progress towards a more malignant phenotype and Gal-1 expression levels affect the invasiveness of cancer cells.

The expression of Gal-1 is upregulated in several different human cancers, including breast, gastric, ovary, lung, prostate, colon, nervous system, liver, myeloid tissue and uterine cervix. There is evidence that the mRNA expression levels of Gal-1 are approximately 7-fold higher in OSCC as compared to normal area of the same tissue. Little is known about the prognostic value of Gal-1 in different stages and different histological grades of OSCC. Therefore, the present study aimed to evaluate the Gal-1 expression in different clinical stages and histological grades of OSCC.

MATERIALS AND METHODS

Forty clinically diagnosed and histologically confirmed cases of OSCC were included. The staging of OSCC was done according to the TNM system. An ethical clearance from the institutional ethical committee and informed consent from the patients was obtained for the present study. The cases were graded according to the histological malignancy grading system given by Bryne et al. (1989).

Two sections of 4 µm were obtained. One section was placed on egg albumin coated slide for routine hematoxylin and eosin stain. Another section was obtained on aminopropyltriethoxysilane coated slide for immunohistochemistry. These were stained with the Gal-1 antibody at a dilution of 1:100 (Mouse Monoclonal Antibody Galectin-1 (C-8), class-IgG2a, Santa Cruz Biotechnology) using Novolink™ Polymer Detection System. Tonsil tissue was used as a positive control.

Evaluation of immunostaining of galectin-1

For the quantitative analysis of Gal-1-positive cells, immunostained slides were examined under high power (magnification: ×400) of a light microscope (Olympus CH 20i). A total of 1000 malignant epithelial cells that had invaded the connective tissue were counted in random high power fields. Those which stained positively for Gal-1 were counted among 1000 cells and expressed in terms of positive percentage. We assessed the expression of Gal-1 in the cytoplasm of cells, which had invaded the connective tissue stroma, and the mean was considered as Gal-1 expression for a particular slide. Gal-1 positivity was determined based on the proportion of stained cells and scored from 0 to 4. Score 0 = 0–5% of stained cells, Score 1 = 6%–25% of stained cells, Score 2 = 26%–50% of stained cells, Score 3 = 51%–75% of stained cells and Score 4 ≥ 75% of stained cells.

The nonparametric Mann–Whitney U-test and Kruskal–Wallis ANOVA test was applied for the evaluation of significant differences among the mean values in different groups. Mann–Whitney U test was applied to compare Gal-1 scores with clinicopathological parameters and histological grades. A \( P < 0.05 \) was considered to indicate statistical significance at 95% of the confidence interval.

RESULTS AND OBSERVATIONS

On comparison of Gal-1 expression with demographic data, we found that Gal-1 expression was higher in males (2.92) than in females (2.75). Mean Gal-1 expression decreased with increasing age groups. The mean expression of Gal-1 was higher in alveolar mucosa (3.25) followed by the tongue (2.93) and then buccal mucosa (2.82) [Table 1]. However, the comparison in the expression of Gal-1 with age, gender and site of OSCC was not found to be statistically significant.

On comparing the expression of Gal-1 score with the nodal status of OSCC, we observed that the mean Gal-1 score increased with regional lymph node involvement.
(N0 = 2.63, N = 3.31) and it was found to be highly significant (P = 0.000) [Table 1 and Figure 1].

We found 3 cases in Stage I, 21 in Stage II and 16 in Stage III. The levels of Gal-1 protein expression was found to be significantly higher in Stage III (3.31) as compared to Stage I (2.67) and Stage II (2.62) (P = 0.001) [Table 1 and Figure 2]. The pairwise comparison showed a statistically significant difference in the Gal-1 expression of Stage II versus III (P = 0.000) [Table 2].

Comparison of expression of Gal-1 with histological grades of OSCC showed the highest Gal-1 expression in Grade III (3.83), followed by Grade II (3.00) and Grade I (2.44). This difference in expression was found to be statistically significant (P = 0.000) [Table 1 and Figure 3]. The pairwise comparison by the Mann–Whitney U-test showed a significant difference between Grade I versus Grade II (P = 0.000), Grade I versus Grade III (P = 0.000) and Grade II versus Grade III (P = 0.000) [Table 3].

**DISCUSSION**

Oral cancer is one of the most common cancers in the world. An estimated 378,500 new cases of intraoral cancer are diagnosed annually worldwide. In parts of India, oral cancer represents more than 50% of all cancers and is the most common cancer among males and the third most common cancer among females.[10]

Clinical stage at the time of diagnosis and anatomic region are the most important predictors of survival in HNSCC patients, but the clinical behavior of these tumors varies in different patients and may be attributed to the biological factors concerned in growth and invasion.[11]

Tumor metastasis is a multistep process that includes changes in cell adhesion, increased invasiveness, angiogenesis and evasion of the immune response. Many lectins are involved in metastasis[12,13] of which Gal-1 has been shown to contribute to all these processes.[13] There is evidence that Gal-1 expression increases as cancer cells progress towards a more malignant phenotype and Gal-1 expression levels affect the invasiveness of cancer cells.[10] A change in the proteolytic degradation of adjacent tissue is required.

**Table 1: Association of Gal-1 expression and Clinicopathological parameters in oral squamous cell carcinoma**

| Parameters         | Number of patients | Mean Gal-1 score | P     |
|--------------------|--------------------|------------------|-------|
| Gender             |                    |                  |       |
| Male               | 36 (90%)           | 2.92             | 0.612 |
| Female             | 4 (10%)            | 2.75             |       |
| Age                |                    |                  |       |
| ≤ 40 years         | 8 (20%)            | 3                | 0.552 |
| > 60 years         | 2 (5%)             | 2.50             |       |
| Site               |                    |                  |       |
| Buccal mucosa      | 22 (55%)           | 2.82             | 0.393 |
| Tongue             | 14 (35%)           | 2.93             |       |
| Alveolar mucosa    | 4 (10%)            | 3.25             |       |
| Tumor size         |                    |                  |       |
| T1                 | 3 (7.5%)           | 2.67             | 0.484 |
| T2                 | 37 (92.5%)         | 2.92             |       |
| Lymph node metastasis |                |                  |       |
| N0                 | 24 (60%)           | 2.63             | 0.000 |
| N1                 | 16 (40%)           | 3.31             |       |
| Stage I            | 3 (7.5%)           | 2.67             | 0.001 |
| Stage II           | 21 (52.5%)         | 2.62             |       |
| Stage III          | 16 (40%)           | 3.31             |       |
| Grade I            | 16 (40%)           | 2.44             | 0.000 |
| Grade II           | 18 (45%)           | 3                |       |
| Grade III          | 6 (15%)            | 3.83             |       |

**Table 2: Pair wise comparison of Gal-1 score among TNM stages by Mann-Whitney U Test**

| Stages         | p-value   |
|----------------|-----------|
| Stage I Vs. II | 0.876     |
| Stage I Vs. III| 0.074     |
| Stage II Vs. III| 0.000*    |

**Table 3: Pair wise comparison of Gal-1 score among Histopathological grades by Mann-Whitney U Test**

| Grades         | p-value   |
|----------------|-----------|
| Grade I Vs. Grade II | 0.000*    |
| Grade I Vs. Grade III | 0.000*    |
| Grade II Vs. Grade III | 0.000*    |

Figure 1: Comparison of galectin-1 score with Nodal status by Mann–Whitney U-test

Figure 2: Comparison of galectin-1 score with TNM stages of oral squamous cell carcinoma by Kruskal–Wallis ANOVA
during tumor invasion. Gal-1-increased tumor invasion is related to the reorganization of the actin cytoskeleton and the upregulation of MMP expression. There is evidence that high Gal-1 expression can be used as a poor prognostic marker for tumors. In the present study, we assessed the immunoexpression of Gal-1 and correlated it with the clinicopathological parameters of OSCC.

Immunohistochemical analysis of Gal-1 in malignant epithelial tumor cells that had invaded the connective tissue stroma demonstrated an overexpression of this protein in all cases of OSCC studied, suggesting that these molecules play an effective role in tumor invasion and progression. On comparison of the immunoexpression of Gal-1 and the demographic data, we did not find statistically significant results. These findings were in concordance with those of Zhong et al., Ding et al. (2008). These findings could be attributed to the fact that the current study is not an epidemiological type of the study; hence, the limited number of the involved cases preclude definitive clinical findings. In the present study, we found Gal-1 expression was significantly raised in patients with positive lymph node status (P = 0.00). Similar results were reported by Noda et al., Zhong et al. On the contrary, Zhong et al. did not find any correlation between Gal-1 expression and regional lymph node metastasis. Gal-1 contributes to change in cell adhesion and increased invasiveness by upregulation of MMPs; hence, we hypothesized that this may contribute to lymph node metastasis.

On comparison of Gal-1 expression with clinical stages, the Gal-1 expression score was higher in advanced clinical stages, and the difference was found to be statistically significant. This was in contrast to the findings of Noda et al., Zhong et al., Ding et al. (2008), who did not find a correlation between Gal-1 expression and clinical stage of OSCC. Gal-1 expression was compared with histological grades of OSCC; we observed that the mean Gal-1 expression score gradually increased as the tumor progressed from Grade I to Grade II to Grade III and was also found to be statistically highly significant (P = 0.000) [Figures 4-6]. Pairwise intergroup comparison showed expression of Gal-1 was statistically significantly higher in Grade III than in Grade II and Grade I, confirming the hypothesis as previously mentioned, that Gal-1 expression has a significant role in tumor invasion and progression.

Our results confirmed the findings of Noda et al., Zhong et al., Ding et al. (2008), who found statistically significant correlation between Gal-1 immunostaining and tumor grade of differentiation and also concluded that higher Gal-1 protein expression indicates a poorer differentiation grade of cancerous tissue and related to poor prognosis. Greer P et al. observed that the Gal-1 is expressed by normal oral keratinocytes (NOK) and OSCC cell lines in vitro. Small molecular inhibitor (OTX008) decreases the cell viability of OSCC and NOK cells in a dose-dependent manner; however, this effect is reduced by higher endogenous levels of Gal-1.

In the present study, Gal-1 expression was noted both in the tumor cells and also in the adjacent stromal cells such as fibroblasts and inflammatory cells. The expression of Gal-1 in the nucleus, as well as the membrane of the cells, was observed in some cases. The reason is that Gal-1 is present in cell nuclei and cytosol and also translocates to the intracellular side of cell membranes. Although Gal-1 lacks a secretion signal sequence and does not pass through the endoplasmic reticulum/Golgi pathway, it is secreted and found on the extracellular side of all cell membranes as well as in the extracellular matrices of various normal and neoplastic tissues.

We also observed that the intensity of the staining was strong in Grade III, moderate in Grade II and weak in Grade I. This finding suggests that the Gal-1 protein expression level increases with increasing grades of OSCC, and it may serve as a candidate marker for pathologic differentiation grade of OSCC.
The role of Gal-1 in tumor invasion opens a new avenue for developing therapeutic approaches to inhibit tumor metastasis. Gal-1 is involved in the mechanisms of tumor immune escape. Gal-1, a negative regulator of T-cell activation and survival, plays a pivotal role in promoting escape from T cell-dependent immunity, thus conferring the immune privilege of tumors by modulating the survival or polarization of effector T cells. The blockade of immunosuppressive Gal-1 in vivo promotes tumor rejection and stimulates the generation of a tumor-specific T cell-mediated response in syngeneic mice. Gal-1 signaling in activated T cells constitutes an important mechanism of tumor-immune escape and that blockade of this inhibitory signal can allow for and potentiate effective immune responses against tumor cells, with profound implications for cancer immunotherapy.

CONCLUSION

In the present study, the upregulation of Gal-1 immunoeexpression was seen in OSCC. Gal-1 expression in tumor cells with regional lymph node metastasis was significantly higher than in those without metastasis. The Gal-1 expression also correlated with the clinical stages of OSCC. Thus, Gal-1 can be considered as a strong prognostic factor for the locoregional spread and clinical behavior of OSCC. As Gal-1 expression correlated significantly with histological grades of OSCC, it may serve as a candidate marker for pathologic differentiation grade of OSCC.

Gal-1 is not only a prognostic marker but also an ideal therapeutic target. The role of Gal-1 in tumor invasion opens a new avenue for developing therapeutic approaches to inhibit tumor metastasis. When considering the potential therapeutic use of Gal-1 inhibitors, however, the potential antitumor response of Gal-1 (i.e., role in tumor immune escape) must be taken into account and attempts must also be made to limit inhibitor interference with physiological Gal-1 function. In the future, multicentric studies with large sample sizes are required to positively correlate Gal-1 expression with clinical stages, histological grades and survival rate in OSCC patients.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Saman DM. A review of the epidemiology of oral and pharyngeal carcinoma: Update. Head Neck Oncol 2012;4:1.
2. Tajmirriahi N, Razavi SM, Shirani S, Homayooni S, Gasemzadeh G. Evaluation of metastasis and 5-year survival in oral squamous cell carcinoma patients in Isfahan (2001-2015). Dent Res J (Isfahan) 2019;16:117-21.
3. Kiran G, Shyam ND, Rao J, Krishna A, Reddy BS, Prasad N. Demographics and histopathological patterns of oral squamous cell carcinoma at a tertiary level referral hospital in Hyderabad India: A 5 year retrospective study. J Orofac Res 2012;2:198-201.
4. Neville BW, Day TA. Oral cancer and precancerous lesions. CA Cancer J Clin 2002;52:195-215.
5. Demydenko D, Berest I. Expression of galectin-1 in malignant tumors. Exp Oncol 2009;31:74-9.
6. Wu MH, Hong TM, Cheng HW, Pan SH, Liang YR, Hong HC, et al. Galectin-1-mediated tumor invasion and metastasis, up-regulated matrix metalloproteinase expression, and reorganized actin cytoskeletons. Mol Cancer Res 2009;7:311-8.
7. Aggarwal S, Sharma SC, Das SN. Galectin-1 and galectin-3: Plausible tumour markers for oral squamous cell carcinoma and suitable targets for screening high-risk population. Clin Chim Acta 2015;442:13-21.
8. Greene FL, Page DL, Fleming ID, Fritz AG, Balch CM, Haller DG, et al., editors. AJCC Cancer staging manual. 6th ed. Chicago, USA: Springer; 2002.
9. Bryne M, Koppang HS, Lilleng R, Stene T, Bang G, Dabelsteen E. New malignancy grading is a better prognostric indicator than Broders' grading in oral squamous cell carcinomas. J Oral Pathol Med 1989;18:432-7.
10. Tondon A, Bordoloi B, Jaiswal R, Srivastava A, Singh RB, Shafique U. Demographic and Clinicopathological profile of oral squamous cell carcinoma patients of North India: A retrospective institutional study. J Oral Sci 2018;60:114-8.
11. Nelson AR, Fingleton B, Rothenberg ML., Matrisian LM. Matrix metalloproteinases: biologic activity and clinical implications. J Clin Oncol 2000;18:1135-49.
12. Sherwani AF, Mohmood S, Khan F, Khan RH, Azfer MA. Characterization of lectins and their specificity in carcinomas-An appraisal. Indian J Clin Biochem 2003;18:169-80.
13. Zhong JP, Wei KJ, Yang X, Pan HY, Ye DX, Wang LZ, et al. Overexpression of Galectin-1 is negatively correlated with pathologic differentiation grade in oral squamous cell carcinoma. J Cancer Res Clin Oncol 2010;136:1527-35.
14. Wu R, Wu T, Wang K, Luo S, Chen Z, Fan M, et al. Prognostic significance of galectin-1 expression in patients with cancer: a meta-analysis. Cancer Cell Int 2018;18:108.
15. Ding YM, Dong JH, Chen LL, Zhang HD. Increased expression of galectin-1 is associated with human oral squamous cell carcinoma development. Oncol Rep 2009;21:983-7.
16. Noda Y, Kishino M, Sato S, Hirose K, Sakai M, Fukuda Y, et al. Galectin-1 expression is associated with tumour immunity and prognosis in gingival squamous cell carcinoma. J Clin Pathol 2017;70:126-33.
17. Greer P, Coates D, Rich A. Galectin-1 inhibition of oral cancer in vitro. J oooo 2019;128:e72.
18. Gupta GS, Gupta A, Gupta R. Animal Lectins: Form, Function and Clinical Application. Vol. 1. India: Springer; 2012.
19. Camby I, Le Mercier M, Lefranc F, Kiss R. Galectin-1: A small protein with major functions. Glycobiology 2006;16:137R-57.
20. Rubinstein N, Alvarez M, Zwirner NW, Toscano MA, Ilarregui JM, Bravo A, et al. Targeted inhibition of galectin-1 gene expression in tumor cells results in heightened T cell-mediated rejection; A potential mechanism of tumor-immune privilege. Cancer Cell 2004;5:241-51.