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Serum Level of Galectin-3 in Patients with Oral Squamous Cell Carcinoma

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Abstract:

Introduction: Galectin-3, a member of the family of β-galactoside-binding animal lectins, has been implicated in tumor invasion and metastasis.

Materials and Methods: Using an ELISA kit, we assessed the circulating levels of galectin-3 in sera from 40 oral squamous cell carcinoma (OSCC) patients and from 43 healthy controls.

Results: Serum galectin-3 levels in OSCC patients were significantly higher (5.1±2.5 ng/ml) when compared with healthy controls (2.6±3.07 ng/ml, $P<0.0005$). There was no apparent correlation between serum galectin-3 concentration and clinico-pathological features such as stage, tumor size, nodal status, distant metastasis and histological grade.

Conclusion: This result suggests that, in addition to other tests, measurement of serum galectin-3 concentrations can be utilized as an adjuvant test for establishing a diagnosis of OSCC.

Keywords: Galectin-3, Serum, Oral squamous cell carcinoma

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Introduction

Lectins are classified into families, among which the galectins are ancient and particularly interesting members. Galectins are a growing family of β-galactoside-binding proteins. A common characteristic of the galectins is the presence of at least one carbohydrate recognition domain (CRD) of approximately 135 amino acids with an affinity for β-galactosides.[1] They have been shown to play roles in diverse biological events such as embryogenesis, adhesion and cell proliferation, apoptosis, mRNA splicing, bacterial colonization and modulation of the immune response.[2,3] Moreover, galectins play a key role in various pathological states including autoimmune diseases, allergic reactions,

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inflammation, tumor spreading, atherosclerosis and diabetic complications.[4,5]

Thus far, 14 mammalian galectins have been identified.[6] Galectin-3 is an approximately 31 KDa unique chimeric gene product which consists of three structural domains, each associated with at least one specific function: (a) an NH2 terminal domain containing a serine phosphorylation site which is important in regulating its cellular signaling; (b) a collagen-α-like sequence cleavable by matrix metalloproteinase; and (c) a COOH terminal containing a single carbohydrate recognition domain and the NWGR anti-death motif.[7]

Galectin-3 is located in the cytoplasm, nucleus, on the cell surface, in the extracellular matrix, and in biological fluids and sera.[8,9] Galectin-3 lacks the classical secretion signal sequence and does not pass through the standard ER/Golgi pathway.[8] Still, it can be transported into the extracellular milieu via a non-classical pathway.[10]

The biological roles of galectin-3 are defined by its cellular localization, which strongly depends on various factors such as cell type, proliferation status, cultivation and neoplastic progression.[11] Cytoplasmic galectin-3 is involved in the modulation of cell proliferation, differentiation, survival and apoptosis. In the nucleus, galectin-3 is involved in pre-mRNA processing and gene transcription. Extracellular galectin-3 mediates cell adhesion through its multivalent properties and ability to bind cell surface glycoproteins and glycosylated components of the extracellular matrix.[12]

Galectin-3 has been detected in activated macrophages, eosinophils, neutrophils, mast cells, the epithelium of gastrointestinal and respiratory tracts, kidneys and some sensory neurons.[8,13] Moreover, galectin-3 displays pathological expression in many tumors; for example, pancreatic, colon, head and neck, thyroid, breast and prostate carcinomas.[14] Galectin-3 expression has recently emerged as a potential diagnostic and/or prognostic marker of some cancers.[5]

The enhanced cytoplasmic expression of galectin-3 has been associated with a decreased disease-free survival of tongue cancer patients.[15] However, the clinical relevance of galectin-3 serum levels in patients diagnosed with oral squamous cell carcinoma (OSCC) is not fully understood. Therefore, we examined galectin-3 serum levels in patients with OSCC.

Materials and Methods

For the purposes of this study, 40 serum samples from patients diagnosed with OSCC (16 males, 24 females; age: 59.58±17.03 years) and 43 serum samples from healthy control subjects (22 males, 21 females; age: 57.14±2.58 years) were collected. All the study patients were admitted to the ENT Department of Shiraz University of Medical Sciences and they had histopathological diagnoses of OSCC. Patients with other malignancies were excluded. Control cases were healthy nonsmoker blood donors who had no evidence of systemic or inflammatory diseases, or infections, who were matched for age and sex. All participants were informed about the research study and agreed to participate by signing an informed consent form.

Serum samples were obtained from clotted blood following centrifugation at 4 °C and stored at -80 °C until analysis. Galectin-3 concentrations were measured by ELISA in accordance with the manufacturer's instructions (BM S 279; Bender Med Systems GmbH, Germany). The sensitivity of the ELISA test was 0.12 ng/ml.

Independent t-test was performed to compare the results of serum galectin-3 concentrations between controls and study participants. Mann-Whitney and Kruskal-Wallis tests were used to define the relation between serum galectin-3 and clinical data. Differences were considered significant at $P<0.05$.

Results

Table 1 shows the clinical data of patients assayed for serum galectin-3. There were 16 males and 24 females diagnosed with OSCC in our study. At the time of presentation, most patients were at stages II (43.6%) and IV (35.9%). Regional lymph node involvement was not present

Table 1. Clinico-pathological profile of 39 oral SCC patients.*

Age (years)	59.58 ± 17.03
Gender	
Male	16 (42.1%)
Female	24 (57.9%)
Tumor size	
T1	2 (5.1%)
T2	26 (66.7%)
T3	11 (28.2%)
Regional lymph node involvement	
N0	21 (53.8%)
N1	4 (10.3%)
N2	12 (30.8%)
N3	2 (5.1%)
Distant metastases	
M0	39 (100%)
M1	0 (0%)
TNM stage	
I	1 (2.6%)
II	17 (43.6%)
III	7 (17.9%)
IV	14 (35.9%)
Histological grade	
I	27 (69.2%)
II	11 (28.2%)
III	1 (2.6%)

*There is missing clinico-pathologic data for one case.

in the majority of the patients (53.8%) and all had localized tumor (M0). A total of 27 tumors (69.2%) were well-differentiated, 11 (28.2%) moderately differentiated and 1 (2.6%) was poorly differentiated. The serum galectin-3 level in OSCC patients was significantly higher (5.1±2.5 ng/ml, n=40) compared with healthy controls (2.6±3.07 ng/ml, n=43, $P<0.0005$).

There was no significant difference in galectin-3 concentration between males and females, nor was there a correlation between serum galectin-3 levels and age.

There was no apparent correlation in serum galectin-3 concentration with the clinico-pathological features such as stage, tumor size, nodal status, distant metastasis and histological grade.

Discussion

Galectin-3 is expressed in a variety of tissues and cell types. It is mainly found in the cytoplasm although, depending on cell type and proliferative state, a significant amount of this lectin can also be detected in the nucleus, on the cell surface or the extracellular environment. Galectin-3 exhibits a pleiotropic biological function, playing a key role in many physiological and pathological processes.[14]

Several reports have indicated its involvement in carcinogenesis.[16,17] All cancers share common characteristics such as uncontrolled proliferation, disturbed adhesiveness and resistance to apoptosis. In regard to the functional properties of galectin-3, it is more than clear that this lectin plays multiple roles in cancer pathogenesis, proliferation and the spread of metastasis.[11]

Galectin-3 expression has been shown to be up-regulated in some cancers such as thyroid carcinoma, hepatocellular carcinoma and lymphoma, and down-regulated in others including breast, uterine and pancreatic carcinomas.[18] Choufani et al. studied the expression of galectin-3 and the expression of ligands for this lectin in head and neck squamous cell carcinoma (HNSCC) and normal tissue

specimens. The results showed that HNSCC exhibited a significantly lower amount of galectin-3 and its ligands than their corresponding normal counterparts. A decrease in the extent of galectin-3 expression correlated with an increasing level of clinically detectable HNSCC aggressiveness.[19]

Honjo et al. analyzed the intracellular expression of galectin-3 in tongue squamous cell carcinoma and normal mucosa. They reported that the nuclear expression of galectin-3 which markedly decreases during neoplastic progression may serve as a prognostic factor for tongue cancer patients.[15]

We used an ELISA kit to measure serum galectin-3 concentrations in patients with OSCC. To the best of our knowledge, this is the first report to do so. We demonstrated that serum galectin-3 concentrations in OSCC patients were statistically higher than normal control patients. However, there was no statistical difference in either histological grade or pathological stage with OSCC patients.

It has been 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, bladder cancer, hepatocellular carcinoma, melanoma and non-Hodgkin's lymphoma as compared with normal individuals. [20,21] Moreover, galectin-3 concentrations in sera from patients with metastatic disease were higher than those in sera from patients with localized tumors.[22]

Iurisci et al. proposed that changes in the level of galectin-3 expression in circulation may favor metastasis by either one or all of the following modalities, such as enhancing the adhesive interactions between tumor cells and the extracellular matrix, promoting tumor cell embolization through increased cell-to-cell adhesion and conferring a selective survival advantage to metastatic cells.[22] In the present study, all patients had localized tumor (M0).

The source of increased serum galectin-3 in cancer patients remains unclear. A diversity of cells involved in the immune system secrete galectin-3[23], therefore, it seems that circulating galectin-3 is generated not only by tumor cells but also from peritumoral inflammatory cells.

In conclusion, the serum galectin-3 concentration in OSCC patients was statistically higher than that of controls. This result suggests that the measurement of serum galectin-3 concentration can be an adjuvant test for establishing the diagnosis of OSCC, in addition to other tests.

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References

1. Barondes SH, Cooper DNW, Gitt MA, Leffler H. Galectins. Structure and function of a large family of animal lectins. *J Biol Chem* 1994;269: 20807-20810.
2. Rabinovich GA, Baum LG, Tinari N, Paganelli R, Natoli C, Liu FT, et al. Galectins and their ligands: Amplifiers, silencers or tuners of the inflammatory response? *Trends Immunol* 2002;23:313-20.
3. Liu FT, Patterson RJ, Wang JL. Intracellular functions of galectins. *Biochim Biophys Acta* 2002;1572:263-73.
4. Wada J, Makino H. Galectins, galactoside-binding mammalian lectins: Clinical application of multi-functional proteins. *Acta Med Okayama* 2001;55:11-17.
5. Danguy A, Camby I, Kiss R. Galectins and cancer. *Biochim Biophys Acta* 2002;1572:285-93.
6. Hirabayashi J, Kasai K. The family of metazoan metal-independent beta-galactoside-binding lectins: Structure, function and molecular evolution. *Glycobiology* 1993;3:297-304.
7. Nangia-Makker P, Balan V, Raz A. Regulation of tumor progression by extracellular galectin-3. *Cancer Microenviron* 2008;1:43-51.
8. Hughes RC. Secretion of the galectin family of mammalian carbohydrate-binding proteins. *Biochim Biophys Acta* 1999;1473:172-185.
9. Gong HC, Honjo Y, Nangia-Makker P, Hogan V, Mazurak N, Bresalier RS, et al. The NH2 terminus of galectin-3 governs cellular compartmentalization and function in cancer cells. *Cancer Res* 1999;59: 6239-45.
10. Nickel W. Unconventional secretory routes. Direct protein export across the plasma membrane of mammalian cells. *Traffic* 2005;6:607-14.
11. Dumic J, Dabelic S, Flogel M. Galectin-3: An open-ended story. *Biochim Biophys Acta* 2006;1760:616-35.
12. Matsuda Y, Yamagiwa Y, Fukushima K, Ueno Y, Shimosegawa T. Expression of galectin-3 involved in prognosis of patients with hepatocellular carcinoma. *Hepatol Res* 2008;38:1098-11.
13. Hughes RC. The galectin family of mammalian

carbohydrate-binding proteins. *Biochem Soc Transact* 1997;25:1194-98.
14. Krzeslak A, Lipinska A. Galectin-3 as a multifunctional protein. *Cell Mol Biol Lett* 2004; 9:305-28.
15. Honjo Y, Inohara H, Akahani S, Yoshii T, Takenaka Y, Yoshida J, et al. Expression of cytoplasmic galectin-3 as a prognostic marker in tongue carcinoma. *Clin Cancer Res* 2000;6:4635-40.
16. Takenaka Y, Inohara H, Yoshi T, Oshima K, Nakahara S, Akahani S, et al. Malignant transformation of thyroid follicular cells by galectin-3. *Cancer Lett* 2003;195:111-19.
17. Yoshi T, Inohara H, Takenaka K, Honjo Y, Akahani S, Nomura T, et al. Galectin-3 maintains the transformed phenotype of thyroid papillary carcinoma cells. *Int J Oncol* 2001;18:787-92.
18. Califice S, Castronovo V, van den Brule F. Galectin-3 and cancer. *Int J Oncol* 2004;25:983-92.
19. Choufani G, Nagy N, Saussez S, Marchant H, Bisschop P, Burchert M, et al. The levels of expression of galectin-1, galectin-3 and Thomsen- Friedenreich antigen and their binding sites decrease as clinical aggressiveness increases in head and neck cancer. *Cancer* 1999;86: 2353-63.
20. Sato S, Hughes RC. Regulation of secretion and surface expression of Mac-2 a galactoside-binding protein of macrophages. *J Biol Chem* 1994;269:4424-30.
21. Castronovo V, Campo E, van den Brule F, Claysmith A, Cioce V, Liu FT, et al. Inverse modulation of steady-state messenger RNA levels of two non-integrin laminin binding proteins in human colon carcinoma. *J Natl Cancer Inst* 1992;84:1161-9.
22. Iurisci I, Tinari N, Natoli C, Angelucci D, Cianchetti E, Iacobelli S. Concentrations of galectin-3 in the sera of normal controls and cancer patients. *Clin Cancer Res* 2000;6:1389-93.
23. Chen HY, Liu FT, Yong RY. Roles of galectin-3 in immune responses. *Arch Immunol Ther Exp* 2005;53:497-504.