SHORT REPORT

Differential expression of galectin 3 in solid cell nests and C cells of human thyroid

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Aims: To provide new insights into characterising solid cell nests and gain information that might help distinguish between solid cell nests and C cells.

Methods: Thyroid tissue specimens from patients who had undergone prophylactic thyroidectomy for familial medullary thyroid cancer were immunostained for calcitonin, carcinoembryonic antigen, and galectin 3.

Results: Solid cell nests displayed a strong and diffuse staining for carcinoembryonic antigen and galectin 3, but not for calcitonin. C cells located at the periphery of solid cell nests and in neighbouring follicles expressed both calcitonin and carcinoembryonic antigen but not galectin 3. These three markers were positive in medullary thyroid carcinoma.

Conclusion: Galectin 3 immunoreactivity permits a better characterisation and differentiation between solid cell nests and C cells, avoiding the misidentification of two biologically and clinically different thyroid structures.

Solid cell nests are found in between 5% and 61% of human thyroid glands, depending mainly on the number of sections subjected to microscopic observation.^{1–3} These structures are thought to be a normal component of the thyroid gland, embryologically originating in the ultimobranchial body.^{1–3} They are composed of cytokeratin and carcinoembryonic antigen (CEA) immunoreactive cells, but calcitonin (CT) positive cells are often associated.² In patients with hereditary C cell disease, hyperplastic and neoplastic foci of C cells may be difficult to differentiate from solid cell nests on conventional histological sections. Recently, we found that galectin 3 (GAL-3) immunostaining was positive in medullary thyroid carcinoma (MTC) and negative in normal and hyperplastic C cells.⁴ Positive GAL-3 staining was also seen in thyroid tumours of follicular origin,⁵ suggesting that this molecule may be a marker of endocrine thyroid malignancy. GAL-3 is a β galactoside binding protein recognised to be a ligand for CEA. It plays an important role in embryogenesis and organogenesis and modulates the process of cell–cell adhesion.⁶

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In our study, we evaluated GAL-3 expression in solid cell nests and in C cells, which share a common origin in the ultimobranchial body.

MATERIALS AND METHODS

Tissue specimens

Formalin fixed, paraffin wax embedded tissue was obtained from 34 patients (17 males and 17 females, with an age ranging from 3 to 39 years). These patients belonged to families affected

by MTC and underwent prophylactic thyroidectomy at the Institute Gustave-Roussy between 1983 and 2001, for RET gene mutation or raised plasma calcitonin concentrations. C cell hyperplasia only was diagnosed in nine patients, a combination of hyperplasia and MTC in 10, and MTC only in 15. Hyperplasia was defined as the presence of three fields containing more than 50 C cells at a magnification of $\times 100$.⁷ Carcinoma was defined by: (1) the presence of fibrous and/or amyloid stroma between C cells; (2) the infiltration of interstitial tissue by C cells; and (3) the coalescence of hyperplastic C cell nodules.

At light microscopy, solid cell nests were found in five samples, and were associated with C cell hyperplasia only in two and with C cell hyperplasia combined with MTC in three.

Solid cell nests were defined as aggregates of polygonal or spindle cells forming isolated areas of solid tissue. These cells are smaller than C cells and exhibit round or ovoid nuclei with dense chromatin.

Our study was performed in accordance with protocols previously approved by the local human studies committee.

Immunohistochemistry

Two samples of each thyroid gland, representative of the histological diagnosis, were selected to perform immunohistochemistry. Tissue sections of 4 μ m were initially dewaxed by serial passages in xylene and in alcohol. Endogenous peroxidase activity was quenched by incubation in 0.03% hydrogen peroxide and in 0.1M Tris/HCl buffer (pH 7.6) for five minutes. Subsequently, microwave/pressure cooker pretreatment (three cycles of five minutes each) was performed in 1mM EDTA buffer (pH 8). Sections were then incubated for 30 minutes at room temperature with either anti-CT (1/75 dilution; monoclonal antibody; Dako, Carpinteria, California, USA), anti-CEA (1/200 dilution; polyclonal antibody; Dako), anti-GAL-3 (1/50 dilution; monoclonal antibody; Novocastra, Newcastle upon Tyne, UK) antiserum. They were washed three times in Tris/HCl buffer for five minutes each time and incubated with a peroxidase conjugated antibody for 15 minutes (peroxidase antimouse/rabbit; Dako). After three additional washes, peroxidase staining was revealed in diaminobenzidine tetrahydrochloride (Polysciences Inc, Warrington, Pennsylvania, USA) with 0.1% hydrogen peroxide, in 0.01M Tris buffer (pH 7.2). Sections were counterstained with haematoxylin, dehydrated, mounted, and examined under a microscope. For negative controls, incubation with the primary antibody was omitted.

RESULTS

Solid cell nests were localised in the middle third of the thyroid lobe in four patients and in the superior third in one. Two samples displayed multifocal localisation. Solid cell nests comprised compact spindle or polygonal cells. In all cases, these cells displayed a strong and diffuse staining for CEA and GAL-3, but

Abbreviations: CEA, carcinoembryonic antigen; CT, calcitonin; GAL-3, galectin 3; MTC, medullary thyroid carcinoma

Figure 1 Expression of (A) galectin 3 (GAL-3), (B) carcinoembryonic antigen (CEA), and (C) calcitonin (CT) in solid cell nests and C cells. (A) GAL-3 and (B) CEA are positive and (C) CT is negative (grey arrow) in solid cell nests; (C) isolated peripheral C cells closely associated with solid cell nests are positively stained for CT (black arrow) and (B) CEA but not for GAL-3 (A). In solid cell nests, GAL-3 staining is positive both in the cytoplasm and nucleus (A, arrow), whereas CEA staining is present only in the cytoplasm (B, arrow). Follicular cells display negative staining for all three markers. (Original magnification, $\times 200$.)

no CT staining was seen (fig 1). Staining for CEA was diffuse in the cytoplasm but was absent in the nucleus and GAL-3 staining was present both in the cytoplasm and in the nucleus.

In contrast, normal and hyperplastic C cells expressed both CT and CEA but not GAL-3 (fig 1), whereas malignant C cells forming MTC showed positive CT, CEA, and GAL-3 immunoreactivity.

DISCUSSION

We describe for the first time GAL-3 expression in solid cell nests. Because normal and hyperplastic C cells do not express this molecule, GAL-3 immunostaining is a useful test to differentiate between solid cell nests and C cells.

Solid cell nests and C cells are two thyroid specific cell types with a common origin in the ultimobranchial body, but with different physiological roles.^{1,2,8} Solid cell nests are often found in both normal and pathological thyroid tissue and they are not related to the presence of thyroid disorder.³ In contrast, C cell hyperplasia can occur in several benign conditions,^{9,10} and is associated with neoplastic foci in subjects with the germinal RET mutation predisposing to hereditary MTC.^{11,12}

CT immunoreactive cells may be closely associated with the solid cell nest cells,¹³ which can make the differential diagnosis difficult. Thus, the selective expression of GAL-3 by solid cell nest cells, with no staining in C cells, provides an extremely useful discriminating criterion. The importance of GAL-3 expression in solid cell nests remains to be investigated. Because GAL-3 has been reported to be a ligand for CEA,⁶ this interaction may play a role in the migration and organisation of C cells inside the thyroid gland.

"The selective expression of GAL-3 by solid cell nest cells, with no staining in C cells, provides an extremely useful discriminating criterion"

From a practical point of view, an important consequence of these results is that it should be recognised that GAL-3 positive immunostaining in thyroid tissue is not exclusively a marker of endocrine malignancy. This finding is a possible source of misdiagnosis, especially when a medullary thyroid carcinoma is suspected, because of the absence of cytological and histological markers of malignancy on conventional histological sections. Therefore, in this case, a combination of GAL-3, CT, and CEA immunostaining should be performed. This combination provides an optimal diagnostic panel, because it produces a different immunohistochemical profile for solid cell nests, benign C cell hyperplasia, and early stage medullary carcinoma.

In summary, GAL-3 expression is found not only in malignant thyroid cells but also in normal epithelial cells forming solid cell nests, and its assessment contributes to distinguishing between solid cell nests and benign and malignant C cell foci.

Take home messages

- This report describes for the first time GAL-3 expression in solid cell nests
- Because normal and hyperplastic C cells do not express this molecule, GAL-3 immunostaining is a useful test to differentiate between solid cell nests and benign and malignant C cell foci

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