GALECTIN-3 EXPRESSION IN PAPILLARY THYROID CARCINOMA: RELATION TO HISTOMORPHOLOGIC GROWTH PATTERN, LYMPH NODE METASTASIS, EXTRATHYROID INVASION, AND TUMOR SIZE

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Abstract: Background. Galectin-3 has been recently recognized as a promising presurgical marker of thyroid malignancy.
Methods. Galectin-3 expression was examined immunohistochemically in 202 specimens of papillary thyroid carcinoma (PTC) in relation to histomorphologic subtypes and clinicopathologic data.
Results. The sensitivity of galectin-3 immunostaining versus conventional histology was 98% (100 of 102) for classical PTC, 85.2% (46 of 54) for follicular variant, and 50% (23 of 46) for follicular/solid variant of PTC. All cases (n = 36) involving lymph node metastases and 42 of 45 cases with extrathyroid invasion expressed galectin-3. However, among the galectin-3–positive cases (n = 169), 133 were without lymph node metastases, and 127 were without extrathyroid invasion. Galectin-3 expression was not related to the size of intrathyroid PTC.
Conclusions. Galectin-3 immunohistochemical expression itself is not an indicator of local metastatic spread or extrathyroid invasion of PTC, thus being irrelevant clinically from this aspect. Galectin-3 is an excellent marker for classical PTC but must be used with caution in diagnosing unconventional variants of PTC because of the possibility of false-negative results. © 2005 Wiley Periodicals, Inc. Head Neck 27: 1049–1055, 2005

Keywords: galectin-3; thyroid; papillary carcinoma; immunohistochemistry; tumor marker

A number of different substances have been evaluated as potential molecular markers in the differential diagnosis of thyroid neoplastic lesions. Among them, galectin-3 seems to be one of the most promising immunohistochemical markers of thyroid malignancy.

Galectins are a family of lectin molecules endowed with a carbohydrate recognition domain that specifically recognizes beta-galactosides.¹ Human galectin-3 is a 31-kDa protein constitutively expressed by immune cells and several epithelial cells. Galectin-3 localization is domi-
nanty cytoplasmic but also can be nuclear, membranous, or extracellular, depending on the cell type and physiologic conditions, which suggests multifunctionality for this molecule.\textsuperscript{1–3} Because of its interactions with carbohydrate and noncarbohydrate ligands, galectin-3 may play a role in a wide spectrum of biologic and pathologic processes, such as differentiation, cell growth and apoptosis,\textsuperscript{4,5} cell adhesion,\textsuperscript{6} immune response,\textsuperscript{7} mRNA splicing,\textsuperscript{8} neoplastic transformation,\textsuperscript{9,10} and metastatic processes.\textsuperscript{11}

Altered expression of galectin-3 in human tumors compared with their normal tissue counterparts has been documented for many tumor types (reviewed by Danguy et al\textsuperscript{9} and van den Brule et al\textsuperscript{10}). Thus, galectin-3 has been extensively studied in thyroid tissue during the past few years.\textsuperscript{12–31} These studies demonstrated immunohistochemical overexpression of galectin-3 in thyroid carcinomas, in both histologic sections\textsuperscript{12–14,17,18,21–25,30,31} and cytologic specimens,\textsuperscript{1,5,16,19,20,26,28} whereas its expression in nonmalignant thyroid cells was absent or weak. Therefore, galectin-3 was identified as a potential presurgical immunohistochemical marker for distinguishing benign from malignant thyroid tumors.

According to the dedifferentiation state, thyroid carcinomas originating from the epithelial follicular cells are in general classified as follows: well-differentiated carcinomas (papillary and follicular carcinomas) and poorly differentiated carcinomas and undifferentiated (anaplastic) thyroid carcinomas. All authors investigating galectin-3 expression in thyroid tumors have found the highest level of expression in welldifferentiated thyroid carcinomas and subsequent downregulation from poorly differentiated toward undifferentiated carcinomas. Furthermore, different patterns of galectin-3 expression were identified for each subhistotype of follicular carcinoma, starting with a high level in the welldifferentiated subtype and ending with a low level in the poorly differentiated follicular carcinoma subtype.\textsuperscript{15,20} Interestingly, the highest level of galectin-3 expression in the spectrum of malignant thyroid tumors was found to be associated with papillary tissue architecture (ie, with the classical [papilliform] variant of papillary thyroid carcinoma [PTC]). However, PTC also occurs in different histomorphologic variants.\textsuperscript{32–37} Whether galectin-3 expression varies in association with the histologic growth pattern of PTC has not been pointed out in previous studies.

In this study, we addressed this issue by examining immunohistochemically galectin-3 expression in a series of 202 cases of paraffin-embedded tissues of PTC in relation to the histomorphologic growth pattern. In addition, because clinical data were available for this relatively large series of PTC, immunostaining results were also correlated with the occurrence of lymph node metastases, extrathyroid invasion, and tumor size.

**MATERIALS AND METHODS**

**Patients and Tissue Specimens.** Formalin-fixed, paraffin-embedded tissues from 202 surgically removed PTCs were used for this immunohistochemical analysis. Tumors were selected from the archival material of the Institute of Endocrinology, Diabetes and Metabolism, Clinical Center of Serbia, Belgrade. Histologic slides from the thyroid tumor tissue stained by hematoxylin-eosin were reevaluated by two pathologists (S. T. and M. H.) to confirm the diagnosis, according to widely accepted histologic criteria\textsuperscript{32–35} and to subclassify the variants of papillary carcinomas. Nuclear features (ground glass nuclei, grooved nuclei, and nuclear pseudo-inclusions, at least two of them), regardless of the growth pattern, were taken as the “gold standard” for confirming the diagnosis of papillary carcinoma. Selected papillary carcinoma cases included different histologic variants, and we divided them into three main categories according to the histologic growth pattern: (1) classical PTC with papillary architecture (CL-PTC, 102 cases), (2) follicular variant of PTC (FV-PTC, 54 cases), and (3) follicular variant of PTC including areas with a solid growth pattern (FV/SOLID-PTC, 46 cases).

Information concerning age, sex, lymph node metastases involvement, extrathyroid invasion, and tumor size was retrieved by reviewing the pathology reports. The patients included 169 females and 33 males (ratio, 5:1), ranging from 10 to 72 years old, with an average age of 46 years. The tumor size ranged from 2 mm to 10 cm. With regard to tumor size, the cases were divided into groups (T1–T4) according to the TNM classification of thyroid tumors\textsuperscript{38}: T1, <10 mm; T2, 10–40 mm; T3, >40 mm (T1–T3 = intrathyroid); and T4, extension outside the thyroid capsule (ie, extrathyroid invasion). In 36 cases, lymph node metastases (LNM) were present at the time of surgery, and extrathyroid invasion (EI) was present in 45 cases. No patient had distant metastatic disease at the time of original diagnosis.
Immunohistochemistry. A rat monoclonal antibody (M3/38) against galectin-3, produced by ATCC TIB-166 (American Type Culture Collection, Rockville, MD) was kindly provided by Dr. M. E. Huflejt, La Jolla Institute for Allergy and Immunology, San Diego, CA. The same antibody was used in our previous reports on galectin-3 expression in thyroid tissue.14,18,27

Immunostaining was performed on 4- to 6-μm thick sections using the avidin-biotin-peroxidase complex (ABC) technique with reagents supplied by Vector Laboratories (Burlingame, CA).

After deparaffinizing and rehydrating, endogenous peroxidase activity was blocked with 0.3% H2O2/methanol followed by nonimmune horse serum for 20 minutes to block nonspecific binding. The sections were then incubated with primary antibody against galectin-3 at 4°C overnight at a dilution of 1:200. This was followed by incubation with biotinylated horse anti-mouse immunoglobulin G (IgG) (which also cross-reacts with the primary rat antibody) for 30 minutes and thereafter with the ABC reagents for 30 minutes. Between each step, sections were washed three times in phosphate-buffered saline (PBS). The reaction was visualized using 3, 3′-diaminobenzidine tetrahydrochloride (DAB) solution.

After counterstaining with hematoxylin, slides were dehydrated, cover-slipped, and examined using a Reichart–Jung microscope supplied with a Photostar automatic camera system (Cambridge Instruments, Buffalo, NY). Controls were incubated with PBS in place of the primary antibody, and no positive staining was observed. The internal positive control was represented by histiocytes.

Scoring of Staining and Statistical Data. Staining was scored as follows: (−), no staining, (+), weak or focal staining, and (++), moderate to strong staining in most epithelial thyroid cells.

The sensitivity of galectin-3 immunohistochemistry in confirming the diagnosis of PTC versus classical histologic analysis (taken as the “gold standard”) was calculated as follows: true positive/true positive + false positive + true negative + false negative).

Statistical comparisons of data were performed with Student’s t test. A value of p < .05 was considered to be statistically significant.

RESULTS

When present, normal or hyperplastic thyroid epithelial cells adjacent to malignant tissue of papillary carcinoma showed no immunoreactivity for galectin-3.

The results of galectin-3 immunohistochemical staining of PTC tissue are given in Tables 1–3, and some representative photographs are shown in Figure 1.

As shown in Table 1, positive immunohistochemical staining for galectin-3 was found in 169 (83.7%) cases of PTC of the 202 cases analyzed in this study. Intensity of staining varied from moderate/strong to weak or focal. Galectin-3 localization was dominantly cytoplasmic but also membranous or nuclear in some cells. Galectin-3 could not be detected immunohistochemically in 33 cases, which means that we obtained 33 false-negative results. Thus, the sensitivity of galectin-3 immunohistochemistry versus conventional histology was 83.7% for the overall series of PTC cases analyzed.

However, apparent differences in galectin-3 immunostaining results were found in relation to the histologic growth pattern. The highest level of galectin-3 expression was associated with the group of classical papilliform PTC cases (CL-PTC), in which 100 of 102 cases (98.0%) showed positive galectin-3 immunostaining (moderate to strong or weak/focal, as detailed in Table 1), whereas only two cases were negative (2.0%), giving 98.0% sensitivity for galectin-3 immunostaining. In the FV-PTC group, 46 of 54 cases showed galectin-3 positive immunostaining, and eight cases were galectin-3 negative (sensitivity, 85.2%). Furthermore, in the FV/SOLID-PTC group, galectin-3 was not detected in 23 (50%) cases, giving a sensitivity of galectin-3 immunostaining of only 50%. Statistically significant differences in the sensitivity of galectin-3 immunohistochemistry versus conventional histology were found between the FV/SOLID-group and the FV and CLV groups of PTC cases (p < .05).

In addition, galectin-3 immunostaining of PTC cases was correlated with available clinical data. Galectin-3 expression in relation to LNM and EI is shown in Table 2. All 36 cases involving LNM
Galectin-3 immunostaining in PTC in relation to histologic growth pattern.

<table>
<thead>
<tr>
<th>PTC histologic growth pattern</th>
<th>Galectin-3 immunostaining*</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL-PTC (classical papilliform)</td>
<td>++ 92 (90.2%) + 8 (7.8%) − 2 (2.0%)</td>
<td>98.0%†</td>
</tr>
<tr>
<td>FV-PTC (follicular variant)</td>
<td>++ 35 (64.8%) + 11 (20.4%) − 8 (14.8%)</td>
<td>85.2%‡</td>
</tr>
<tr>
<td>FV/SOLID-PTC (follicular variant with solid areas)</td>
<td>++ 12 (26.1%) + 11 (23.9%) − 23 (50.0%)</td>
<td>50.0%§</td>
</tr>
<tr>
<td>Total (n = 202)</td>
<td>++ 139 (68.8%) + 30 (14.9%) − 33 (16.3%)</td>
<td>83.7%</td>
</tr>
</tbody>
</table>

Positive: 169 (83.7%) Negative: 33 (16.3%)

Table 1.

Abbreviations: PTC, papillary thyroid carcinoma; CL-PTC, classical papillary thyroid carcinoma with papillary architecture; FV-PTC, follicular variant of papillary carcinoma; FV/SOLID-PTC, follicular variant of papillary carcinoma including areas with a solid growth pattern.

Note. p < .05 for § vs † and vs ‡.

were galectin-3 positive (sensitivity, 100%). In the group of PTC cases without LNM, there were 133 (80.1%) cases showing galectin-3 positivity and 33 (19.9%) cases negative for galectin-3 immunostaining (specificity, 19.9%). Although it seemed that higher levels of galectin-3 expression were associated with primary tumors involving LNM, galectin-3 expression itself did not indicate local metastatic spread of PTC, because among the total of 169 cases expressing galectin-3 there were more cases without (n = 133) than with (n = 36) LNM involvement. The diagnostic accuracy of galectin-3 immunodetection in distinguishing LNM-positive from LNM-negative cases was only 34.2%.

Galectin-3 immunostaining in PTC in relation to EI of PTC is also shown in Table 2. Galectin-3 was immunohistochemically detected in 42 of 45 cases (93.3%) with EI (sensitivity, 93.3%) and in 127 of 157 cases (80.9%) without EI (specificity, 19.1%). Thus, in a similar way to LNM involvement, galectin-3 expression itself did not indicate the presence of EI. The diagnostic accuracy of galectin-3 immunodetection in distinguishing PTC cases with and without EI was only 35.6%.

Galectin-3 immunorepression was also correlated with the tumor size of PTC. The cases were divided into four groups (T1–T4), as detailed in “Material and Methods.” Group T4 (extension beyond the gland), consisting of the cases with EI, was analyzed in Table 2. Table 3 shows galectin-3 expression in relation to the size of intrathyroid PTC (groups T1–T3). There were no significant differences in galectin-3 expression among groups T1, T2, and T3, which indicates that galectin-3 is not associated with the size of intra-thyroid PTC.

DISCUSSION

During the past few years, galectin-3 has been widely investigated as a potential immunohistochemical marker of thyroid malignancy.12–31 Because of its high expression in thyroid carcinomas and the absence or weak expression in normal and benign thyroid tissue, the findings indicate the diagnostic value of galectin-3 immunohistochemistry in distinguishing benign from malignant thyroid tumors.

The highest levels of galectin-3 immunohistochemical expression are associated with papillary

Table 2. Galectin-3 immunostaining* in PTC in relation to lymph node metastases and extrathyroid invasion.

<table>
<thead>
<tr>
<th>Galectin-3 immunostaining</th>
<th>LNM (+)</th>
<th>LNM (−)</th>
<th>EI (+)</th>
<th>EI (−)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(++/++), number</td>
<td>36 (n = 36)</td>
<td>133 (n = 166)</td>
<td>42 (n = 45)</td>
<td>127 (n = 157)</td>
</tr>
<tr>
<td>(−/−), number</td>
<td>0</td>
<td>33</td>
<td>3</td>
<td>30</td>
</tr>
</tbody>
</table>

Sensitivity, % 100.0 93.3
Specificity, % 19.9 19.1
Diagnostic accuracy, % 34.2 35.6

Table 3.

Abbreviations: PTC, papillary thyroid carcinoma; LNM, lymph node metastases; EI, extrathyroid invasive.

*Staining was scored as follows: (−) no staining, (+) weak or focal staining, and (++) moderate to strong staining in the majority of thyroid epithelial cells.

†According to Hermanek and Sobin.38 Details in “Material and Methods.”
‡Differences not statistically significant (p > .05) for T1 vs T2, T2 vs T3 and T1 vs T3.

Table 3. Galectin-3 immunostaining in PTC in relation to tumor size (T1).

<table>
<thead>
<tr>
<th>Staining intensity</th>
<th>Positive (++/++), total</th>
<th>Negative (−), total</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 (n = 60)</td>
<td>33 (55.0%) 14 (23.3%)</td>
<td>47 (78.3%) 13 (21.7%)</td>
</tr>
<tr>
<td>T2 (n = 83)</td>
<td>57 (68.7%) 13 (15.7%)</td>
<td>70 (84.3%) 13 (15.7%)</td>
</tr>
<tr>
<td>T3 (n = 14)</td>
<td>8 (57.1%) 2 (14.3%)</td>
<td>10 (71.4%) 4 (28.6%)</td>
</tr>
</tbody>
</table>
architecture of thyroid carcinoma. PTC is defined from the histologic features (essentially papillary growth pattern intermingling with a follicular growth pattern to various degrees), cytologic features (ground glass nuclei, grooved nuclei, and intranuclear inclusions), and biologic behavior (slow growth and propensity to metastasize to lymph nodes). The follicular variant is the most common subhistotype of PTC. In addition, some PTCs also contain focal areas of a more cellular, solid growth pattern. According to widely accepted histologic criteria, PTC is solely defined by its characteristic nuclear features regardless of its growth pattern. However, sometimes PTC shows a multifocal rather than a diffuse distribution of nuclear features and can mimic hyperplastic nodule or follicular adenoma of the thyroid.

In previous studies on galectin-3 expression in thyroid tumors, galectin-3 was found to be expressed in all or almost all PTCs. The sensitivity of galectin-3 immunohistochemistry versus conventional histology was reported to be in the range of 92% to 100%, with the exception of one study in which galectin-3 sensitivity for PTC was found to be 82%. However, this type of thyroid malignancy was mainly analyzed as part of a spectrum of thyroid tumors, with the consequence that in most studies a small number of PTC cases were involved. Furthermore, it is not clear whether the PTC cases analyzed included different histomorphologic subtypes of PTC, because that was not mentioned in these reports. Only the group of LiVolsi noticed weaker galectin-3 expression in five of 15 cases of the follicular variant of PTC versus classical PTC, whereas Weber et al recently reported 100% sensitivity for typical PTC, but the sensitivity for a follicular variant of PTC was found to be 83%.

This is the first study focused on galectin-3 immunohistochemical expression in PTC in relation to different histologic growth patterns. Thus,
we observed that galectin-3 expression varies in relation to this parameter. In fact, our results clearly show that galectin-3 is immunohistochemically expressed at the highest levels in classical PTC with a papillary architecture, whereas its expression is slightly decreased in the follicular variant of PTC and further decreased in the follicular variant of PTC including solid growth pattern areas. Thus, although the sensitivity of galectin-3 immunostaining for the classical and the pure follicular variant of PTC was mainly similar to the values reported in previous studies, the number of false-negative results in FV/SOLID PTC variants included in this study pointed to unsatisfactory sensitivity of galectin-3 immunostaining in confirming the conventional diagnosis of PTC.

In this study, because the relatively large series of PTC cases was collected together with the clinical data, we also examined the association of galectin-3 expression with LNM, EI, and tumor growth. Although all the PTC cases involving LNM and most cases presented with EI (91.4%) were galectin-3 positive in this study, we concluded that galectin-3 immunohistochemical expression itself is not an indicator of local metastatic spread or EI of PTC, because galectin-3 immunopositivity was found in a larger proportion of cases without LNM or EI involvement. Thus, from the results of this study, we cannot completely confirm the finding of Kawachi et al., who reported that primary PTC tumors involving LNM contained significantly higher concentrations of galectin-3 than tumors without metastases. In certain types of human tumors, such as head and neck, gastric, and colon cancers, the level of galectin-3 expression has been positively correlated with tumor progression and acquisition of metastatic capabilities, whereas in contrast, in some tumors, such as breast, ovarian, and prostate cancer, the expression of galectin-3 is inversely correlated with metastatic potential (reviewed by Takenaka et al11). From the results of this study, it could be suggested that galectin-3 immunohistochemical expression is not related to aggressiveness (local metastatic spread or EI) of PTC.

The suggestion for a possible role of galectin-3 in metastatic processes is based on its participation in cell adhesion,5,6,11 which requires galectin-3 to be expressed on the cell surface. However, as shown in this and previous studies, galectin-3 localization in thyroid tumor cells is dominantly cytoplasmic, and only occasionally membranous or nuclear, suggesting other roles for galectin-3 in thyroid tumor biology, such as proapoptotic activity or regulation of the cell cycle (proliferative activity). In this work, galectin-3 expression was not found to be related to the growth of intrathyroid PTC, indicating that this protein probably does not contribute to the proliferative capacity of PTC. Further efforts using methods of cell biology are required to explain the exact function of galectin-3 in malignantly transformed thyroid cells.

In summary, from the aspect of clinical practice, our results could be of importance, because they indicate that galectin-3 immunohistochemistry is an excellent marker for the classical papilliform variant of PTC, but not for the unconventional cases of PTC, which are a diagnostic problem. In our opinion, galectin-3 immunohistochemistry need not be used for easily diagnosed cases of thyroid tumors (such as classical PTC with papillary architecture, which is easily diagnosed by routine histopathologic analysis) but primarily for doubtful cases as an aid to conventional histologic diagnostics.

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REFERENCES
Galectin-3 in Papillary Thyroid Carcinoma

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