Differential expression of galectin-3 in papillary projections of malignant and non-malignant hyperplastic thyroid lesions

D. Cvejic, S. Savin, I. Petrovic, I. Paunovic, S. Tatic, M. Havelka

1 Institute for the Application of Nuclear Energy, University of Belgrade, Zemun - Belgrade
2 Center for Endocrine Surgery, Institute of Endocrinology, Diabetes and Diseases of Metabolism, Clinical Center of Serbia, Belgrade

**Rationale**

Galectin-3 is a beta-galactoside binding protein recently proposed to be a promising presurgical molecular marker for distinguishing benign from malignant thyroid neoplasms. We analyzed galectin-3 expression immunohistochemically in papillary areas of hyperplastic lesions of benign thyroid tissue in comparison with malignant papillary projections of papillary thyroid carcinoma (PTC). A monoclonal antibody to galectin-3 and ABC immunohistochemical technique were used to evaluate galectin-3 expression in 26 cases of benign papillary hyperplasia (8 cases of hyperplastic adenoma, 8 cases of hyperplastic colloid goiter, 10 cases of Graves disease) in comparison with 25 cases of PTC. Immunohistochemical results showed no reactivity for galectin-3 in papillary areas of benign hyperplastic lesions. Strong cytoplasmic galectin-3 immunoreactivity was found in all 25 cases of PTC. These results show that galectin-3 expression is a feature of malignant papillary projections but not of benign papillary hyperplasia. Thus, the immunohistochemical evaluation of galectin-3 might contribute to differential diagnosis between malignant and benign thyroid lesions with papillary projections.

Key words: galectin-3, papillary thyroid carcinoma, benign papillary hyperplasia, presurgical tumor marker, immunohistochemistry

**Introduction**

Lectins, or carbohydrate binding proteins, recognize specific oligosaccharide structures on glycoproteins and glycolipids. Galectins are a lectin family defined by two properties: affinity for beta-galactosides and significant sequence homology in the carbohydrate binding site. To date, fourteen different galectins have been characterized and numbered according to the chronology of their discovery. These lectins are found both in the extracellular compartment (on the cell surface and within the extracellular matrix) and in the intracellular compartment (in the cytoplasm and in the cell nucleus), which suggests multifunctionality of these molecules.

The most extensively studied galectin is galectin-3 (Mr 29-31 KDa). This lectin has been implicated in various biological and pathophysiological processes such as differentiation, growth regulation, adhesive interactions, apoptosis, RNA splicing, modulation of the immune response, neoplastic transformation and invasiveness.

Clinical and experimental evidence appears to support a correlation between galectin expression and tumorigenesis. Thus, galectin expression has been shown to correlate with neoplastic transformation and tumor progression in some types of cells (reviewed in ref. 10).

An initial study on galectin-1 and galectin-3 expression in surgical specimens of thyroid tissue demonstrated increased expression of both galectins in all types of thyroid malignant neoplasms of epithelial origin, while neither benign thyroid adenomas nor normal thyroid tissue expressed galectin-1 or galectin-3. These results suggested that these galectins were associated with malignant transformation of thyroid epithelium and might serve as markers for distinguishing benign from malignant thyroid lesions.

Further studies on thyroid neoplasia have been focused on galectin-3, which appears to be a promising presurgical marker of thyroid malignancy. Furthermore, all these studies showed that among thyroid malignant tumors, papillary carcinoma of the thyroid (PTC), either of papillary or follicular architecture, displayed an intense and consistent expression of galectin-3, but especially strong intensity was found in papillary projections of PTC. However, papillary projections may be encountered in benign papillary hyperplasia (multinodular goiter, follicular benign neoplasms, Hashimoto thyroiditis, Graves disease).
The following question was not precisely addressed in all previous studies, but was considered to be interesting for future investigation (15,18): whether strong galectin-3 expression is an important hallmark of malignant papillary projections of PTC or papillary projections in thyroid hyperplastic non-malignant conditions also express galectin-3.

Thus, the aim of this study was to investigate galectin-3 expression immunohistochemically in papillary areas of hyperplastic non-malignant thyroid lesions in comparison with the expression of this lectin in malignant papilae of papillary thyroid carcinoma.

MATERIALS AND METHODS

Tissue samples

Formalin-fixed paraffin-embedded tissues of 26 cases of hyperplastic non-malignant thyroid lesions showing papillary projections (including 8 cases of hyperplastic adenoma, 8 cases of colloid hyperplastic goiter and 10 cases of Graves disease) and 25 cases of classical papillary thyroid carcinoma were obtained from the archival material of the Institute of Endocrinology, Diabetes and Diseases of Metabolism, KCS, Belgrade. The selection of material was based primarily on the prior diagnosis made by routine histopathological analysis (22).

Immunohistochemistry

A rat monoclonal antibody M3/38 (ATCC TIB-166) against a mouse macrophage cell surface antigen identical to galectin-3 (23) was kindly provided by Dr. M. E. Hufbauer, La Jolla Institute for Allergy and Immunology, San Diego, CA. Immunostaining was performed on 4-6 μm thick sections using the avidin-biotin peroxidase complex (ABC) technique (24) with reagents supplied by Vector Laboratories (Burlingame, CA). Following deparaffinization and rehydration, endogenous peroxidase activity was blocked with 0.3% H2O2/methanol followed by non-immune horse serum for 20 min to block non-specific 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 IgG (which also cross-reacts with the primary rat antibody) for 30 min and thereafter with avidin-biotin-peroxidase complex (ABC reagents) for 30 min. 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, coverslipped and examined using a Reichart-Jung microscope supplied with a Photostar automatic camera system. Controls were incubated with PBS in place of the primary antibody and no positive staining was observed.

RESULTS

The results of immunohistochemical staining for galectin-3 in papillary projections of non-malignant and malignant (PTC) thyroid tissue are presented in Table 1.

<table>
<thead>
<tr>
<th>Thyroid tissue</th>
<th>Total No of cases</th>
<th>Positive staining for galectin-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperplastic adenoma</td>
<td>8</td>
<td>0.8</td>
</tr>
<tr>
<td>Hyperplastic colloid goiter</td>
<td>8</td>
<td>0.8</td>
</tr>
<tr>
<td>Graves disease</td>
<td>10</td>
<td>0.10</td>
</tr>
<tr>
<td>Papillary carcinoma</td>
<td>25</td>
<td>25.25</td>
</tr>
</tbody>
</table>

In all specimens of non-malignant thyroid tissue (including 8 cases of hyperplastic adenoma, 8 cases of hyperplastic colloid goiter and 10 cases of Graves disease) the thyroid epithelium was negative for galectin-3 staining throughout the tissue sections, including the hyperplastic areas with papillary projections. Some positivity was found in endothelial and muscle cells of vessels, in nerve fibers and also in polymorphonuclear inflammatory cells. Thus, galectin-3 was not expressed in the follicular epithelium of benign papillary hyperplasia.

In all twenty five cases of papillary thyroid carcinoma intense and diffuse cytoplasmic staining for galectin-3 was found in malignant epithelial cells covering papillae. The cell membrane of some cells was also stained and scattered nuclei were found to be positive.

Malignant epithelial cells of the remaining follicles also expressed galectin-3.

DISCUSSION

In recent years several researchers have proposed galectin-3, a beta-galactoside binding protein, as a potential presurgical marker for distinguishing malignant from benign follicular thyroid lesions (11-21). However, in some of these studies, galectin-3 expression was also found in a small proportion of follicular adenomas (13,14,20,21). Such findings indicated that the use of galectin-3 immunodetection as a molecular marker for thyroid follicular carcinoma must be interpreted with caution. Although this issue is still debatable, it is tempting to postulate that follicular adenomas with positive expression of galectin-3 could be considered to be potential early cancer.

On the other hand, all these studies agree that among the thyroid neoplasms galectin-3 expression is the most prominent and consistent in papillary thyroid carcinoma (PTC). Papillary thyroid carcinoma is the most common malignant thyroid tumor. It is characterized by slow growth and long term survival, even if it is associated with metastases in the cervical lymph nodes. Despite that, some patients die from PTC. The diagnosis of PTC is based on the clas-
sical histocytological features: fine papillae covered by overlapping ground glass nuclei with or without psammoma bodies (25-29). However, papillary architecture and ground glass nuclei may be encountered in hyperplastic areas of follicular neoplasms, multinodular goiter, diffuse hyperplasia, Graves disease, Hashimoto thyroiditis and thyroiditis.

In this study we examined whether strong galectin-3 expression is a feature of malignant papillary projections of PTC or this galectin is also expressed in papillae of non-malignant hyperplastic thyroid lesions. We found that galectin-3 is uniformly and strongly expressed in papillary projections of PTC, while it is not expressed in papillae of non-malignant thyroid lesions, such as hyperplastic adenomas, hyperplastic goiter or Graves disease.

CONCLUSIONS

These results further support the statement that galectin-3 expression in thyroid tissue is a feature of malignancy. In addition, although the distinction of PTC from benign papillary hyperplasia can usually be made by routine (H-E) staining, the immunohistochemical evaluation of galectin-3 may contribute to the differential diagnosis in doubtful cases.

REZIME

RAZLIČITA EKSPRESIJA GALEKTINA-3 U PAPILARNIM PROJEKCIJAMA MALNIGNIH I NE-MALNIGNIH HIPERPLASTI NA NH TIREOIDNE LEZIJA

Galektin-3, protein koji vezuje beta-galaktozide, smatra se perspektivnim molekularnim markerom u razlikovanju benignih i malignih tireoidnih tumora. U ovom radu analizirali smo imunohistokemijski ekspresiju galektina-3 u papilarnim regijama hiperplastičnih lezija benignog tireoidnog tkiva u poređenju sa malignim papilarnim projekcijama u papilarnom tireoidnom karcinomu (PTC). Monoklonalno antitelo na galektin-3 i ABC histokemijska metoda korišćeni su za ispitivanje ekspresije galektina-3 u 26 slučajeva benigne papilarno hiperplazije (8 slučajeva hiperplasti nog adenoma, 8 slučajeva hiperplastične koloidi drume i 10 slučajeva Gravesove bolesti) u poređenju sa 25 slučajeva PTC. Rezultati imunohistokemijskog bojenja pokazali su odsustvo reaktivnosti na galektin-3 u papilarnim zonama benignih hiperplazija. U svih 25 slučajeva PTC nadjeno je intenzivno citoplazmatsko bojenje na galektin-3. Ovi rezultati pokazuju da je ekspresija galektina-3 karakteristika malignih papilarnih projekcija, ali i benignih papilarnih hiperplazija, tako da se imunohistokijska analiza galektina-3 mogla biti od koristi u njihovoj diferencijalnoj dijagnostici.

 Ključne reči: papilarni tireoidni karcinom, galektin-3, benigna papilarna hiperplazija, tumorski marker, imunohistohemija

REFERENCES


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