Evaluation of galectin-3, β-catenin, and nuclear morphometry in different thyroid lesions
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Purpose This study aimed to evaluate the expression pattern of galectin-3 and β-catenin and assess the nuclear morphometric features of different thyroid lesions in order to investigate their diagnostic significance.

Materials and methods Sixty specimens of benign and malignant thyroid lesions, in addition to 10 cases of normal thyroid tissue used as control, were immunohistochemically stained with antibodies against galectin-3 and β-catenin. Hematoxylin and eosin-stained slides were examined using image analyzer Proplus V software. Morphological parameters measured included mean nuclear diameter, mean nuclear perimeter, and mean nuclear area.

Results Cytoplasmic immunoreactivity for galectin-3 was strongest in papillary thyroid carcinomas (88.9%), whereas staining was less intense in the follicular variant of papillary thyroid carcinoma and in follicular (60 and 75%, respectively) and poorly differentiated carcinomas (28.6%); it was expressed focally and weakly in follicular adenoma cases and even more weakly in the reactive follicular epithelium of nodular goiter (P<0.5). Membrane β-catenin expression was decreased in only two of 13 (15.4%) adenomas and in 21 of 38 (55.3%) carcinomas (P<0.5). Among carcinomas, reduced membrane β-catenin was associated with progressive loss of tumor differentiation and increased invasiveness (P<0.5). The mean nuclear diameter and the mean nuclear perimeter were higher in undifferentiated carcinomas when compared with other subtypes and were the least for follicular neoplasms.

Conclusion Galectin-3 immunostaining and analysis of β-catenin dysregulation may be useful as an adjunct to distinguish benign from malignant thyroid lesions. Quantitative estimation of nuclear features can play a role in assessment of the morphological features and thus help in the diagnosis of thyroid lesions. Egypt J Pathol 33:129—138 © 2013 Egyptian Journal of Pathology.

Keywords: galectine-3, β-catenin, nuclear morphometry, thyroid lesions

Introduction Thyroid cancer is a common endocrine malignancy with an apparent increasing incidence. A survey sponsored by the WHO in 2010 revealed that there are around 44,670 new cases and 1,690 deaths caused by this disease every year (Jemal et al., 2010). Among thyroid malignancies, papillary thyroid carcinoma (PTC) is the most common malignant tumor, accounting for 80% of all thyroid cancers in the USA (Ringel and Ladenson, 2004; Erdem et al., 2011).

According to the Egyptian National Cancer Institute, malignant thyroid gland tumors constituted 65.31% of endocrine malignant tumors and the most common tumor was PTC, which constitutes 67.59% (Mokhtar et al., 2007).

For uncertain reasons, the incidence of thyroid cancer appears to be increasing, although the outcome remains excellent with long-term disease-free survival rates. Appropriate clinical management and prognosis largely depend on the diagnostic reliability of histopathological examination of surgically removed thyroid tissue (Ringel and Ladenson, 2004).

However, even with the application of the diagnostic criteria, such as characteristic nuclear appearances in PTC, distinguishing it from thyroid papillary hyperplasia is extremely challenging because of tumor heterogeneity. Occasionally, cases of papillary thyroid hyperplasia, in particular solitary nodules with papillary change, can simulate PTC and cause a diagnostic dilemma (Casey et al., 2003).

In addition, interobserver variations still lead to low diagnostic reproducibility, especially in the diagnosis of follicular carcinoma (FCA) (Hirokawa et al., 2002; Franc et al., 2003; De Matos et al., 2012; Papale et al., 2013). Also, the morphologic distinction between benign and malignant thyroid follicular lesions can sometimes be challenging; therefore, an immunohistochemical marker to aid in this distinction would be useful (Matsuo et al., 2004).

Galectins are structurally related proteins of the lectin family, defined by having at least one characteristic carbohydrate recognition domain with an affinity for β-galactosides (Barondes et al., 1994a, 1994b; Lee and Lee, 2013).

Fourteen different galectins have been characterized. Because of the potential of galectins to participate in cell–cell and cell–matrix adhesion, growth regulation, and internal processes, it is deduced that this galectin family is involved in pathological expression (Perillo et al., 1998; Hughes, 2001; Lee and Lee, 2013).

Among various galectins, Gal-1, Gal-3, and Gal-7 are of interest in thyroid malignancy (Danguy et al., 2002). It was mentioned that a combination of Gal-7 expression together with cytokeratin-19 markers has some important diagnostic value in distinguishing the encapsulated papillary thyroid...
carcinoma follicular variant (PTCFV) from microfollicular adenomas (Ronive et al., 2002). Gal-3 is a galactoside-binding protein that is expressed in many cell types (Liu and Rabinovich, 2005) and is found inside cells, extracellularly (but still cell-surface-associated), and in the circulation. Intracellular Gal-3 is an apoptosis inhibitor and mRNA splicing promoter, whereas extracellular cell-surface-associated Gal-3 acts as an adhesion molecule during cell–cell interactions and is associated with metastasis (Takenaka et al., 2004; Liu and Rabinovich, 2005).

β-Catenin is part of a membrane-bound cell growth-signaling complex that plays a role in cell adhesion, as well as in promotion of growth through activation of the Wnt-signaling pathway. Oncogenic signaling occurs when β-catenin is released, accumulates in the cytoplasm, translocates into the nucleus, and promotes transcription of genes including bcl-1 (cyclin D1) and c-myc, which induces cell proliferation (Rezk et al., 2004; Giunti et al., 2013).

β-Catenin dysregulation has been shown to play an important role in human tumorigenesis (Peifer and Polakis, 2000; Romitti et al., 2013). Activating β-catenin mutations were discovered in a variety of human neoplasms ranging from colonic adenocarcinoma, where they are present in approximately half of the tumors with wild-type adenomatous polyposis coli gene (Sparks et al., 1998), to malignant fibrous histiocytoma (Miller et al., 1999). Although a reduction in β-catenin bound to the cell surface has been demonstrated in thyroid carcinoma (Garcia-Rostan et al., 1999; Huang et al., 1999), the biological and clinical relevance of β-catenin dysregulation in thyroid neoplasia is primarily unknown.

Computerized morphometry is a scientific tool for evaluating cellular changes enhancing the interpretation of morphological features by the transformation of pathological changes in cells to a qualitative form (Wang et al., 2005). Nuclear morphometric features of malignant cells differ from those observed in nonmalignant cell nuclei. This observation led to the hypothesis that such changes occur before the emergence of clinically detectable disease and that nuclear morphometry can be used as a biomarker for estimating an individual’s risk for cancer (Boone et al., 2000).

Morphometry may complement cytological diagnosis and provide useful information. The potential significance of this technique is to distinguish between benign, borderline, and malignant lesions, for objective grading of invasive tumors, prediction of prognosis, and therapeutic response (Priya and Sundaram, 2011). Morphometry has been described for more than a century because the histological characteristics of normal and abnormal cells have been used as a measure of prognosis and as a way of predicting the cause of the disease (Shih et al., 2013). The usefulness of quantitative morphometric analysis has helped in cytological grading of breast lesions but has not yet received widespread acceptance in thyroid lesions owing to limited references and subjectivity (Baloch et al., 2002).

The aim of this study was to examine the expression patterns of Gal-3 and β-catenin, assess the nuclear morphometric features in different thyroid lesions, clarify their roles in diagnosis, and explore their correlations with clinicopathological parameters.

**Materials and methods**

**Materials**

A retrospective, controlled study comprising formalin-fixed, paraffin-embedded blocks of tissues from patients with thyroid lesions were retrieved from the archives of the Departments of Pathology, National Cancer Institute, Cairo University, and Benha Faculty of Medicine, Benha University. The patients were unknown to us as there was no direct contact with them. Tissue blocks with sufficient thyroid tissue, including capsular components, were selected. A total of 60 specimens of benign and malignant thyroid lesions, in addition to 10 cases of normal thyroid tissue adjacent to lesional tissue used as the control group, were utilized in this study. All patients had undergone surgical removal of thyroid lesions between the years 2007 and 2011.

**Histopathological study**

Serial sections were prepared from each block. Histological sections, 4-μm thick, were stained by hematoxylin and eosin (H&E) for histopathological and nuclear morphometric study of different thyroid lesions. The studied H&E sections were reviewed and were also used to select representative areas of the tumor for subsequent immunohistochemical study.

**Nuclear morphometry study**

Morphometric analysis of cases studied was carried out with the Olympus soft imaging system, Germany (Analysis Lifescience Program) to measure the nuclear area, the minimum diameters (min D), and maximum diameters (max D) of the examined nuclei of thyroid carcinoma cases with an optical magnification ×400 on routine H&E-stained sections.

According to this method, 60 tumor cell nuclei – in randomly chosen fields within a well-preserved and highly cellular area of the tumor – were selected for each case. The mean nuclear area (MNA), that is, a cross-sectional area, was determined in μm². Both min D and max D were used to calculate the mean nuclear elongation factor (MNEF) (min D/max D). The elongation factor expresses the degree of nuclear ellipticity. The value of 1.0 represents a circle and less than 1.0 an elliptical structure (Bakr et al., 2000; Nativ et al., 2001). The ratio of max D/min D [long diameter/small diameter (LD/SD)] was also calculated (Kefeli et al., 2010).

**Immunostaining**

From each paraffin block 4-μm-thick sections mounted onto positively charged slides were taken for immunohistochemical staining. Monoclonal antibodies (for Gal-3 and β-catenin) were used. Dilution and incubation times are listed in Table 1. The streptavidin–biotin immunoperoxidase method was used (Universal LSAB_2 Kit; Dako, Egypt). 3,3 ′-Diaminobenzidine tetrahydrochloride solution was used as the final chromogen, and sections were counterstained with Mayer’s hematoxylin before mounting.
Negative controls for nonspecific binding, which were incubated with secondary antibodies only, were processed and revealed no signals. Positive controls (breast tissue for Gal-3 and for β-catenin) (Castronovo et al., 1996) recommended by the manufacturer were used to confirm correct immunohistochemical staining. Slides were screened and observed by two pathologists who had no prior access to the H&E report of the specimens by using code numbers for each block in order to avoid bias. Morphology and cytological appearances were recorded. Scoring was done on the basis of the intensity of staining characteristics on a scale of 1–3: a score of 1 indicates focal/weak staining, a score of 2 indicates moderate staining, and a score of 3 indicates strong positive staining. The mean of the two scores was calculated. Data analysis was performed with the statistical package for social sciences (version 12.0.1; SPSS Inc., Chicago, Illinois, USA). Descriptive analysis of the variables and statistical significance of the tests were expressed in $P$-value.

### Immunostaining interpretation

Gal-3 reactivity was classified according to Collet et al. (2005), as follows:

1. **Score 1**: 1–25% of tumor cells showed cytoplasmic staining of Gal-3.
2. **Score 2**: 25–50% of tumor cells showed cytoplasmic staining of Gal-3.
3. **Score 3**: over 50% of tumor cells showed cytoplasmic staining of Gal-3.

### β-Catenin

Immunoreactivity was expressed according to the method of Garcia-Rostan et al. (2001) as the percentage of positively stained target cells in each of four intensity categories (0, no staining; 1+, weak but detectable above control; 2+, distinct; and 3+, intense). For each tissue section, a numerical value (the H or histology score) was derived by summing the percentages of cells staining at each intensity multiplied by the weighted intensity of staining after random high-power field observations ($\times 400$) corresponding to at least 4000 tumor cells. The intense membranous staining of normal thyroid follicles adjacent to the tumor was used as an internal standard for the scoring of β-catenin expression. Membranous, cytoplasmic, and nuclear immunoreactivity were evaluated by separate H scores; however, given the relative paucity of tumor cells with nuclear or cytoplasmic immunostaining and the resulting low H scores, nuclear and cytoplasmic immunoreactivity patterns were simply recorded as positive or negative with no cutoff values for statistical analysis.

### Results

The cases studied included nine cases of PTC, 10 cases of PTCFV, 12 cases of FCA, seven cases of poorly differentiated carcinoma (PDCA), 13 cases of follicular adenoma (FA), and nine cases of nodular goiter. The age in adenoma cases ranged from 34 to 74 years, with a mean age of 46.6 years. For malignant cases, the ages ranged from 20 to 79 years, with a mean age of 53.2 years.

### Galectin-3 immunostaining results

Cytoplasmic Gal-3 immunostaining has been found overexpressed in all malignant thyroid neoplasms (Figs 1 and 2). However, it was absent or weakly detectable in normal and nonmalignant tissue and FA. It was expressed in nodules with cytological atypia. The nonmalignant hyperplastic papilla stained weakly compared with malignant papillae, in the PTCs studied, showing strong expression of Gal-3. Gal-3 was also expressed focally and weakly in reactive follicular epithelium.

Immunoreactivity was strongest in PTCs (100% had scores 2 and 3), whereas staining was less intense in the PTCFV (90% had scores 2 and 3), and was even weaker in PDCA (57.2% had scores 2 and 3). Staining was weak in three cases (25%) of follicular thyroid carcinoma (Table 2). In several tumors, staining was stronger at the advancing invasive edge of the lesion (Fig. 3) than in the central portion of the tumor. Also, a characteristic positive staining reaction in the capsular invading cells in FCA was also observed.

Gal-3 expression was also observed in fibroblasts, endothelial cells, macrophages, histiocytes, red blood cells, and inflammatory infiltrates in the thyroid tissue.

A statistically significant correlation ($P < 0.05$) was found between galectin score and type of thyroid tumor. All hyperplastic and benign lesions had low score (1), whereas most carcinomas showed high expression (scores 2 and 3).

All cases with positive lymph node (LN) metastases showed high expression of galectin (scores 2 and 3); this was statistically significant ($P < 0.05$).

Of 25 cases with positive capsular invasion, 22 (74%) showed high expression for galectin (scores 2 and 3), another statistically significant correlation ($P < 0.05$).

Of 21 cases with positive blood vessel invasion, 20 (95.2%) showed high expression of galectin (scores 2 and 3), which was also a statistically significant correlation ($P < 0.05$).

High galectin expression is related to the presence of thyroid carcinomas, regardless of the type of carcinoma. It is also related to the presence of LN metastases and capsular and blood vessel invasion (Table 2).

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>Dilution</th>
<th>Incubation time (h)</th>
<th>Source</th>
<th>Staining pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galectin-3</td>
<td>Ready to use</td>
<td>2</td>
<td>Neomarkers</td>
<td>Cytoplasmic</td>
</tr>
<tr>
<td>β-Catenin</td>
<td>1 : 50</td>
<td>2</td>
<td>Neomarkers</td>
<td>Membranous, nuclear, or cytoplasmic</td>
</tr>
</tbody>
</table>
**β-Catenin immunostaining results**

Normal thyroid follicular cells from perilesional tissue showed strong membrane β-catenin immunoreactivity with no nuclear or cytoplasmic localization. This strong membranous staining of normal tissue provided an internal control for staining distribution and intensity. Three patterns of immunoreactivity were observed in the thyroid tumors: (i) membranous (Fig. 4), (ii) nuclear, and (iii) cytoplasmic (Fig. 5).

Membrane β-catenin expression was decreased in all of the thyroid carcinomas. Of all 38 malignant cases, 44.7% showed membranous β-catenin expression compared with 84.6% of 13 adenoma cases that showed membranous expression. However, 55.3% of malignant cases showed nuclear or cytoplasmic expression. Analysis of variance of the means of the raw β-catenin H scores demonstrated a significant difference between FAs and all carcinomas \((P < 0.01)\) or with the well-differentiated FCA group \((P < 0.05)\). Membrane β-catenin reduction correlated with progressive loss of tumor differentiation \((P < 0.01)\) (Table 3). Nuclear β-catenin expression was observed only in undifferentiated anaplastic carcinomas (four of seven cases, 57.1%), indicating that aberrant nuclear localization of β-catenin also is a marker for loss of tumor differentiation \((P < 0.01)\). Nuclear expression was associated with marked reduction or complete loss of membrane immunoreactivity among thyroid carcinomas \((P < 0.01, \text{analysis of variance})\). In the other two cases there was focal but distinct cytoplasmic immunoreactivity. There was significant difference in β-catenin expression among different histological types of thyroid

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**Table 2 Relation of galectin-3 to tumor type, lymph node metastases, and capsular and blood vessel invasion**

<table>
<thead>
<tr>
<th>Type of lesion</th>
<th>Score of galectin-3 expression ([n %])</th>
<th>Number of cases</th>
<th>1 (1–25%)</th>
<th>2 (26–50%)</th>
<th>3 (51–100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NG</td>
<td>9</td>
<td>9 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>FA</td>
<td>13</td>
<td>13 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>FCA</td>
<td>12</td>
<td>3 (25)</td>
<td>0 (0)</td>
<td>9 (75)</td>
<td></td>
</tr>
<tr>
<td>PTC</td>
<td>9</td>
<td>0 (0)</td>
<td>1 (11.1)</td>
<td>8 (88.9)</td>
<td></td>
</tr>
<tr>
<td>PTCFV</td>
<td>10</td>
<td>1 (10)</td>
<td>3 (30)</td>
<td>6 (60)</td>
<td></td>
</tr>
<tr>
<td>PDTCA</td>
<td>7</td>
<td>4 (42.8)</td>
<td>2 (28.6)</td>
<td>1 (28.6)</td>
<td></td>
</tr>
<tr>
<td>Lymph node metastases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>13</td>
<td>8 (32)</td>
<td>4 (16)</td>
<td>13 (52)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>13</td>
<td>0 (0)</td>
<td>2 (15.4)</td>
<td>11 (84.6)</td>
<td></td>
</tr>
<tr>
<td>Capsular invasion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>13</td>
<td>13 (100)</td>
<td>1 (7.7)</td>
<td>5 (38.5)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>25</td>
<td>1 (4)</td>
<td>5 (20)</td>
<td>19 (76)</td>
<td></td>
</tr>
<tr>
<td>Blood vessel invasion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>17</td>
<td>8 (47.1)</td>
<td>5 (29.4)</td>
<td>4 (23.5)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>21</td>
<td>0 (0)</td>
<td>1 (4.8)</td>
<td>20 (95.2)</td>
<td></td>
</tr>
</tbody>
</table>

FA, follicular adenoma; FCA, follicular carcinoma; NG, nodular goiter; PDTCA, poorly differentiated thyroid carcinoma; PTC, papillary thyroid carcinoma; PTCFV, papillary thyroid carcinoma follicular variant.
carcinoma. As 66.7% of well-differentiated papillary versus 33.3% of FCA showed membranous \(\beta\)-catenin expression (Table 3).

Cytoplasmic and/or nuclear expression was significantly associated with invasive tendency of the thyroid carcinoma (vascular invasion, capsular invasion, and lymph node metastasis) \((P < 0.05)\).

A statistically significant correlation \((P < 0.05)\) between \(\beta\)-catenin expression and type of thyroid tumor, LN metastases, and capsular and blood vessel invasion was found.

\(\beta\)-Catenin expression is membranous in all hyperplastic cases, in 84.6% of benign FA, and in 63.2% of carcinoma cases with papillary features, whereas it was mainly cytoplasmic in FCA and poorly differentiated anaplastic carcinomas (65.7 and 85.7%, respectively).

Membranous expression is more common in cases (60%) with negative LN metastases, negative capsular invasion (76.9%), and negative blood vessel invasion (76.5%), whereas cytoplasmic and/or nuclear expression is related to cases with positive LN metastasis, positive capsular invasion, and positive blood vessel invasion (84.6, 72, and 81%, respectively).

### Nuclear morphometric results

A statistically significant correlation between MNA and type of thyroid tumor \((P < 0.05)\) was found. Benign lesions had a significantly lower MNA compared with carcinomas (Figs 6–8). Also, PDCA had higher LD/SD \((P < 0.05)\) compared with other types of thyroid carcinomas (Table 4).

However, correlations between MNA and other variables (LN metastasis, capsular invasion, and vascular invasion) were statistically insignificant \((P > 0.05)\).

Other calculated morphometric measures (MNEF and LD/SD) had statistically insignificant correlations with the clinicopathological variables examined \((P > 0.05)\).

### Discussion

One of the critical steps in cancer metastasis is the adhesion of disseminating tumor cells to the blood vessel endothelium in distant organs. This process is thought to be regulated by the mechanical properties of the cancer cells and also by the specific expression of various adhesion molecules and/or ligands to adhesion molecules on the surface of cancer cells and endothelial cells (Miles et al., 2008).

Gal-3 is a \(\beta\)-galactoside-binding protein that regulates many biological processes, including cell adhesion, migration, cell growth, tumor progression, metastasis, and apoptosis (Bartolazzi et al., 2008; Chiu et al., 2010). The expression of Gal-3 was studied immunohistochemically in thyroid...
lesions, with the aim of studying its reliability as a diagnostic indicator, particularly in differentiating problematic cases that are inconclusive with the routine H&E staining technique. These include PTCFV and minimally invasive FCA. By doing so, it is supposed to facilitate surgical management and treatment.

In this study, Gal-3 was found overexpressed in all malignant thyroid neoplasms. However, it was absent or was weakly detectable in normal and nonmalignant tissue including FA, supporting the results of Xu et al. (1995), Inohara et al. (1996), Orlandi et al. (1998), Gasbarri et al. (1999), Kawachi et al. (2000), Coli et al. (2002), and Than et al. (2008). On the basis of all these results, it can be concluded that Gal-3 could be a useful marker for differentiating benign from malignant thyroid neoplasms especially differentiating follicular thyroid adenoma from carcinoma, which is also parallel to the results of Paunovic et al. (2012). Also, Chiu et al. (2010), Shankar et al. (2012), and Lee and Lee (2013) demonstrated that Gal-3 is the single most accurate marker for the diagnosis of differentiated thyroid cancer.

These different expression patterns of Gal-3 among thyroid lesions may be related to their different biological behaviors (Danguy et al., 2002).

In the present study, Gal-3 was expressed in nodules with cytological atypia and could thus provide a valuable clue in the detection of lesions of undetermined malignant potential, like in nodules with an overall benign appearance but with focal areas suspicious for malignancy. It was also observed that Gal-3 may be valuable in differentiating a nonmalignant hyperplastic papilla from a papilla formed in PTC in which a strong expression of Gal-3 was detected, suggestive to be useful in the early detection of occult PTC in hyperplastic goiter.
This is supported by the results of Gong et al. (2012) and Paunovic et al. (2012) who found that the expression of this individual marker was most helpful for the diagnosis of papillary carcinoma.

With a strong expression of Gal-3 in PTC, we believe that Gal-3 is a good indicator in early detection of malignant transformation. Cytoplasmic predominance expression of Gal-3 has been related to the progression of normal tissue to adenoma and carcinoma in the colon carcinoma model (Lotan et al., 1991). Our data and previous studies suggest that the same pattern of cellular staining can be used with thyroid neoplasm.

Controversial work performed by Jakubiak-Wielganowicz et al. (2003) and Mehrotra et al. (2004) showed that Gal-3 is not a reliable marker for distinguishing benign from malignant thyroid lesions, and that it is not a highly specific marker in differentiating follicular benign from malignant tumors. Also, Davies et al. (2004) concluded that Gal-3 does not distinguish between FAs and carcinomas; it is neither specific nor sensitive enough to be used satisfactorily in clinical practice as a marker of thyroid malignancy. Furthermore, Papale et al. (2013) found that Gal-3 alone, as a molecular marker of thyroid cancer, can still have a limited application in presurgical diagnosis.

As observed by Coli et al. (2002), this study found a high expression of Gal-3 in fibroblasts, endothelial cells, macrophages, histiocytes, red blood cells, and inflammatory infiltrates. This observation could be applicable as an internal positive control.

A characteristic finding in the current study of high positive Gal-3 staining reaction, particularly in the capsular invading cells in FCA, supports the study by Kawachi et al. (2000) and Cvejic et al. (2005) confirming that this marker has a possible role in the invasive capacity of the tumor and in metastasis formation.

Saggiorato et al. (2001) revealed that Gal-3 is a reliable presurgical immunocytochemical diagnostic marker in minimally invasive FCA, improving the accuracy of conventional fine-needle aspiration biopsy (FNAB). This is in accordance with the findings of the present study that Gal-3 is a valuable adjunct diagnostic indicator, preferably in PTC, and could be of great value in preoperative diagnostic FNAB.

In the current work, all cases with positive LN metastases and with positive blood vessel invasion showed high expression of Gal-3 (scores 2 and 3), which was statistically significant. Of 25 cases with positive capsular invasion, 24 cases (96%) showed high expression for Gal-3, another statistically significant correlation. High Gal-3 expression is related to the presence of thyroid carcinomas, regardless of the type of carcinoma.

The explanation for this can be found in the study by Zhao et al. (2009) showing that Gal-3, whose concentration is greatly increased in the circulation of cancer patients, increases cancer cell adhesion to macrovascular and microvascular endothelial cells under static and flow conditions, increases transendothelial invasion, and decreases the latency of experimental metastasis in athymic mice. These effects of Gal-3 are shown to be a consequence of its interaction with cancer-associated MUC1, which breaks the ‘protective shield’ of the cell surface MUC1 by causing MUC1 polarization leading to exposure of smaller cell surface adhesion molecules/ligands including CD44 and ligand(s) for E-selectin. Thus, the interaction in the bloodstream of cancer patients between circulating Gal-3 and cancer cells expressing MUC1 bearing the Gal-3 ligand TF (Galβ1, 3GalNAc−) promotes metastasis.

However, Gong et al. (2012) and Lee and Lee (2013) found that there was no difference in the expressions of Gal-3 in relation to lymph node status.

The present work supports previous findings that Gal-3 detection is a simple, cheap, and an important diagnostic support in distinguishing malignant from benign thyroid neoplasms. The immunocytochemical method is preferred to other molecular biology-based methods, which may show false-positive results (Coli et al., 2002). Morphological evaluation associated with immunocyto-staining with Gal-3 should be the best approach for presurgical evaluation for thyroid nodules.

β-Catenin is part of a membrane-bound cell growth-signaling complex that plays a role in cell adhesion, as well as in promotion of growth (Rezk et al., 2004).

Thyroid carcinomas in the current work are associated with decreased membrane expression of β-catenin. The reduction is significantly higher in carcinomas compared with FAs. Although the number of cases analyzed is small, this observation may prove useful in the distinction between FA and well-differentiated FCA, a differential diagnosis that is extremely difficult on FNAB specimens and can be problematic also in histological sections. β-Catenin can be of diagnostic utility for thyroid lesions.
because it shifts from a membranous localization to a cytoplasmic localization in malignant lesions.

In the present study there was a marked downregulation of membrane-bound β-catenin in poorly differentiated anaplastic thyroid cancer that represents the least differentiated form of carcinoma and the endpoint of tumor progression. This study expands the preliminary observation of Garcia-Rostan et al. (2001) and Rezk et al. (2004) who demonstrated that reduced membranous immunoreactivity closely parallels tumor formation and progressive loss of tumor differentiation. Reduced membrane β-catenin has been documented in numerous human cancers (Wijnhoven and Pignatelli, 1999), where it was sometimes observed in high-grade or poorly differentiated tumors.

The close parallel between dysregulation of β-catenin and neoplastic progression is consistent with the concept of a continuum in the spectrum of thyroid tumor differentiation (Rezk et al., 2004).

In contrast with neoplasms of most other epithelial organs, thyroid carcinomas of follicular cell origin have traditionally been viewed in terms of extremes – that is, well-differentiated carcinomas with papillary or follicular morphology and undifferentiated carcinomas – and this approach is still reflected in the current staging system for thyroid cancer (Fleming et al., 1997).

The correlations shown in this study between loss of tumor differentiation and β-catenin dysregulation suggest that this marker may prove an objective and useful adjunct to the diagnosis of these thyroid tumors.

In the present study, membranous expression is more common in cases (60%) with negative LN metastases, negative capsular invasion (76.9%), and negative blood vessel invasion (76.5%), whereas cytoplasmic and/or nuclear expression is related to cases with positive LN metastasis, positive capsular invasion, and positive blood vessel invasion. In a few studies (Ramesh et al., 1999) the correlation has been shown to be statistically significant. This reduction is consistent with progressive deregulation of intercellular adhesion in cancer that promotes tumor detachment from the primary site and facilitates tumor spread (Wijnhoven and Pignatelli, 1999). In this series, reduced membrane β-catenin immunoreactivity parallels high tumor stage and an increased propensity of the cancer to spread locally outside the thyroid, to invade blood vessels, or give rise to distant metastases. These findings validate experimental models showing an inverse correlation between β-catenin levels and hematogenous spread of murine carcinoma cells (Akimoto et al., 1999) and are similar to reports on other tumor types in which reduced β-catenin expression was also associated with the capacity to invade (Lou et al., 1999) and metastasize (Fujimoto et al., 1997). This must be related to the location and mechanism of action of β-catenin. This study identifies β-catenin dysregulation as a pathway in the progression of thyroid tumors. Altered β-catenin is a marker for aggressive tumor phenotypes among neoplasms of thyroid follicular cell derivation because both reduction of β-catenin membrane immunoreactivity and its aberrant cytoplasmic and/or nuclear localization closely parallel loss of tumor differentiation and poor prognosis. The analysis of β-catenin expression or mutation status may ultimately be very useful to objectively subtype thyroid neoplasms and predict outcomes.

Computerized morphometry can enhance the interpretation of morphological features by the transformation of pathological changes in cells to a qualitative form (Wang et al., 2005). Nuclear morphometric features of malignant cells differ from those observed in nonmalignant cell nuclei. This observation has led to the hypothesis that such changes occur before the emergence of clinically detectable disease and that nuclear morphometry can be used as a biomarker for estimating an individual’s risk for cancer (Boone et al., 2000).

In this study, there was a statistically significant correlation between MNA and type of thyroid tumor (P < 0.05). Benign lesions had a significantly lower MNA compared with carcinomas. However, correlations between MNA and other variables (LN metastasis, capsular invasion, and vascular invasion) were statistically insignificant (P > 0.05).

Bak et al. (2000) reported that the MNA and MNEF are statistically significant discriminators between thyroid carcinomas and adenomas and the combination of both parameters constitutes an optimal discriminatory potential. Also, Wright et al. (1987) detected significant differences in MNAs and nuclear perimeters between multinodular goiters and follicular and papillary neoplasms and significant differences between FAs and follicular and papillary carcinomas. Furthermore, Janković et al. (2011) concluded that preoperative morphometry is a useful method in the differential diagnosis of thyroid carcinoma from benign lesions, and considered it as a complementary method to conventional cytodiagnostics.

In contrast, Kefeli et al. (2010) reported that there were no significant differences between the benign lesion and follicular neoplasia groups for any nuclear parameters. Also, Rajesh et al. (2004) reported that there was overlap of morphometric parameters between FA, FCA, and papillary carcinoma.

We believe that morphometric assessment alone is inadequate for predicting malignancy in thyroid tumors.

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Conflicts of interest
There are no conflicts of interest.

References


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