EXPERIMENTAL STUDY

The investigation of galectin-3 in diseases of the thyroid gland

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Abstract

Objective: Malignant tumors of the thyroid gland exhibit a variety of histopathologies and clinical behavior. Immune markers are gaining more and more importance in diagnostic pathology, especially in the differential diagnostics and in the grading of thyroid gland tumors.

Design: The authors investigated the immunohistochemical reaction of galectin-3 (gal3) in patients with various thyroid gland diseases. They tested the diagnostic value of gal3 in determining the benign or malignant nature of various lesions, especially in lesions of follicular origin, because previous results have indicated nearly 100% specificity and sensitivity in this regard.

Methods: Gal3 immunoperoxidase reaction was carried out on 91 sections of thyroid gland samples fixed in formalin and embedded in paraffin.

Results: While gal3 expressed itself strongly and diffusely in papillary carcinomas (19 of 20 cases), in other malignant lesions it showed weaker, focal or variable positivity. Focal positivity was found in four of 19 follicular adenomas, and negativity was found in three of 10 follicular carcinomas. In all cases of inflammation a focal positivity was observed (eight of eight cases). All nodular goiter and normal thyroid tissue were negative (25 of 25 cases).

Conclusions: Based on our results, the gal3 immunohistochemical reaction seems to be reliable in the diagnosis of papillary carcinomas. However, in the case of solitary thyroid nodules and follicular lesions, although it is still a useful supplementary marker, it is not of absolute value (as stated in previous studies) in determining whether a tumor is benign or malignant.

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Introduction

Galectin-3 (gal3) is a beta-galactosidase-binding polypeptide (31 kDa molecular weight), a member of the lectin family. It seems to play an important role in a number of biological and pathological processes. It is a regulating component of the cell cycle (an apoptosis inhibitor, it stimulates cell proliferation in which process gal3 shows an increased expression and nuclear localization) (1–5), it regulates cell–cell and cell–matrix interaction, adhesion (major non-integrin laminin binding protein) (2, 5–7) and migration, it has a role in inflammation (8), neoplastic transformation (9), metastatization and in reparation of the damaged cell. Gal3 is expressed in various tissues and cell types (e.g. epithelial cells, cells of the immune system) in which it is localized in the nucleus and/or cytoplasm, the cell surface or in the matrix around the cell. Based on recent investigations, gal3 expression correlates with the invasive and metastatic potential of various tumors (10). As an adhesive molecule, it plays a role in the various stages of embryogenesis (11, 12) and tumor progression. In this way, for example in colorectal and breast cancer, the

down-regulation of gal3 is likely to make the interaction of tumor cells and laminin possible, thereby promoting invasion and metastatization (13–15). Gal3 has also been detected in anaplastic large cell lymphomas in which it can provide help in their characterization (16). Finally, based on the results described in recent publications benign and malignant thyroid neoplasms as well as malignant tumors and other tumor-like lesions (17–19) are more likely to be differentiated if simultaneous examination of CD-44v6 and/or cytokeratin 19 (CK-19) markers (20, 21) is undertaken. Some authors in their prospective analysis proved that gal3 immunodetection of thyroid lesions concerning preoperative diagnostics had about 100% accuracy in differentiating benign and malignant lesions (22). In their study, Bartolazzi *et al.* found that the sensitivity and specificity in fine needle aspiration biopsy smear (FNAB-smear) were over 99% and 98% respectively. Prediagnostic value and diagnostic accuracy were 92% and 99% respectively (22).

In order to confirm these findings (and to test whether it is valid for use with histology) we investigated retrospectively 91 thyroid lesions with the gal3 reaction, using paraffin sections.

Materials and methods

We carried out the gal3 immunoperoxidase reaction on 91 sections of thyroid gland samples fixed in formalin and embedded in paraffin. The histopathological classification of the lesions was as follows: 20 papillary carcinomas, 19 follicular adenomas, 10 follicular carcinomas, five medullary carcinomas, one mucoepidermoid carcinoma, seven Hashimoto's thyroiditis, one deQuervain thyroiditis, three pseudo-papillary hyperplasias and 25 nodular goiters in connection with normal thyroid tissue. In setting up the diagnosis of the aforesaid lesions, we put special emphasis among the basic histomorphological criteria on the following: in cases of follicular carcinomas, the simultaneous vascular and capsular invasion was the absolute criterion, capsular invasion alone was not accepted. In cases of papillary carcinomas, nuclear overlapping, optically clear (ground glass) nuclei, intranuclear pseudoinclusions and grooves as well as the characteristic structure such as papillary grounds and psammoma bodies were considered to be diagnostic – with the exception of the follicular variant.

After deparaffining the representative sections, a citric microwave antigen exploration followed at 1000 W for 30 min using DAKO (Denmark A/S, Glostrup, Denmark) target retrieval solution in a 1:10 dilution was used. Endogenous peroxidase activity was inhibited by immersing the sections in 3% hydrogen peroxidase for 10 min. Then by means of indirect avidin–biotin immunoperoxidase techniques (Vectastain ABC kit) the gal3 reaction was carried out (Ventana Nexes, Ventana Medical Systems, Inc., AZ, USA, fully automated immunostaining system), following the instructions of Novocastra Laboratories Ltd. Each section was treated with a 1:50 dilution of purified monoclonal primary mouse antibody to gal3 (Novocastra Laboratories Ltd, Newcastle upon Tyne, UK, 9C4 clone, IgG1 subclass) for 30 min. The visualization of enzyme activity was carried out by AEC (amino-ethyl-carbasol). Negative controls of all cases were performed by omitting the primary gal3 antibody. No positive staining was observed at all in the controls.

The following criteria were used for the interpretation of the results: negative, no reaction or scattered cells showing nuclear reaction; focal positivity, < 20% cytoplasmic (and nuclear) reaction; positive, > 20% cytoplasmic (and nuclear) reaction.

Results

A summary of the results is shown in Table 1.

All papillary carcinomas showed definitive, strong positivity (Figs 1 and 2) with one exception where only focal positivity was observed (sensitivity: 100%). The reaction was stronger in the classical papillary thyroid gland carcinomas than in the follicular variant

Table 1 Number of cases with positive, focally positive and negative gal3 immunohistochemical reactions in 91 thyroid gland samples from different thyroid diseases.

Thyroid disease	Total no. of cases	No. of cases		
		Positive	Focally positive	Negative
Papillary carcinoma	20	19	1	0
Follicular adenoma	19	0	4	15
Follicular carcinoma	10	2	5	3
Medullary carcinoma	5	3	1	1
Mucoepidermoid carcinoma	1	0	1	0
Hashimoto's thyroiditis	7	0	7	0
DeQuervain thyroiditis	1	0	1	0
Pseudo-papillary hyperplasia	3	0	0	3
Normal + nodular goiter	25	0	0	25

Figure 1 Intensive cytoplasmic immunoreactivity of gal3, partly appearing also in the cell nucleus, in a classical papillary carcinoma (magnification $\times 200$).

Figure 2 Note the strongly positive gal3 reaction at the edge of the invasive papillary carcinoma (magnification $\times 100$).

of papillary carcinoma and in the Hurthle-cell (oxyphil, follicular) carcinoma. Two of ten follicular carcinomas were positive for gal3, five of them showed focal reactivity. We found three gal3-negative follicular carcinomas (30% false negativity). Among the 19 follicular adenomas, we found focal positivity in four cases (Fig. 3) (21% false positivity), the remaining 15 were negative. Concerning follicular neoplasm, the sensitivity was 70% and the specificity was 78.9%. Of the five medullary carcinomas, three were positive, one focally positive and one negative. The only mucopidermoid carcinoma showed focal positivity.

The expressed gal3 was found mostly in the cytoplasm; nuclear positivity was observed in only a few cells. These cells proved to be positive also with the Ki-67 proliferation marker. In the thyroid stroma, macrophages, polymorphonuclear inflammatory cells,

follicular dendritic reticulum cells, fibroblasts, and smooth muscle cells all equally show gal3 expression.

In our material, we found focal positivity in each of the inflammatory lesions (seven Hashimoto's and one deQuervain-thyroiditis). In Hashimoto's thyroiditis we observed definitive gal3 expression in follicular cells showing oncocyctic changes and localized around a lymphoid follicle (Fig. 4). The follicular cells entrapped in areas of great chronic lymphocyte inflammation were also positive. The gal3 positivity of the non-tumorous thyrocytes in the inflamed area was seen in both benign and malignant tumors.

Normal thyroid tissue (even that next to a tumor), as well as the 25 nodular goiters and each of the three pseudo-papillary hyperplasias were negative (specificity of papillary carcinoma versus pseudo-papillary hyperplasia: 100%). In cystic goiters, however, weak and membranous positivity was sporadically observed in a part of the foamy cells (macrophages).

Figure 3 Focal cytoplasmic gal3 positivity in follicular adenoma (magnification $\times 400$).

Discussion

Regarding papillary carcinomas, our results are identical with data found in the literature. All tumors (including the follicular variant of papillary carcinoma) showed a strong reactivity for gal3, with the exception of a single case (which was only focally positive). According to previous publications, follicular adenoma and minimally invasive follicular carcinoma show a significant difference in the cytoplasmic expression of gal3 both in histological and cytological samples (23). Cytoplasmic expression is the sign of a possible malignant transformation (while nuclear localization is connected only with cell proliferation) (1). The focal positivity of some follicular and Hurthle-cell adenomas can predict that these benign lesions may later become malignant

Figure 4 Hurthle-cells showing intensive gal3 positivity in Hashimoto's thyroiditis (magnification $\times 400$).

(24). Several authors emphasize that the gal3-positive and morphologically suspect follicular adenomas (with cellular atypia) can be considered, potentially, as early carcinomas, in which capsular and vascular invasion cannot be observed as yet, but transformation at the molecular level has already occurred (22, 25).

According to Kawachi *et al.* (26), gal3 down-regulation in papillary thyroid carcinoma may promote the release of some tumor cells from the primary tumor, resulting in metastasis. In our single case of known metastatic papillary carcinoma we found strong gal3 positivity.

The immunohistochemical examination and localization of gal3 may be a useful aid in the differential diagnosis of solitary thyroid gland lesions – it can serve as a marker in recognizing follicular carcinoma, especially the minimally invasive form. In previous studies, variable expression of gal3 was described in medullary carcinoma (27).

Gal3 is also expressed in places where the follicular cells are in a highly inflamed area: both in cells without cytological atypia or with Hurthle-cell transformation. The most likely explanation for the gal3 expression of non-neoplastic follicular cells in an inflamed area are neosynthesis effected by cytokines secreted by inflammatory cells or the simple permeation of gal3 abundantly shed by lymphocytes into the neighboring follicular cells (28).

The question may be asked whether the gal3-positive cells in Hashimoto's thyroiditis are cells with possibly early neoplastic transformation?

Due to the benign feature of follicular cells, the gal3 positivity in inflammatory lesions will not create any diagnostic problem; however, it may be problematic in cytological smears especially if the inflammatory elements are not prominent.

Since cytology in itself is not a reliable method to distinguish between malignant and benign forms of follicular tumors, these patients have to undergo surgery, although less than 20% of these lesions are malignant. This is why a reliable marker is necessary by which the malignantly transformed thyrocytes can be identified preoperatively. With regard to papillary carcinomas, gal3 immunohistochemistry can provide a sensitive and reliable approach in the pre-operative diagnosis by FNAB (18, 29), although most forms of papillary carcinomas and inflammations can also be recognized with tolerable certainty by their characteristic morphological and/or cytological features. The two main problems are the differentiation of minimally invasive follicular carcinoma and follicular adenoma, and the identification of the follicular variant papillary carcinoma.

The application of the gal3 reaction can be of help (i) in recognizing the follicular variant of papillary carcinoma (because the strength and distribution of positivity differs from the follicular carcinoma), (ii) in the case of positivity, differentiating (mostly minimally

invasive) follicular carcinoma from follicular adenoma, and (iii) in preneoplastic cases, when no vascular or capsular invasion can be observed but a transformation at the molecular level can be considered.

Problems of interpretation can be caused by the focal positivity of inflammatory, cystic and other benign lesions such as nodular goiter (30, 31) and by false negativity in some cases of follicular carcinoma.

Connected with this, we wish to highlight three of our cases (follicular carcinomas) in which – in spite of the classical morphological criteria – gal3 was negative, although each of them were of the minimally invasive type and were well differentiated. (A well-known fact in the literature is that poorly-differentiated follicular carcinoma subtypes show a weaker gal3 expression or have absolutely no expression compared with the well-differentiated carcinomas. The anaplastic carcinoma is usually negative (32).) In these three cases we cannot give an acceptable explanation for the gal3 negativity. This result emphasizes that to make the distinction between follicular adenomas and carcinomas based on gal3 positivity may be very dangerous. It is very likely that other authors have also met similar doubts because in their studies they completed the gal3 examination with other markers simultaneously, such as CD44v6 and CK 19 (20, 21). This combination increases the sensitivity and specificity in deciding whether the lesion is benign or malignant. The expression of cell adhesion molecule-isoform – similar to gal3 – is down-regulated during the malignant transformation of epithelial cells. As regards thyroid gland tumor, CK 19 shows expression only in follicular carcinomas; therefore it can provide help in differentiating the follicular-variant papillary carcinoma from follicular adenomas and nodular hyperplasia. According to a recent study (33), serum gal3 increased significantly in patients with breast, gastrointestinal, lung and ovarian cancer, as well as in patients with melanoma and non-Hodgkin's lymphoma. These authors also reported that the level of serum gal3 of patients with metastatic disease was higher than in patients with a localized tumor. This proves that circulating gal3 may have a role in tumor progression and, based on this, assay of gal3 carried out in an early stage could predict the metastatic potential of the tumor.

In conclusion, although gal3 can be regarded as an important supplementary marker in the histological and cytological diagnosis of thyroid gland tumors, it cannot replace the conventional morphological and clinical examinations. Only the histological examination based on classical morphological criteria is able to give a satisfactory and final diagnosis in deciding the question of whether a lesion is benign or malignant. Because the specificity was 78.9% and the sensitivity was 70% concerning follicular neoplasm, it seems that gal3 analysis is not a reliable method to distinguish follicular adenoma from follicular carcinoma, especially on aspiration cytological material.

Based on our own study and findings we are of the opinion that the nearly 100% accuracy of gal3 immunohistochemistry which has been reported previously cannot be maintained in differentiating benign and malignant nodular thyroid gland lesions.

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