Role of pro-angiogenic marker galectin-3 in follicular neoplasms of thyroid

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Received 17 April 2012; revised 06 August 2012

The role of pro-angiogenic marker galectin-3 (GAL-3) was examined in differential diagnosis of follicular neoplasms of thyroid into histological subsets of follicular adenoma (FA), follicular carcinoma (FC) and follicular variant of papillary thyroid carcinoma (FVPTC). The study included 22 cases from January 2006 to June 2011 comprising of FA (n = 12), FC (n = 3) and FVPTC (n = 7). Immunohistochemical evaluation of GAL-3 was performed on representative histologic sections from the resected thyroid specimens. The proportion of stained cells and intensity of staining in tumor blood vessels were evaluated. GAL-3 expression showed that angiogenesis was prominent in malignancy (FC and FVPTC) and negative in non-neoplastic thyroid parenchyma and benign condition (FA). GAL-3 expression was found to differentiate benign from malignant follicular neoplasms. Focal and diffuse positivity for GAL-3 was found to be associated with FC and FVPTC respectively, thus GAL-3 can be used as a immunohistochemical marker in the differential diagnosis of follicular neoplasms of thyroid. Limitation of this study was relatively less number of cases studied; however, this data need to be corroborated in larger cohort.

Keywords: Follicular adenoma, Follicular carcinoma, Follicular variant of papillary thyroid carcinoma, Immunohistochemistry, Galectin-3.

In follicular neoplasms of thyroid, there are three histologically distinct classes — follicular adenoma (FA), follicular carcinoma (FC) and follicular variant of papillary thyroid carcinoma (FVPTC). Diagnostic difficulties are quite often encountered while reporting these follicular neoplasms. Apart from histopathology, different tools, such as immunohistochemistry, molecular biomarkers etc. are being used for the differential diagnosis of follicular neoplasms of thyroid. However, studies correlating angiogenesis and follicular thyroid neoplasms are lacking.

In this study, we have attempted to explore galectin-3, a biomarker of angiogenesis as a surrogate marker for the differential diagnosis of follicular neoplasms of thyroid.

Materials and Methods

The study was conducted at Jawaharlal Nehru Institute of Post-graduate Medical Education & Research (JIPMER), Puducherry, India. The study material was retrieved from the Pathology archives over five and half years from January 2006 to June 2011 after obtaining the informed consent from the patients. The study protocol was approved by the Ethics Committee of JIPMER. We included archives of 22 cases, comprising of follicular adenoma (n = 12), follicular carcinoma (n = 3) and follicular variant of papillary thyroid carcinoma (n = 7). All these surgical specimens were fixed in 10% buffered formalin, routinely processed, paraffin-embedded and the sections were stained with hematoxylin & eosin (H & E) stain. Histologic examination was carried out using high resolution microscope. The final histologic diagnosis was made based on the well-established histological criteria. Immunohistochemical (IHC) evaluation of pro-angiogenic marker galectin-3 (GAL-3, Novocastra) was performed on representative histologic sections from the resected thyroid specimens and also using positive and negative controls. IHC was performed by the standard streptavidin-biotin-peroxidase complex technique on 3 µm thick sections mounted on poly-L-lysine-treated glass slides using DAB (diaminobenzidine) as a chromogen (brown color). Unambiguous histologic diagnosis made on the routine H & E-stained sections was taken as ‘the gold standard’. IHC marker was interpreted based on the guidelines recommended by the Saggiorato et al. The proportion of stained cells and intensity of staining were evaluated as follows: Negative: absent staining...
or <10% of cells with weak or moderate positivity; focally positive: 10-50% of cells with moderate or strong positivity; and diffusely positive: >50% of cells with strong positivity.

**Statistical analysis**

The presence or absence of GAL-3 expression in benign and malignant tumors was computed in 2 × 2 contingency table, odds ratio (OR) and 95% confidence interval (CI) were calculated. P<0.05 was considered statistically significant. For all the statistical analysis, Graphpad Instat Version 3.0 software was used.

**Results**

In FA, GAL-3 expression was absent in 83.3% cases, while focal positivity was observed in 16.7% cases. In FC, all three cases exhibited focal positivity for GAL-3 with only the tumor cells in the infiltrating margins showing strong positivity. In FVPTC, one case showed focal positivity for GAL-3, while remaining cases exhibited diffuse positivity (Table 1) (Fig. 1A–L).

GAL-3 expression was found to be positively associated with malignant neoplasm, compared to benign cases (OR: 95%, CI: 4.59, p<0.0001). FC (OR: 95%, CI: 1.11, p<0.05) and FVPTC (OR: 95%, CI: 3.06, p = 0.001) cases showed increased GAL-3 expression, compared to FA.

Increased focal positivity of GAL-3 was observed in FC (OR: 95%, CI: 1.11, p<0.05) compared to FA, while increased diffuse positivity of GAL-3 was found in FVPTC (OR:95%, CI: 2.56, p = 0.003).

**Table 1—Immunexpression of GAL-3 in follicular thyroid neoplasms**

<table>
<thead>
<tr>
<th>Galectin-3</th>
<th>FA</th>
<th>FC</th>
<th>FVPTC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Focally positive</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Diffusely positive</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>3</td>
<td>7</td>
</tr>
</tbody>
</table>

FA, Follicular adenoma; FC, Follicular carcinoma; FVPTC, Follicular variant of papillary thyroid carcinoma

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![Fig. 1](image)

**Fig. 1**—(A & B): Positive (Nodular prostatic hyperplasia) and negative control (Normal thyroid) for galectin-3 (GAL-3) (400x, IHC); (C & D): Follicular adenoma (FA) (100x, H & E stain); (E): GAL-3 staining in FA (negative<10% staining); (F & G): Follicular carcinoma (FC) H & E stain, 100x (F) with capsular invasion (G, 400x); (H & I): GAL-3 staining in FC (Tumor cells infiltrating the capsule focally, showing strong positivity); (J & K): Follicular variant of papillary thyroid carcinoma (FVPTC) (H & E stain, 100x & 400x, respectively) and (L): Gal-3 staining in FVPTC (strong and diffuse positivity)
Discussion

Thyroid gland shows increased vascularity in non-neoplastic conditions like hyperplastic goiter and Graves’ disease, as well as in various thyroid malignancies. Few studies have explored angiogenesis in thyroid malignancies using microvessel density (MVD) as a marker. These studies have suggested positive correlation of MVD with disease-free survival in papillary thyroid carcinoma and intrathyroidal tumor spread in follicular carcinoma. There are well known angiogenic promoters and inhibitors that have been studied in great details in animal models and in human beings in different malignancies. GAL-3 is a carbohydrate-binding proteins having affinity for beta-galactoside containing glyco-conjugates and is one such pro-angiogenic marker. It plays a vital role in cell growth, adhesion, differentiation, inflammation, apoptosis and metastasis. It is found to be directly related to the stage of tumor progression in colon, gastric, thyroid, breast and head and neck carcinomas. In the present study, GAL-3 was used to differentiate benign and malignant thyroid follicular neoplasms.

Endothelial cell morphogenesis is a carbohydrate-dependent process and GAL-3, in particular induces intracellular signaling cascade, leading to the differentiation and angiogenesis of endothelial cells. It also stimulates capillary tube formation in vitro and angiogenesis in vivo. It had been found that in thyroid follicular neoplasms and benign neoplasms such as FA did not have prominent vascularity close to the capsule. On the other hand, in malignancy such as FC, cells infiltrating the capsule and the stroma adjacent to it have shown significantly increased vascularity. An increased expression of VEGF-C has been observed in FVPTC, which correlates well with lymphatic invasion.

There are conflicting results on using GAL-3 expression as surrogate marker for differential diagnosis of follicular neoplasms of thyroid. Aron et al have suggested that GAL-3 is of limited value as a pre-surgical marker, while in another study it has not been considered to be an absolute marker for differentiating benign and malignant follicular lesions, though the study has indicated that GAL-3 could serve as a ‘useful adjunct’ for pre-surgical cytologic diagnosis. On the contrary, another study has reported a sensitivity and specificity of 99% and 98% for GAL-3 alone in discriminating the benign from malignant thyroid lesions. These findings were consistent with our current observation.

In our study, GAL-3 was found to be very useful not only for distinguishing the benign and malignant follicular lesions, but also in differentiating an FA from FC. In contrast to FVPTC that expressed diffuse GAL-3 positivity, FC showed an intense focal GAL-3 staining, only in the neoplastic cells infiltrating the tumor capsule.

Earlier, Saggiorato et al demonstrated the maximum utility of the three well-studied markers GAL-3, CK-19 and HBME-1 in differentiating malignant and benign follicular neoplasms. They found that a sequential combination of GAL-3 and CK-19 could distinguish between benign and malignant Hürthle cell neoplasms, while a sequential combination of GAL-3 and HBME-1 is useful in differentiating the benign and malignant non-Hürthle cell neoplasms.

In conclusion, this study demonstrated that presence or absence of GAL-3 expression could differentiate and malignant follicular of thyroid and type of expression focal or diffuse could differentiate between follicular carcinoma and follicular variant of papillary thyroid carcinoma. However, as the number of cases studied was relatively less, this data need to be corroborated in relatively large cohort.

Acknowledgement

Our sincere thanks to immunohistochemistry (IHC) technical supervisor Mrs. Girija Natarajan and laboratory technicians Mr. K Veerappan and Miss D Semmalar for their continuous guidance for IHC.

References