



Galectin-3 expression in medullary thyroid carcinoma in relation to tumor progression

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BACKGROUND: *Galectin-3, a lectin with specificity for beta galactosides, is believed to be implicated in multiple biological processes through interactions with complementary glycoconjugates. Alterations in galectin-3 expression are observed in a variety of human tumors. In thyroid, this lectin has been found to be highly expressed in malignancies of epithelial origin. We analyzed galectin-3 expression in medullary thyroid carcinoma (MTC).*

MATERIALS AND METHODS: *An immunohistochemical study using monoclonal antibody was performed on paraffin sections of twenty cases of sporadic MTC, comprising ten cases without and ten cases with lymph node metastases at the time of surgery.*

RESULTS: *Positive cytoplasmic staining for galectin-3 was found in 16/20 cases, but varied in intensity and distribution from weak/focal (7/16) to moderate (7/16) or strong (2/16). Advanced stage of MTC (with lymph node metastases at the time of surgery) showed moderate to strong galectin-3 expression more frequently (8/10) than cases without lymph node metastases (1/10).*

CONCLUSION: *These findings suggest that galectin-3 expression is associated with the advanced stage of disease and that this lectin might play a role in the pathobiology of MTC.*

KEY WORDS: *Thyroid Neoplasms; Lectins; Immunohistochemistry, Carcinoma, Medullary; Tumor Markers, Biological*

INTRODUCTION

Galectins are a family of lectins (carbohydrate binding proteins) defined by two properties: affinity for beta galactosides and significant sequence homology in the carbohydrate binding site (1,2). Galectin-3, a ~30kDa protein, is the most extensively studied member of the family. This lectin is expressed in various kinds of cells, localized in intracellular compartment (in the cytoplasm and nucleus), and in extracellular compartment (on the cell surface and within the extracellular matrix) (3,4). It is assumed that through binding to complementary glycoconjugates galectin-3 plays important roles in various biological and pathological processes such as adhesion (5), cell growth and differen-

tiation (6,7), apoptosis (6), immunomodulation (8), neoplastic transformation, and tumor progression (9-11).

Alterations in galectin-3 expression were observed in tumor cell lines and in spontaneous human tumors including colon, breast, gastric and skin carcinomas, lymphomas and glioblastomas (reviewed recently in ref. 11). In some of these tumors galectin-3 expression correlated with tumor progression and acquisition of metastatic capabilities. However, the exact role of galectin-3 in neoplastic transformation is still unknown.

Recent studies on galectin-3 expression in human thyroid tissue (12-22) demonstrated galectin-3 up-regulation in thyroid carcinomas, while its expression in normal and benign thyroid tissue was absent or weak. These studies were mainly focused on thyroid malignancies originating from thyroid follicular epithelium (papillary, follicular and anaplastic carcinomas), giving only limited information about galectin-3 expression in medullary thyroid carcinoma (MTC).

MTC originates from calcitonin producing parafollicular (C) cells of the thyroid and accounts for 5-10% of all thyroid cancers. Sporadic forms of MTC comprise about 80% of these carcino-

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mas, whereas about 20% has a familial basis. MTC shows unique biological and genetic features, which differ from those of thyroid carcinomas of follicular cell origin. Its prognosis is still debatable due to the wide variation in the course of the disease (22-25).

Thus, the aim of this study was to analyze immunohistochemically the expression of galectin-3 in a series of 20 cases of sporadic MTC considering clinicopathological data at the time of surgery.

MATERIALS AND METHODS

Tissue samples

Formalin-fixed and paraffin-embedded tissues of twenty cases of sporadic medullary thyroid carcinoma were obtained from the archival material of the Institute of Endocrinology, Diabetes and Diseases of Metabolism, Clinical Centre of Serbia, Belgrade. The selection of material was primarily based on the prior diagnosis made by routine histopathological analysis (26) and positive calcitonin staining.

Immunohistochemistry

A rat monoclonal antibody M3/38 (ATCC TIB-166) against mouse macrophage cell surface antigen Mac-2, which is identical to galectin-3 (27), was kindly provided by Dr M. E. Huflejt, La Jolla Institute for Allergy and Immunology, San Diego, California.

Immunostaining was performed on 4-6 µm thick sections using the avidin-biotin peroxidase complex (ABC) technique (28) with reagents supplied by Vector Laboratories (Burlingame, CA). Following deparaffination 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 the primary antibody (anti-galectin-3, at a dilution of 1/200) at 4 °C overnight. This was followed by incubation with horse anti - mouse biotinylated IgG (which also cross-reacts with the primary rat antibody) for 30 min and thereafter with streptavidin-biotin-peroxidase complex (ABC reagents) for 30 minutes. After each step the sections were washed three times in phosphate buffered saline (PBS). The reaction was visualized using diaminobenzidine tetrahydrochloride (DAB) solution.

After counterstaining with hematoxylin, slides were dehydrated, coverslipped and examined using a Reichert -Jung microscope supplied with a Photostar automatic camera system.

For each case, the primary antibody was replaced with PBS, as a negative control, and no positive staining was observed.

Staining was scored as follows: (-) = no staining, (+/-) = very weak or very focal staining, (+) = moderate staining in the majority of cells, and (++) = strong staining in the majority of cells.

RESULTS

The results of the immunohistochemical analysis of galectin-3 expression in relation to the histopathological appearance of the tumor and clinical data available at the moment of surgery are presented in Table 1.

Table 1. Clinical data (age, sex, pTNM) and histological pattern for twenty cases of sporadic MTC in relation to immunohistochemical staining for galectin-3

Case No.	Age (Year)	Sex	Histological pattern (Type of cells)	Tumor stage* (pTNM)	Immunostaining results** Gal-3
1.	46	F	polygonal	T ₂ N ₀ M ₀	-
2.	55	F	polygonal	T ₂ N ₀ M ₀	+/-
3.	39	F	polygonal / spindle	T ₃ N ₀ M ₀	-
4.	51	F	polygonal	T ₃ N ₀ M ₀	-
5.	40	F	polygonal / spindle	T ₃ N ₀ M ₀	+
6.	74	F	polygonal / spindle	T ₃ N ₀ M ₀	+/-
7.	66	F	polygonal	T ₃ N ₀ M ₀	+/-
8.	55	F	polygonal	T ₃ N ₀ M ₀	-
9.	53	F	polygonal	T ₂ N ₀ M ₀	+/-
10.	70	F	polygonal	T ₂ N ₀ M ₀	+/-
11.	21	F	polygonal / spindle	T ₃ N ₁ M ₀	+
12.	67	F	polygonal	T ₄ N ₁ M ₀	+
13.	64	M	polygonal / spindle	T ₄ N ₁ M ₀	+
14.	30	M	polygonal	T ₄ N ₁ M ₀	++
15.	68	F	polygonal / spindle	T ₄ N ₁ M ₀	+/-
16.	42	F	polygonal	T ₄ N ₁ M ₀	+
17.	34	F	polygonal	T ₄ N ₁ M ₀	++
18.	63	F	polygonal	T ₃ N ₁ M ₀	+
19.	47	F	polygonal / spindle	T ₄ N ₁ M ₀	+
20.	41	F	polygonal / spindle	T ₄ N ₁ M ₀	+/-

* According to Hermanek and Sobin (29)

** - no staining

+/- very weak or very focal staining

+ moderate staining in the majority of cells

++ strong staining in the majority of cells

Some representative findings are shown in Figure 1 (A-D).

Light microscopy examination revealed that twelve out of the twenty investigated cases of MTC had round and polygonal tumor cells with round or oval hyperchromatic nuclei. Small areas with spindle-shaped cells were seen in eight cases. All but three cases contained amyloid in variable amounts, from minimal to abundant. In ten cases lymph node metastases were present at the time of surgery.

Immunohistochemical staining using monoclonal antibody against galectin-3 showed positive staining in sixteen out of twenty cases (80%), varying from weak/focal (7/16 cases; 44%) to moderate (7/16 cases; 44%) and strong reactivity in most tumor cells (2/16 cases; 12%). Galectin-3 was localized in the cytoplasm of malignant cells. No relation was seen between histological parameters (type of cells, amount of amyloid) and galectin-3 expression. However, when galectin-3 expression was analyzed in relation to tumor stage it was observed that the advanced stage of MTC (with lymph node metastases at the time of surgery, pN1) showed moderate to strong (+ and ++) galectin-3 expression more frequently (8 out of 10 cases, 80 %) than cases without lymph node metastases (1 out of 10 cases, 10 %), i.e. there was a visible trend towards higher galectin-3 expression from stage II (intrathyroid) to stage III (with regional lymph node metastases).

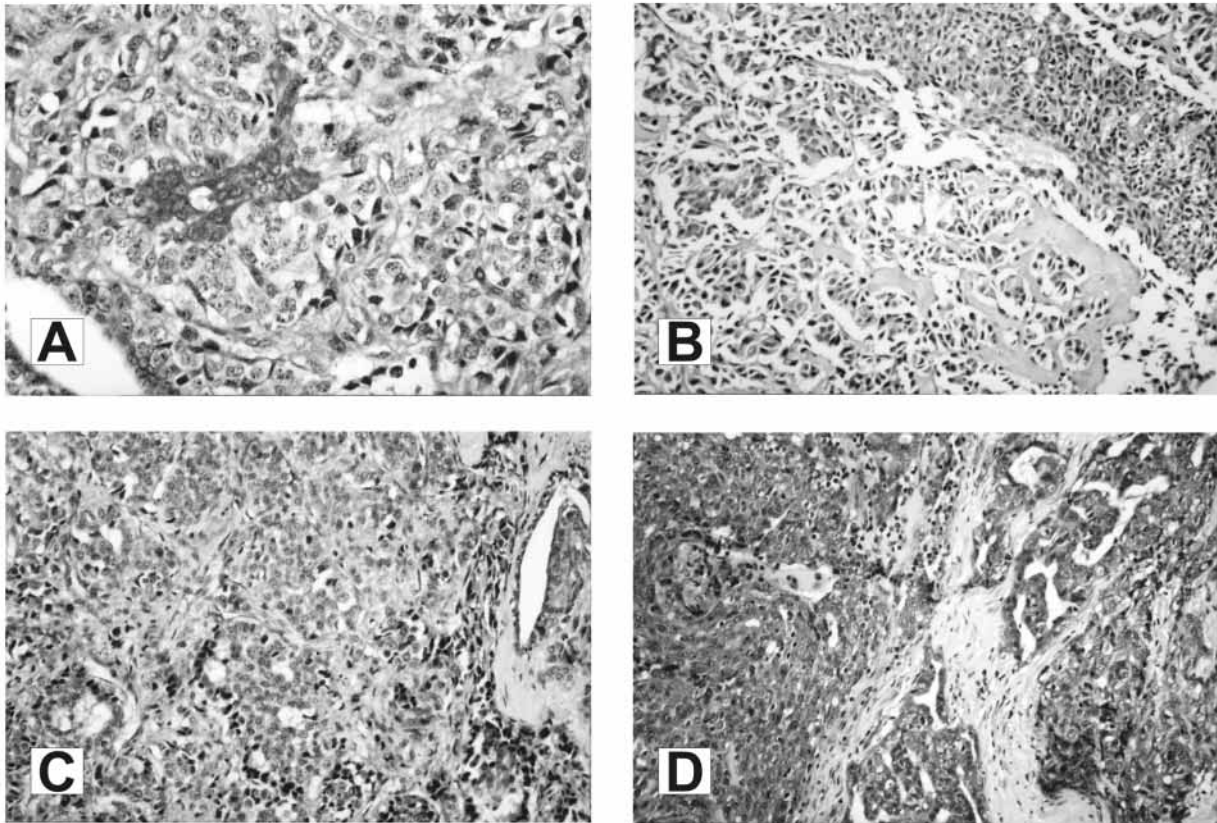


Figure 1. Immunohistochemical staining for galectin-3 in medullary thyroid carcinoma. Weak/ focal staining for galectin-3 in MTC cases No 2 (A) and No 6 (B) without lymph node metastases at the time of surgery (pNo). Moderate or strong galectin-3 immunostaining in MTC cases No 14 (C) and No 17 (D) with lymph node metastases at the time of surgery (pN1). Original magnifications x 40 (A) and x 20 (B, C, D).

DISCUSSION

In the latest years the diagnostic value of galectin-3 immunodetection in thyroid neoplasms has been widely discussed. Several groups of researches have identified galectin-3 as a promising presurgical marker for distinguishing benign from malignant follicular thyroid tumors (12,13,15,17-19). However, variable staining deviating from the general pattern in follicular adenomas and follicular carcinomas deserve attention for reaching a general conclusion (14,16,20,21).

All of these studies were focused on thyroid tumors originating from thyroid epithelium while in some of them only limited information about heterogeneous galectin-3 expression in medullary thyroid carcinoma was given.

In this immunohistochemical study we analyzed galectin-3 expression in MTC in relation to some clinicopathological data at the time of surgery.

Thus, we found that most of the twenty cases of sporadic MTC examined (16/20, i.e. 80%) expressed galectin-3 at the protein level. This indicates that the galectin-3 gene is up-regulated during neoplastic transformation of parafollicular thyroid cells, as has been reported already for follicular thyroid cells (12-21).

Galectin-3 immunostaining pattern showed great variability in the degree and intensity both within and between analyzed specimens and seemed to be independent of histopathological appearance of tumor tissue. Cytoplasmic localization of galectin-3 in malignant cells indicates that the role of galectin-3 in this kind of tumor is more likely to involve a mechanism in the intracellular compartment than on the cell surface. The pathological significance of galectin-3 gene up-regulation in tumor cells and the mechanisms of galectin-3 regulation are still not understood.

The most interesting observation was that moderate to strong galectin-3 expression was mainly associated with advanced stage of disease (stage III), suggesting that galectin-3 might be an indicator of lymph node spread. However, definitive conclusions about galectin-3 clinical implication in MTC prognosis must await the collection of data from a large number of clinically correlated specimens.

CONCLUSION

Since galectin-3 expression seems to increase with MTC progression from stage II (intrathyroid) to stage III (with regional lymph node metastases), it could be suggested that galectin-3 expression is associated with advanced stage of disease and that this lectin might play a role in the pathobiology of MTC.

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