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Increased expression and altered intracellular distribution of adhesion/growth-regulatory lectins galectins-1 and -7 during tumour progression in hypopharyngeal and laryngeal squamous cell carcinomas

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Aims: To examine the level of expression of the pleiotropic regulators galectins-1 and -7 in relation to neoplastic progression of hypopharyngeal (HSCCs) and laryngeal (LSCCs) squamous cell carcinomas.

Methods and results: The presence of galectins-1 and -7 was investigated using quantitative immunohistochemistry in (i) a series of 78 HSCCs by comparison with 17 normal epithelia (N_E), 26 low-grade dysplasia (low_D) and 27 high-grade dysplasia (high_D) and (ii) a series of 56 LSCCs by comparison with 50 N_E, 23 low_D and 29 high_D. Galectin-1 positivity expressed as a percentage of cells was significantly higher in carcinomas (HSCCs and LSCCs) than in N_E, low_D or high_D ($P < 10^{-6}$). Galectin-7 expression was elevated in low_D ($P = 0.0004$) compared with N_E and in carcinomas (HSCC) compared with high_D ($P = 0.0002$). Tumour progression from high_D to carcinomas was associated with a shift of galectin-1 localization from the nucleus towards the cytoplasm. Increased expression of galectin-7 in dysplasias was accompanied by a shift from the cytoplasmic compartment (N_E) to the nucleus (low_D and high_D).

Conclusions: Our data reveal an association between the level of presence of galectins-1 and -7 and neoplastic progression of HSCCs and LSCCs. Moreover, inverse shifts between nuclear and cytoplasmic positivity intimating functional divergence were detected.

Keywords: dysplasia, epithelium, lectin, malignancy, preneoplasia

Abbreviations: ABC, avidin–biotin–peroxidase complex; CA, carcinoma; high_D, high-grade dysplasia; HSCC, hypopharyngeal squamous cell carcinoma; LI, labelling index; low_D, low-grade dysplasia; LSCC, laryngeal squamous cell carcinoma; MOD, mean optical density; N_E, normal epithelium; PBS, phosphate-buffered saline; SSC, squamous cell carcinoma

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Introduction

(i) Head and neck squamous cell carcinomas (SCC) is the sixth most common form of cancer worldwide and has a dismal prognosis. Insights into the biochemical nature of effectors in tumour progression provide an opportunity to develop new strategies to interfere with this process.[1] With this end in mind, attention is turning to the study of galectins, a family of endogenous lectins with a wide range of extra- and intracellular functions mediated by protein–carbohydrate and protein–protein interactions.[2–4] For the following reasons it is promising and timely to investigate the closely related homodimeric galectins-1 and -7, which figure as potent pleiotropic regulators: expression of genes for both proteins appear to be under the control of tumour suppressors, with $p16^{INK4a}$ acting as the activator in Capan-1 pancreatic carcinoma cells in the case of galectin-1, and p53 in DLD-1 colonic carcinoma cells and UVB-exposed epidermal keratinocytes for galectin-7.[5–7] In fact, galectin-7 has been referred to as p53-induced gene 1;[5]

(ii) galectins-1 and -7 share caspase-independent growth-inhibitory activity on SK-N-MC neuroblastoma cells triggered by binding to ganglioside GM_1 and potent pro-apoptotic activity with different caspase profiles on activated T cells.[8–10] At the clinical level, the latter activity accounts for clonal expansion of CD4 + CD7− T leukaemic cells in Sézary syndrome, which are protected from galectin-1-triggered cell death;[11]

(iii) galectin-1 as a pleiotropic effector is present in the nucleus involved in pre-mRNA splicing and in the cytoplasm, e.g. acting as stabilizer of oncogenic H-Ras-GTP and enhancing cell transformation;[12–14]

(iv) modulation of gene expression levels for both galectins has been described to cause a marked series of secondary effects detected by microarrays, i.e. 44 changes more than threefold in Hela cells overexpressing galectin-7 and 86 changes more than twofold in U87 glioblastoma cells when down-regulating galectin-1 expression;[15,16]

(v) butyrate increases galectin-1 expression concurrently with morphological changes and growth inhibition in MDA-886LN head and neck SCC cells (from laryngeal lymph node metastasis).[17] Initial data from a murine model of skin carcinogenesis have revealed low-level expression of galectin-7 in squamous or spindle cell carcinoma, whereas SCC of the buccal mucosa is associated with a 4.1-fold up-regulation of galectin-7;[18,19]

(vi) the relationship of galectin-7 expression to tumorigenesis or aggressiveness appears to depend on the cell type: in bladder SCC it is related to degree of differentiation, in chemical induction of rat mammary carcinogenesis it serves as a positive marker, in lymphoma it is related to dissemination and in thyroid cancer development it shows bell-shaped down- and up-regulation.[20–23]

Our previous report concerning stage IV hypopharyngeal squamous cell carcinoma (HSCC) was the first to demonstrate that high levels of galectin-7 expression could be associated with rapid recurrence rates and a dismal prognosis, features weakly observed with galectin-1.[24] We have also previously investigated the prognostic value of these same galectins in a series of laryngeal squamous cell carcinomas (LSCCs) and shown that high levels of galectin-1 expression are associated with a poor relapse-free and overall survival rate.[25] In the present study we have used quantitative immunohistochemistry in a series of 78 cases of stage IV HSCC and 56 cases of LSCC (stages I, II and IV) in comparison with normal controls and dysplastic tissues from peritumoral regions to address the following questions: do the levels of expression of galectins-1 and -7 change with the course of the disease? What are the cellular sites of positivity? Does the course of the disease alter this parameter?

Materials and methods

PATIENTS' CHARACTERISTICS

A total of 143 cases, including 81 patients with HSCC and 62 patients with LSCC who underwent surgery aimed at curative tumour resection, were studied (see Table 1 for clinical data). These cases were obtained by retrospective compilation (January 1989 to December 2001) from the records of the Ear, Nose and Throat Department of the Hôpital Claude Huriez (Lille, France). The description of tumour status was based on the histological stage of tumour differentiation (criteria defined in[26]) and the TNM classification.[27] Detailed information was available on extent of surgical resection, response to treatment at the primary tumour site, as well as follow-up data up to the last contact with the patient and the status of the disease at that moment. Patients suffering from SCCs localized at other sites of the head and neck area were excluded from the study.

ANTIBODIES

Human galectins-1 and -7 were produced in bacteria, purified to homogeneity as shown by one- and two-dimensional gel electrophoresis and mass spectrometry and used as antigens for raising polyclonal antibodies. The resulting IgG fractions were rigorously checked for

Table 1. Clinical data

Variable	High-stage HSCCs	Low-stage LSCCs	High-stage LSCCs
Age (years)			
Range	40–78	36–88	43–78
Average	55	57	57
Sex (cases)			
Male	75	40	16
Female	3		
Site (cases)			
Supraglottic area		5	9
Glottic area		30	
Supraglottic and glottic areas		5	4
Subglottic and glottic areas			3
Piriform sinus	60		
Postcricoid area	16		
Posterior wall	2		
Histological grade (cases)			
Well differentiated	40	34	10
Moderately differentiated	27	5	6
Poorly differentiated	11	1	
Stage (cases)			
Stage I		30	
Stage II		10	
Stage IV	78		16
Tumour treatment (cases)			
CO_2 laser cordectomy		8	
Frontolateral laryngectomy		2	
Vertical partial laryngectomy		4	
Supracricoid partial laryngectomy		23	3
Supraglottic laryngectomy		3	
Total laryngectomy			13
Partial pharyngolaryngectomy	9		
Total pharyngolaryngectomy	51		
Circular pharyngolaryngectomy	8		
Oesopharyngolaryngectomy	10		

cross-reactivity among this lectin family using human galectins-1, -2, -3, -4, -7, -8 and -9 in Western blot and enzyme-linked immunosorbent assays and chromatographic affinity depletion was performed in any case of positivity, followed by quality controls to ascertain elimination of cross-reactivity as described.[28]

Table 1. (*Continued*)

Variable	High-stage HSCCs	Low-stage LSCCs	High-stage LSCCs
Treatment of the neck (cases)			
Functional neck dissection		19	23
Radical neck dissection		–	2
Recurrence (cases)			
Local recurrence	17	3	4
Distant recurrence	11	3	7
Follow-up			
Range (months)	2–122	2–130	5–74
Average (months)	37	43	30

HSCC, hypopharyngeal squamous cell carcinoma; LSCC, laryngeal squamous cell carcinoma.

IMMUNOHISTOCHEMISTRY

All tumour samples were fixed for 24 h in 10% buffered formaldehyde, dehydrated and routinely embedded in paraffin. Immunohistochemistry was performed on 5 μm thick sections mounted on silane-coated glass slides, as detailed previously.[24] Before starting the immunohistochemistry protocol, dewaxed tissue sections were briefly subjected to microwave pretreatment in a 0.01 M citrate buffer (pH 6.0) for 2 × 5 min at 900 W. The sections were then incubated with a solution of 0.4% hydrogen peroxide for 5 min to block endogenous peroxidase activity, rinsed in phosphate-buffered saline (PBS; 0.04 M Na_2HPO_4, 0.01 M KH_2PO_4 and 0.12 M NaCl, pH 7.4) and successively exposed for 20 min to solutions containing avidin (0.1 mg/ml in PBS) and biotin (0.1 mg/ml in PBS) to avoid false-positive immunoreactivity resulting from endogenous biotin. After thorough washing with PBS, the sections were incubated for 20 min with a solution of 0.5% casein in PBS and sequentially exposed at room temperature to solutions of (i) the specific primary anti-galectin antibodies; (ii) the corresponding biotinylated secondary antibody (polyclonal goat antirabbit IgG); and (iii) the avidin–biotin–peroxidase complex (ABC kit). Incubation steps were separated by thorough washing to remove unbound proteins. Antigen-dependent presence of the peroxidase complex in the sections was visualized by incubation with the chromogenic substrates containing diaminobenzidine and H_2O_2. After rinsing, the sections were counterstained with luxol fast blue and mounted with a synthetic medium. To exclude antigen-independent staining, the incubation step with primary/secondary antibodies was omitted from the protocol in controls. In all cases these controls were negative. The biotinylated secondary antibodies and ABC kit came from DakoCytomation (Glostrup, Denmark).

DEFINITION OF LOW- AND HIGH-GRADE EPITHELIAL DYSPLASIA

Morphological characteristics of dysplasia include increased cellular density associated with a large number of mitotic figures in the vicinity of the basal layer, irregular maturation, loss of polarity, and dyskeratosis. Cytologically, dysplasia is characterized by an increased ratio of nuclear to cytoplasmic area, anisocytosis, poikilocytosis, nuclear polymorphism, chromatin condensation and large nucleoli, features sometimes associated with atypical mitotic figures. Low-grade dysplasia, comprising mild and moderate dysplasia, presents atypical features extending over the lower or middle third of the epithelium.[29] High-grade dysplasia, including severe dysplasia, and carcinoma *in situ* extends over the entire thickness of the epithelium.[29]

COMPUTER-ASSISTED MICROSCOPY

Following immunohistochemistry, the quantitative levels of galectin expression were determined using a computer-assisted KS 400 imaging system (Carl Zeiss Vision, Hallbergmoos, Germany) connected to a Zeiss Axioplan microscope, as detailed previously.[24] For each microscopic field, analysis was focused on the epithelial region using computer-assisted morphometry after interactive identification. In each case, 15 fields were scanned covering a surface area ranging from 60 000 to 120 000 μm^2. In each case, quantitative analysis of

the immunohistochemistry for a given marker yielded data on two variables: (i) the labelling index (LI), i.e. the percentage of positive cells, and (ii) the mean optical density (MOD), i.e. intensity of immunoreactivity of positive cells.[24] For each case of dysplasia (low or high grade), the respective fields within peritumoral areas were defined by one of us (X.L.) specialized in this diagnostic procedure.

DATA ANALYSIS

Independent groups of quantitative data were compared using the non-parametric Kruskall–Wallis (more than two groups) or Mann–Whitney *U*-tests (two groups). In the case of more than two groups, *post hoc* tests (Dunn procedure) were used to compare pairs of groups (to avoid multiple comparison effects). The relationships between the qualitative (or ordinal) variables analysed (e.g. cytoplasmic location versus nuclear and cytoplasmic location) were studied by means of contingency tables. The significance of the potential associations was evaluated by means of the χ^2 or the exact Fisher's test (in the 2×2 cases). The standard survival time analyses were performed using the Kaplan–Meier curves and the Gehan generalized Wilcoxon test. Statistical analyses were carried out using Statistica software (Statsoft, Tulsa, OK, USA).

Results

EXPRESSION OF GALECTIN-1 DURING TUMOUR PROGRESSION OF HYPOPHARYNGEAL AND LARYNGEAL SCCS

The monitoring of different tissue samples revealed that the clinical course toward hypopharyngeal and laryngeal SCCs is characterized by different patterns of immunolabelling (Figure 1). In normal epithelium (N_E), galectin-1 was found in the cytoplasm of the basal layer (71% of cases of hypopharyngeal N_E and 78% of cases of laryngeal N_E) (Figures 1A,B and 3A,B). The immunoreactivity of galectin-1 was clearly different in carcinomas (HSCCs and LSCCs) compared to N_E, low-grade dysplasia (low_D) or high-grade dysplasia (high_D) (Figure 1). For clarity, we describe below the comparison of all groups [N_E, low_D, high_D and carcinoma (CA)] using the non-parametric Kruskall–Wallis test and *post hoc* comparisons used for pairs of groups are described in Figures 2 and 4.

In the case of hypopharyngeal lesions, the use of two different quantitative variables revealed a disparate

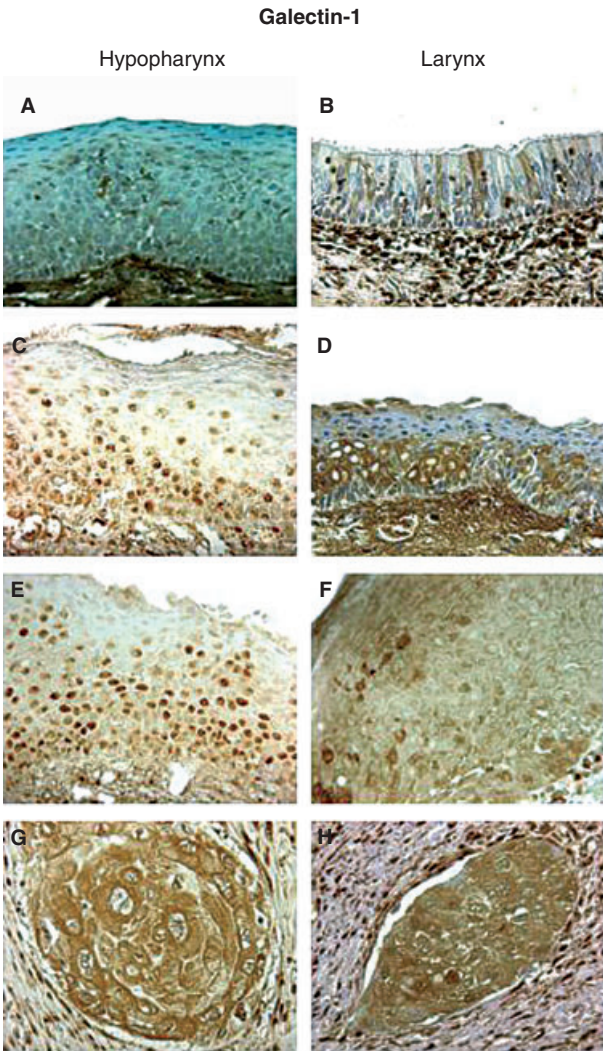

Figure 1. **A–F**, Immunopositivity profile for galectin-1 in hypopharynx (**A**,**C**,**E**,**G**) and in larynx (**B**,**D**,**F**,**H**), respectively. The antigen was localized in normal epithelium (N_E) (**A**), low-grade dysplasia (low_D) (**C**), high-grade dysplasia (high_D) (**E**) in areas surrounding hypopharyngeal carcinoma (CA) (**G**). **B**,**D**,**F**, Galectin-1 positivity in N_E (**B**), low_D (**D**), high_D (**F**) in areas surrounding laryngeal CA (**H**).

relationship to the clinical course. The MOD variable showed no significant difference (Figure 2A), in contrast to the increased presence of galectin-1 when measuring the percentage of immunopositive epithelial cells (LI variable, Kruskall–Wallis: $P < 10^{-6}$) (Figure 2B). Interestingly, the progression was associated with a shift of the immunolabelling from the nucleus to the cytoplasmic compartment exclusively (Figure 1C,E,G) when comparing dysplasias (grouping low_D or high_D) with hypopharyngeal carcinomas (Fisher: $P = 0.02$) (Figure 3A). In quantitative terms,

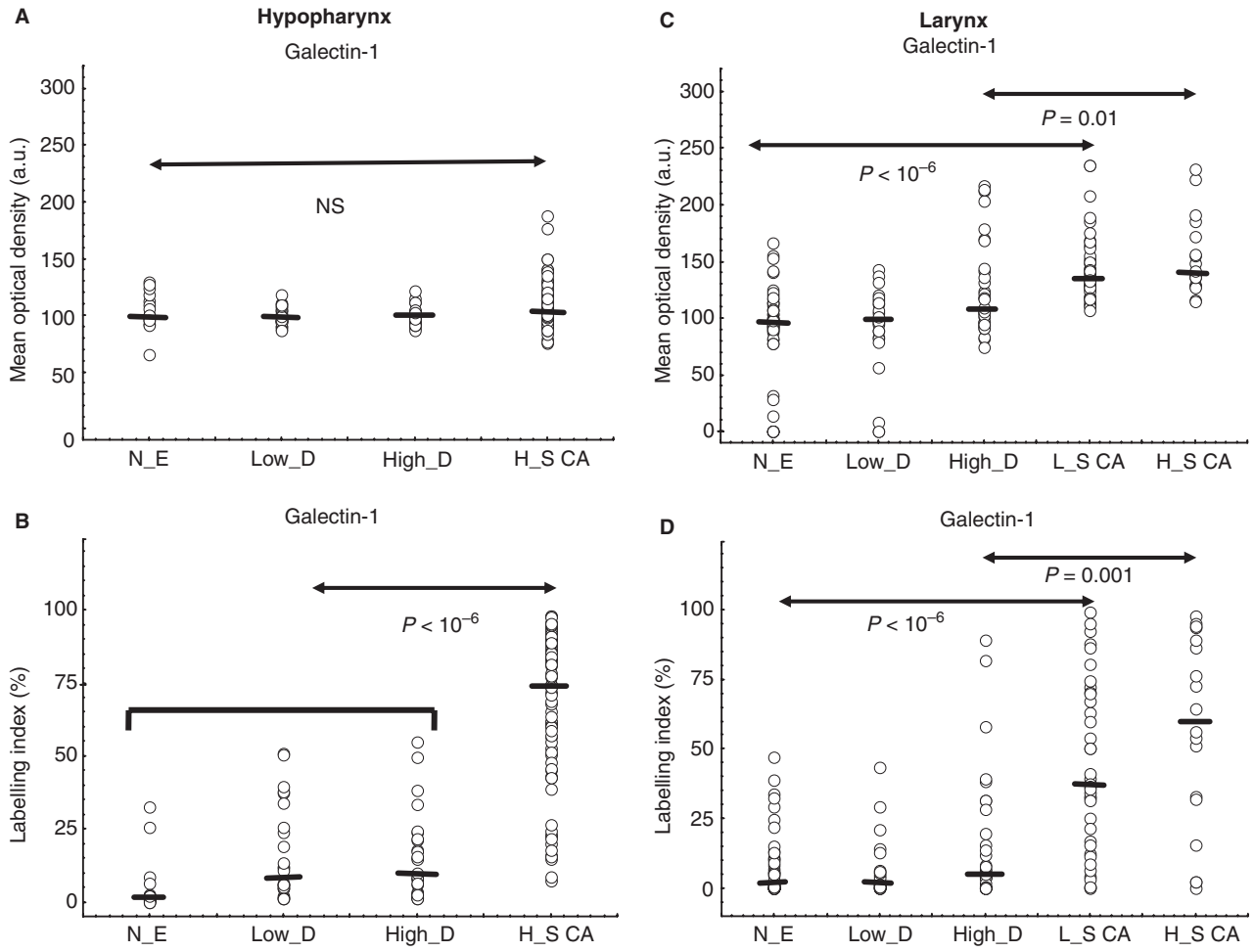

Figure 2. Quantitative determination (by computer-assisted microscopy) of the percentages of galectin-1-immunopositive cells [the labelling index (LI)] (**B**,**D**) and the extent of galectin-1 presence detected immunohistochemically [the mean optical density (MOD)] (**A**,**C**) in a series of 17 normal epithelia (N_E), 26 low-grade dysplasia (low_D), 27 high-grade dysplasia (high_D) and 78 H_CA (stage IV hypopharyngeal squamous cell carcinomas) (**A**,**B**). **C**,**D**, Data on the same parameters in a series of 50 N_E, 23 low_D, 29 high_D and 56 laryngeal squamous cell carcinomas (40 L_S CA: stages I and II, 16 H_S CA: stage IV) (**C**,**D**). *Post hoc* comparisons were used to compare pairs of groups indicated by the arrow. If data of two or three cohorts are grouped together, these are indicated by a bracket. Each symbol (○) represents an individual value and the horizontal line the median.

46% of low_D (12/26) and 41% of high_D (11/27) cases displayed nuclear expression of galectin-1, significantly decreasing to 23% in HSCCs (18/78 cases). We thus investigated if this latter group of cases showing nuclear galectin-1 location could be statistically associated with particular clinical features. Our analysis showed no significant association with any of the clinical features detailed in Table 1 (data not shown).

In the case of laryngeal lesions, both immunohistochemical variables describing galectin-1 expression were higher in carcinomas than in high_D, low_D or N_E, reaching highly significant *P*-values for both MOD and LI (Kruskall–Wallis: $P < 10^{-6}$ for both) variables (Figure 2C,D). Again, a shift of galectin-1 expression was observed from the nucleus toward the cytoplasmic compartment exclusively when comparing N_E with dysplasias and LSCCs (Figure 3C). In fact, 22% of N_E (8/37) displayed nuclear expression of galectin-1 against 6% (1/15) of low_D, 7% (2/29) of high_D and 5% of LSCCs (3/56 cases), with a significant difference appearing after grouping these latter three (preneoplastic and neoplastic) categories (Fisher: $P = 0.01$; Figure 3C). No significant relation was observed between nuclear expression of galectin-1 in CA and any clinical features (data not shown).

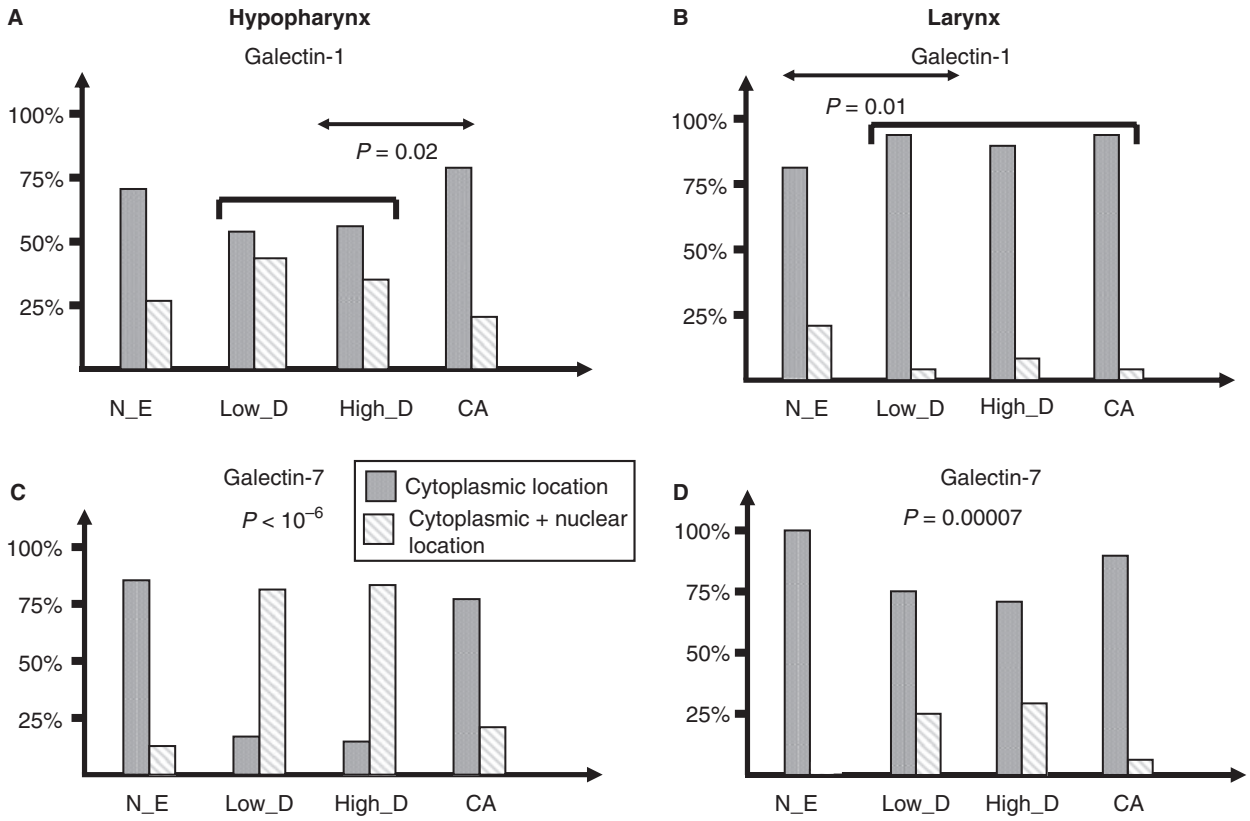

Figure 3. Dysplasias (low_D and high_D) present a galectin-1-dependent nuclear labelling that decreases significantly in hypopharyngeal carcinoma (CA) (**A**). In laryngeal squamous cell carcinomas, dysplasias and CA reveal a significant shift of immunoreactivity from the nucleus to the cytoplasm (**C**). Increased intensity of positivity for galectin-7 is associated in dysplasias with the presence of nuclear labelling (**B**,**D**).

EXPRESSION OF GALECTIN-7 DURING TUMOUR PROGRESSION OF HYPOPHARYNGEAL AND LARYNGEAL SCCS

By using home-made antibody preparations rigorously controlled to lack cross-reactivity among human galectins, we were able to determine unambiguously the presence of the homodimeric galectin-7 closely related to galectin-1. Globally, its presence in tissue was characterized by immunolabelling patterns different from those of galectin-1, established in the course of tumour progression of hypopharyngeal and laryngeal SCCs (Figure 4). In N_E, weak to moderate galectin-7 immunolabelling was observed essentially distributed in the cytoplasm of basal and suprabasal layers (87% of hypopharyngeal N_E and 100% of laryngeal N_E) (Figure 3B,D). Early in the course of tumour development, the intensity of positivity and/or LI increased from N_E to low_D and high_D associated with the appearance of nuclear labelling (Figures 3B,D, 4C–F and 5). Whereas the nuclear labelling decreased in SCCs, galectin-7 expression levels remained high in carcinomas and even increased in high-stage HSCCs (Figure 5B).

In hypopharyngeal lesions, increased expression of galectin-7 is associated with both the percentage of immunopositive epithelial cells (LI variable, Kruskall–Wallis: $P < 10^{-6}$) and the intensity of reactivity of positive cells (MOD variable, Kruskall–Wallis: $P = 0.002$) (*post hoc* comparisons are detailed in Figure 5A,B). Similar observations were recorded for laryngeal lesions (LI variable, Kruskall–Wallis: $P < 10^{-6}$; MOD variable, $P < 10^{-6}$) (Figure 5C,D). When we compared the nuclear/cytoplasmic distribution of galectin-7 immunolabelling, highly significant variations were observed among the different groups for both hypopharyngeal (χ^2: $P < 10^{-6}$) and laryngeal (χ^2: $P = 0.00007$) lesions (Figure 3B,D). Particularly among hypopharyngeal lesions, 82% of the cases of low_D (18/22) and 84% of the cases of high_D (21/25) were characterized by nuclear expression of galectin-7. The situation was different in tumours. The extent of nuclear expression was greatly decreased in the cases of hypopharyngeal carcinoma, in which only

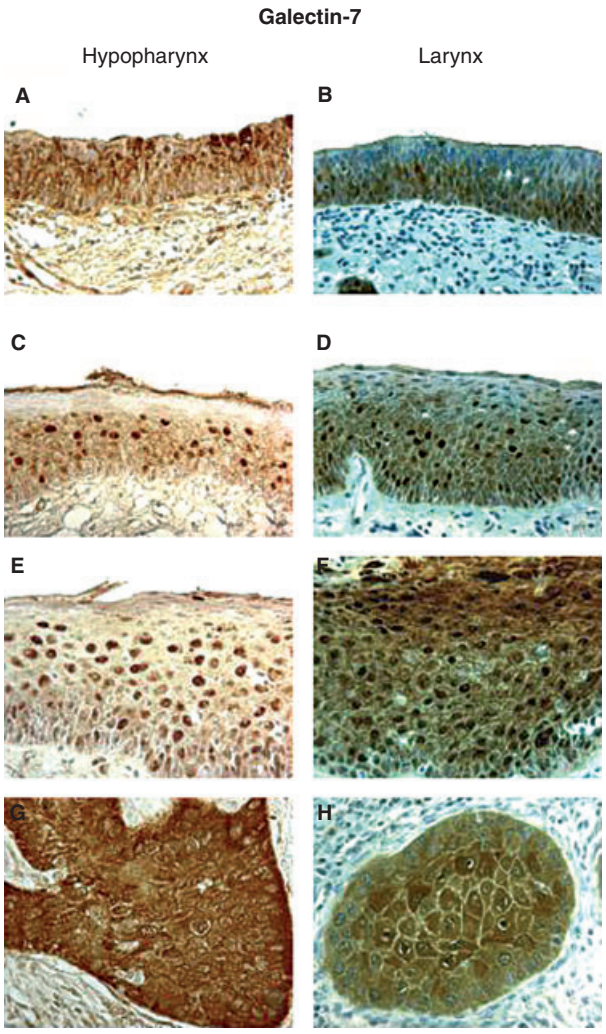

Figure 4. Immunopositivity profile for galectin-7 in the hypopharynx (**A**,**C**,**E**,**G**) and larynx (**B**,**D**,**F**,**H**), respectively. Nuclear immunolabelling of galectin-7 is characteristic of low-grade dysplasia (low_D) and high-grade dysplasia (high_D) (**C**–**F**). A high level of expression in tumour progression of hypopharyngeal squamous cell carcinomas and laryngeal squamous cell carcinomas was detectable (**A**–**H**).

24% displayed nuclear positivity (19/78) (Figure 3B). As described in our previous report[24], the percentages of nuclear galectin-7-immunopositive cells decreased significantly in parallel with the loss of histological differentiation (Kruskal–Wallis test: $P = 0.004$) and with the presence of nodal metastases (Mann–Whitney test: $P = 0.01$). Our observations were similar for laryngeal CA, with 26% of the cases of low_D and 28% of high_D displaying nuclear reactivity, whereas 0% of N_E and 7% of CA exhibited such reactivity (Figure 3D). In laryngeal CA, analysis showed no significant relationship between nuclear expression of galectin-7 in CA and any clinical features (data not shown).

Discussion

This study has combined the quantitative immunohistochemical analysis of two pleiotropic regulators from a family of endogenous lectins and yielded data about significant up-regulation of galectin in the course of tumour progression. Our results confirm the crucial implications of galectins-1 and -7 during the progression of head and neck SCC (see review[30]). Previously, we have determined the immunohistochemical expression of galectins-1, -3 and -7 in a series of stage IV HSCCs and observed that high levels of galectin-7 expression are associated with rapid recurrence rates and a dismal prognosis.[24] These data suggest that the immunohistochemical determination of galectin-7 expression in the case of high-risk hypopharyngeal cancers is a meaningful tool to identify patients who might benefit from aggressive postsurgical adjuvant therapy.[24] Moreover, the level of expression of galectin-1 contributes to the prognosis of the recurrence of laryngeal tumours and, to a lesser extent, of pharyngeal tumours after surgery, and to patients' survival prospects.[24,25] Elevation of galectin-1 levels in LSCCs could contribute to the process of tumour development by killing activated T cells and by promoting motility or activity of oncogenic H-Ras proteins.[25] The quantitative determination of galectin-1 in LSCCs is an independent prognostic marker and could potentially identify patients unlikely to benefit from T-cell-mediated immunotherapy. Answering the second and third questions posed at the end of the Introduction, the intracellular profiles of immunoreactivity of galectins-1 and -7 were found to depend on disease status, with disparate shifts between cytoplasmic and nuclear positivity. Thus far, focus on nuclear presence had been given to galectins-1 and -3, and cell transfectants have been the main source of information on the nuclear presence of galectin-7.[15,31] Herein, we have documented that initiation of tumorigenesis in this cancer type is associated with nuclear localization of galectin-7. In carcinomas it is shifted back to cytoplasmic sites. In contrast, the presence of galectin-1 occurred in cell nuclei in the course of malignant progression. Backed by evidence from colonic cancer progression and the study of epithelial dysplasia of bile ducts and intrahepatic cholangiocarcinoma, increase in intracellular binding activity for β-galactosides or in the presence of galectin-1 thus appears to be a factor also favouring malignancy in HSCCs and LSCCs.[32–35] This result is in marked contrast to

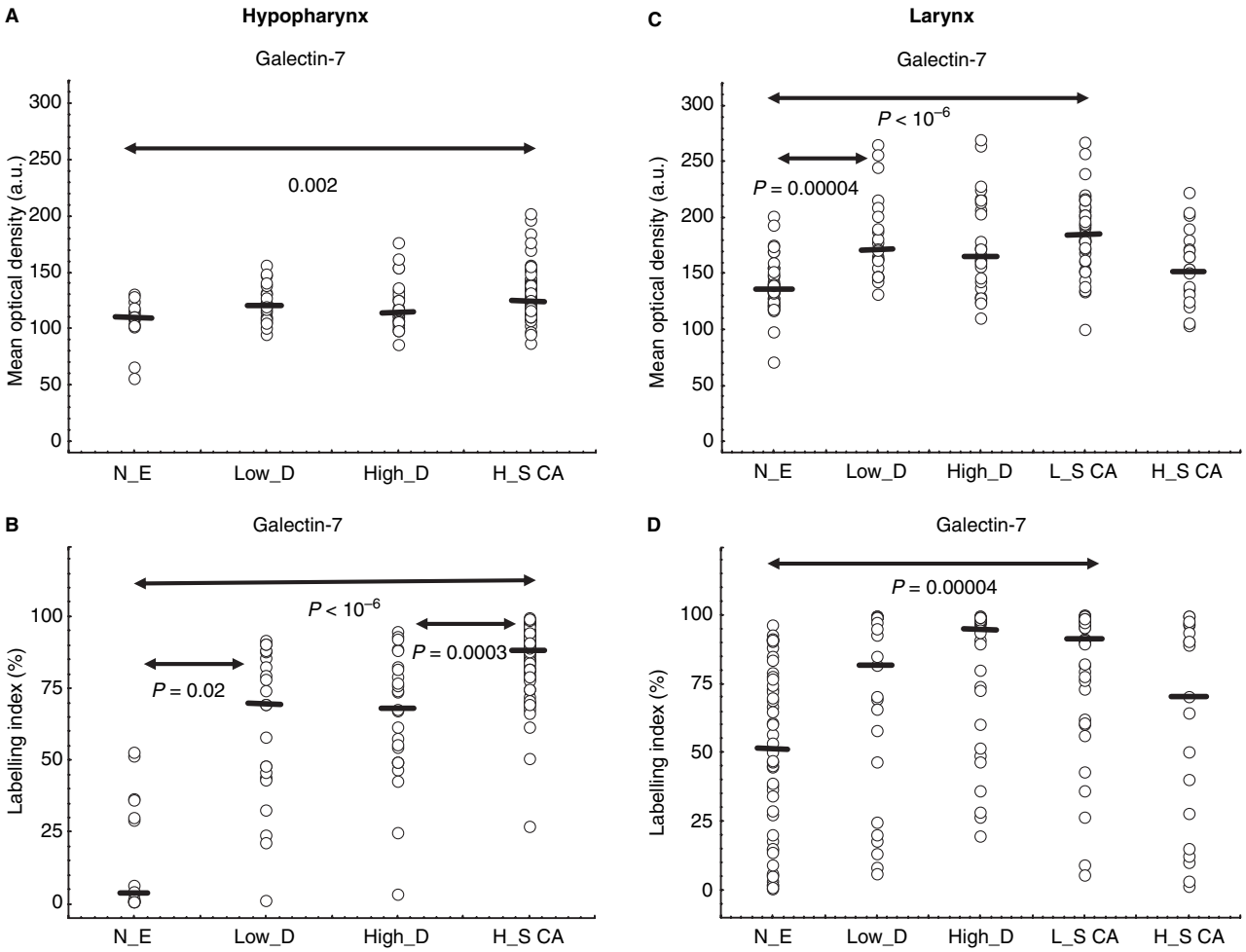

Figure 5. Quantitative determination of the percentages of galectin-7-immunopositive cells [the labelling index (LI)] (**B**,**D**) and the extent of galectin-7 presence detected immunohistochemically [the mean optical density (MOD)] (**A**,**C**) in a series of 78 H_CA (**A**,**B**) and 56 laryngeal squamous cell carcinomas (**C**,**D**) and their surrounding normal epithelium (N_E), low-grade dysplasia (low_D) and high-grade dysplasia (high_D). The rest of the legend is identical to that of Figure 2.

carbohydrate-dependent growth-inhibitory cell surface activities.[7–11,35] Whether a pro-progression interplay between nuclear p53 and galectin-7 may be involved, as intimated by the increased presence of p53 in oral and laryngeal lesions,[36,37] warrants further study.

Our results and the previously detected bell-shaped down- and up-regulation of galectin-7 expression in thyroid cancer[22] explain the increasing interest in the biochemical pathways underlying a role in tumour progression. Interestingly, galectin-7, by virtue of its lectin activity, induces increases in matrix metalloproteinase-9 production in lymphoma cells and marked effector activity on gene profiles is known for both galectin-1 and -7 from genetically engineered cell models.[15,16,23] It seems likely on the basis of the present results that these galectins may be engaged in protein–protein recognition, in turn defining new targets for intervention. Chemical library approaches have already proven their value in developing peptide-based inhibitors.[38] They can be introduced for tests in cell models of this tumour class. Because work with cell lines and clinical material can lead to results which are not readily reconcilable, as illustrated recently for galectin-3 and breast cancer,[39] it is crucial to select models with properties in line with the data of this analysis using clinical material, also considering intratumoral heterogeneity. In this sense, our study provides a guideline for further work and also underscores the need to bear in mind the network character of galectin functionality.

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References

1. Hunter KD, Parkinson EK, Harrison PR. Profiling early head and neck cancer. *Nat. Rev. Cancer* 2005; **5;** 127–135.
2. Gabius H-J. Animal lectins. *Eur. J. Biochem.* 1997; **243;** 543–576.
3. Liu FT, Rabinovich GA. Galectins as modulators of tumour progression. *Nat. Rev. Cancer* 2005; **5;** 29–41.
4. Villalobo A, Nogales-González A, Gabius H-J. A guide to signaling pathways connecting protein–glycan interaction with the emerging versatile effector functionality of mammalian lectins. *Trends Glycosci. Glycotechnol.* 2006; **18;** 1–37.
5. Polyak K, Xia Y, Zweier JL, Kinzler KW, Vogelstein B. A model for p53-induced apoptosis. *Nature* 1997; **389;** 300–305.
6. Bernerd F, Sarasin A, Magnaldo T. Galectin-7 overexpression is associated with the apoptotic process in UVB-induced sunburn keratinocytes. *Proc. Natl. Acad. Sci. U.S.A.* 1999; **96;** 11329–11334.
7. André S, Sanchez-Ruderisch H, Nakagawa H *et al.* Tumour suppressor $p16^{INK4a}$: modulator of glycomic profile and galectin-1 expression to increase susceptibility to carbohydrate-dependent induction of anoikis in pancreatic carcinoma cells. *FEBS J.* 2007; **274;** 3233–3256.
8. Kopitz J, von Reitzenstein C, André S *et al.* Negative regulation of neuroblastoma cell growth by carbohydrate-dependent surface binding of galectin-1 and functional divergence from galectin-3. *J. Biol. Chem.* 2001; **276;** 35917–35923.
9. Kopitz J, André S, von Reitzenstein C *et al.* Homodimeric galectin-7 (p53-induced gene 1) is a negative regulator for human neuroblastoma cells. *Oncogene* 2003; **22;** 6277–6288.
10. Sturm A, Lensch M, André S *et al.* Human galectin-2: novel inducer of T cell apoptosis with distinct profile of caspase activation. *J. Immunol.* 2004; **173;** 3825–3837.
11. Rappl G, Abken H, Muche JM *et al.* $CD4^+CD7^-$ leukemic T-cells from patients with Sézary syndrome are protected from galectin-1-triggered T-cell death. *Leukemia* 2002; **16;** 840–845.
12. Vyakarnam A, Lenneman AJ, Lakkides KM, Patterson RJ, Wang JL. A comparative nuclear localization study of galectin-1 with other splicing components. *Exp. Cell Res.* 1998; **242;** 419–428.
13. Park JW, Voss PG, Grabski S, Wang JL, Patterson RJ. Association of galectin-1 and galectin-3 with Gemin4 in complex containing SMN protein. *Nucleic Acids Res.* 2001; **27;** 3595–3602.
14. Rotblat B, Niv H, André S *et al.* Galectin-1(L11A) predicted from a computed galectin-1 farnesyl-binding pocket selectively inhibits Ras-GTP. *Cancer Res.* 2001; **64;** 3112–3118.
15. Kuwabara I, Kuwabara Y, Yang RY *et al.* Galectin-7 (PIG1) exhibits pro-apoptotic function through JNK activation and mitochondrial cytochrome c release. *J. Biol. Chem.* 2002; **277;** 3487–3497.
16. Camby I, Decaestecker C, Lefranc F, Kaltner H, Gabius H-J, Kiss R. Galectin-1 knocking down in human U87 glioblastoma cells alters their gene expression pattern. *Biochem. Biophys. Res. Commun.* 2003; **335;** 27–35.
17. Gillenwater A, Xu X-C, Estrov Y *et al.* Modulation of galectin-1 content in human head and neck squamous carcinoma cells by sodium butyrate. *Int. J. Cancer* 1998; **75;** 217–224.
18. Magnaldo T, Fowlis D, Darmon M. Galectin-7, a marker of all types of stratified epithelia. *Differentiation* 1998; **63;** 159–168.
19. Chen J, He QY, Yuen APW, Chin JF. Proteomics of buccal squamous cell carcinoma: the involvement of multiple pathways in tumorigenesis. *Proteomics* 2004; **4;** 2465–2475.
20. Lu J, Pei H, Kaeck M, Thompson HJ. Gene expression changes associated with chemically induced rat mammary carcinogenesis. *Mol. Carcinog.* 1997; **20;** 204–215.
21. Østergaard M, Rasmussen HH, Nielsen HV *et al.* Proteome profiling of bladder squamous cell carcinomas: identification of markers that define their degree of differentiation. *Cancer Res.* 1997; **57;** 4111–4117.
22. Rorive S, Eddafali B, Fernandez S *et al.* Changes in galectin-7 and cytokeratin-19 expression during the progression of malignancy in thyroid tumors: diagnostic and biological implications. *Mod. Pathol.* 2002; **15;** 1294–1301.
23. Demers M, Magnaldo T, St-Pierre Y. A novel function for galectin-7: promoting tumorigenesis by up-regulating MMP-9 gene expression. *Cancer Res.* 2005; **65;** 5205–5210.
24. Saussez S, Cucu R, Decaesteker C *et al.* Galectin-7 (p53-induced gene-1): a new prognostic predictor of recurrence and survival in stage IV hypopharyngeal cancer. *Ann. Surg. Oncol.* 2006; **13;** 999–1009.
25. Saussez S, Lorfebvre F, Cucu D *et al.* High level of galectin-1 expression is a negative prognostic predictor of recurrence in laryngeal squamous cell carcinomas. *Int. J. Oncol.* 2007; **30;** 1109–1117.
26. Hyams VJ, Batsakis JG, Michaels L, eds. Tumors of the upper respiratory tract and ear. *Atlas of tumor pathology*. Washington, DC: Armed Forces Institute of Pathology, 1988.
27. Wittekind C, Greene FL, Hutter RVP, Klimpfinger M, Sobin LH. *TNM Atlas*, 5th edn. Berlin: UICC Springer, 2004.
28. Lohr M, Lensch M, André S *et al.* Murine homodimeric adhesion/growth-regulatory galectins-1, -2 and -7: comparative profiling of gene/promoter sequences by database mining, of expression by RT-PCR/immunohistochemistry and of contact sites for carbohydrate ligands by computational chemistry. *Folia Biol (Praha)* 2007; **53;** 109–128.
29. Galle N, Pilch BZ, Sidransky D, Westra WH, Califano J. *Epithelial precursor lesions. World Health Organization Classification of Tumours. Pathology & Genetics. Head and neck tumours.* Lyon: IARC Press, 2006.
30. Saussez S, Camby I, Toubeau G, Kiss R. Galectins as modulators of tumor progression in head and neck squamous cell carcinomas (HNSCCs). *Head Neck* 2007; **29;** 874–884.
31. Smetana K Jr, Dvořánková B, Chovanec M *et al.* Nuclear presence of adhesion-/growth-regulatory galectins in normal/malignant cells of squamous epithelial origin. *Histochem. Cell Biol.* 2006; **125;** 171–182.
32. Brinck U, Bosbach R, Korabiowska M, Schauer A, Gabius H-J. Histochemical study of expression of lectin-reactive carbohydrate epitopes and glycoligand-binding sites in normal human appendix vermiformis, colonic mucosa, acute appendicitis and colonic adenoma. *Histol. Histopathol.* 1996; **11;** 919–930.

33. Shimonishi T, Miyazaki K, Kono N *et al.* Expression of endogenous galectin-1 and galectin-3 in intrahepatic cholangiocarcinoma. *Hum. Pathol.* 2001; **32;** 302–310.
34. Hittelet A, Legendre H, Nagy N *et al.* Upregulation of galectins-1 and -3 in human colon cancer and their role in regulating cell migration. *Int. J. Cancer* 2003; **103;** 370–379.
35. Fischer C, Sanchez-Ruderisch H, Welzel M *et al.* Galectin-1 interacts with the α5β1 fibronectin receptor to restrict carcinoma cell growth via induction of p21 and p27. *J. Biol. Chem.* 2005; **280;** 37266–37277.
36. Kerpdon D, Rich AM, Reade PC. Expression of p53 in oral mucosal hyperplasia, dysplasia and squamous cell carcinoma. *Oral Dis.* 1997; **3;** 86–92.
37. Hussein MR. Alterations of p53 and Bcl-2 in protein expression in the laryngeal intraepithelial neoplasia. *Cancer Biol. Ther.* 2005; **4;** 213–217.
38. André S, Maljaars CE, Halkes KM, Gabius HJ, Kamerling JP. Discovery of galectin ligands in fully randomized combinatorial one-bead-one-compound (glyco)peptide libraries. *Bioorg. MEs. Chem Lett.* 2007; **17;** 789–793.
39. Moisa A, Fritz P, Eck A *et al.* Growth/adhesion-regulatory tissue lectin galectin-3: stromal presence but not cytoplasmic/nuclear expression in tumor cells as negative prognostic factor in breast cancer. *Anticancer Res.* 2007; **27;** 2131–2140.

