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Immunohistochemical expression of Galectin-3 and HBME-1 in granular cell tumors: a new finding

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Summary. Granular cell tumor (GCT) is a relatively rare neoplasm, usually located in the upper aerodigestive tract, skin and soft tissue. Because of its uncertain histogenesis, GCT has been the object of many immunohistochemical and ultrastructural studies that have suggested a Schwann cell origin. Our recent observation of a case of GCT immunoreactive for Galectin-3 and HBME-1 led us to further investigate the immunohistochemical profile of these neoplasms.

We evaluated the immunohistochemical expression of the traditional markers for GCT (S-100, CD68) along with new markers (Galectin-3, HBME-1, Calretinina and Inhibin-α) in 22 granular cell tumors. Our results showed, in all cases, a constant diffuse positivity for S-100 protein, CD68 and Galectin-3. HBME-1 was positive in 95% of cases.

The present study gives a new immunophenotypic profile for GCT, which could help pathologists in distinguishing morphologically ambiguous granular lesions in unusual sites.

Key words: Granular cell tumor, Galectin-3, HBME-1, Immunohistochemistry

Introduction

Granular cell tumor (GCT) is an uncommon neoplastic lesion which typically occurs in the skin, soft tissue and mucosa of the upper aero-digestive tract, particularly tongue and vocal cords (Cavaliere et al., 1994; Le et al., 2004). This tumor was first described by Abrikossoff in 1926 (Abrikosoff, 1926) who coined the term "granular cell myoblastoma" to emphasize the tumor's proposed origin from skeletal muscle. Since then it has been the cause of scientific discussion due to its unclear histogenesis.

Owing to this uncertainty, GCT has been investigated by extensive immunohistochemical and ultrastructural studies which have provided sufficient evidence to support a Schwann cell origin.

Generally, GCT shows characteristic histological features on routine hematoxylin-eosin stained sections. However, in some small biopsies and in unusual sites, where distinction with other similar neoplasms could be important, the use of a panel of immunohistochemical markers, including overall S-100 and CD68, may be needed. Another recent finding is that nestin also, a novel neural marker, is expressed in granular cell tumors, especially in the gastrointestinal tract (Parfitt et al., 2006).

Recently, we observed a case of paratracheal GCT with a surprising immunoreactivity also for Galectin-3 and HBME-1 (Colella et al., 2007). Galectin-3 is implicated in many biological processes and has been identified in a number of tumors, particularly thyroid neoplasms, where it is used to distinguish between papillary thyroid carcinoma and other thyroid neoplasms. HBME-1 is a mesothelial cell marker.

On this basis, to address diagnostic issues and to shed light on the histogenesis, we performed on twenty-two cases of granular cell tumor a panel of immunohistochemical stains, including Galectin-3 and HBME-1, along with the classic markers (S-100 and CD68), and some recently described positive markers for GCT (α-inibin and calretinin) (Fine and Li, 2003; Le et al., 2004).

Materials and methods

Twenty-two cases of GCT were retrieved from the archives of the Institute of Pathological Anatomy and Histology, University of Perugia, Italy. The hematoxylin-eosin stained sections were reviewed in each case by G.B. and R.C. to confirm the diagnosis. Following the criteria established by Fanburg-Smith (Fanburg-Smith et al., 1998), all the tumors were classified as benign. Clinical data (patient age at diagnosis, sex, anatomic site, and follow-up) were collected from the patient's medical records.

Four micron-thick sections of formalin-fixed, paraffin-embedded tissue were deparaffinized,

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rehydrated and endogenous peroxidase activity was blocked by treating with 0.5% hydrogen peroxide in methanol for 5 minutes. Slides were incubated with the primary antibodies used (listed in Table 1) for 30-60 minutes at room temperature. A biotin-free polymeric-horseradish peroxidase (HRP)-linker antibody conjugate system (Bond Polymer Define Detection, Vision BioSystems Ltd, Aus) was used. Slides were subsequently counterstained with Gill's hematoxylin and mounted for microscopic evaluation. Appropriate negative and positive control slides were performed in parallel.

All immunohistochemical stained sections were semiquantitatively scored as follows: 0, no detectable immunoreactivity; 1, 1% to 10% of tumor cells reactive; 2, 11% to 50% of tumor cells reactive; 3, more than 50% of tumor cells positive.

Results

Clinical features

The salient clinical features are summarized in Table 2. Age at presentation ranged from 15 to 76 years (mean 44 years, median 41 years). There were 13 females and 9 males. The anatomic sites were skin (15 cases), upper aerodigestive tract mucosa (tongue 4 cases, lip 1 case, larynx 1 case) and soft tissue of paratracheal region (1 case), which occurred simultaneously with a thyroid carcinoma.

At the last follow-up, ranging from 12 to 216 months (mean 122, median 51), no recurrences had been reported and none of the tumors had metastasized. No postoperative therapy was administered.

Pathological features

The tumors ranged in dimension between 4 and 40 mm (mean 11; median 9).

Histologically, all the cases showed nests and sheets of large polygonal cells with abundant, eosinophylic and granular cytoplasm (Fig. 1). Nuclei were small round to oval with inconspicuous nucleoli.

Pseudoepitheliomatous hyperplasia was present in 4/15 cases in which overlying epithelium was present. Neither necrosis, nuclear pleomorphism, spindled cells,

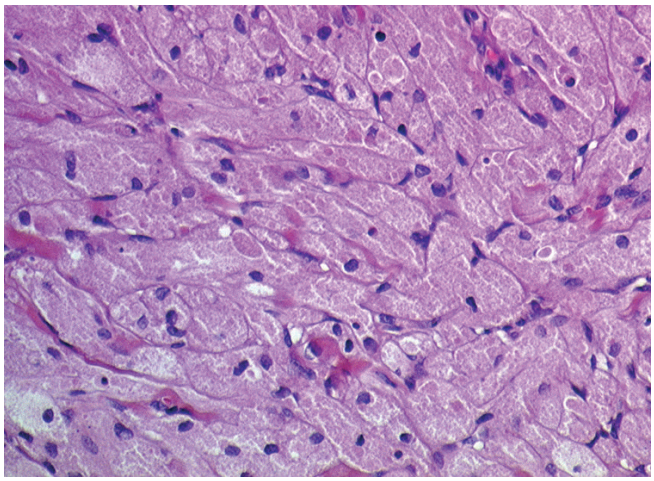

Fig. 1. GCT showing sheets of large polygonal cells with abundant, eosinophylic and granular cytoplasm. Nuclear pleomorphism was minimal or absent. Hematoxylin-eosin. x 200.

Table 2. Case details and sites of the granular cell tumors.

Case n.	Sex/age	Site	Size (mm)
1	F/51	Lip	40
2	F/76	Skin/shoulder	15
3	M/40	Skin/neck	10
4	F/32	Skin/vulva	9
5	M/38	Tongue	5
6	F/36	Skin/back	16
7	F/33	Skin/breast	5
8	F/64	Tongue	9
9	F/64	Skin/vulva	12
10	M/22	Skin/thigh	23
11	F/15	Skin/hand	4
12	M/49	True vocal cord	5
13	M/41	Skin/finger	4
14	M/40	Skin/forearm	10
15	F/54	Skin/shoulder	10
16	F/41	Tongue	6
17	M/41	Soft Tissue/neck	8
18	F/59	Skin/vulva	20
19	F/55	Skin/vulva	13
20	M/53	Skin/thigh	4
21	M/41	Skin/neck	13
22	F/44	Tongue	7

F: female; M: male.

Table 1. Antibodies used.

Antibody	Clone	ilution	Antigen retrieval	Source
Galectin-3	9C4	1:100	Heat-induced	Ylem (Rome, Italy)
S-100	Polyclonal	1:500	None	Dako (Glostrup, DK)
CD-68	PGM-1	pre-diluted	None	(Courtesy of Prof. Falini)
Mesothelial Cell	HBME-1	1:50	Heat-induced	Dako (Glostrup, DK)
Calretinin	CRTO-1	ready to use	Heat-induced	Labvision Neomarkers (Fremont, CA)
Pan-keratin	MNF116	1:150	protease digestion	Dako (Glostrup, DK)
Ki-67	Mib-1	1:100	Heat-induced	Dako (Glostrup, DK)
Inhibin-α	R-1	1:50	Heat-induced	Serotec (Oxford, UK)

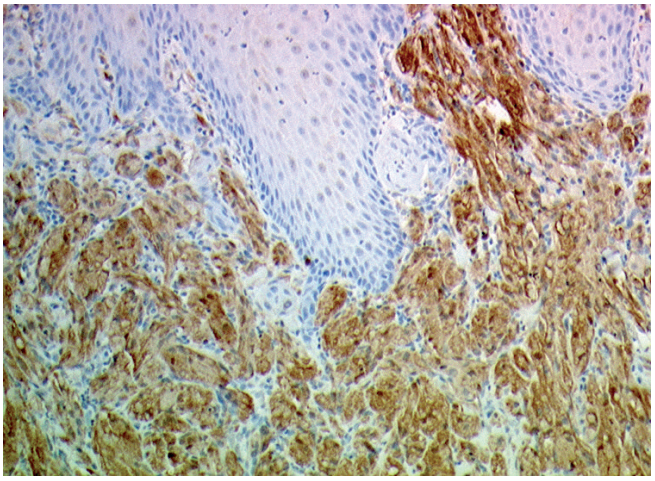

Fig. 2. Intense and diffuse staining for Galectin-3 (score 3). x 250.

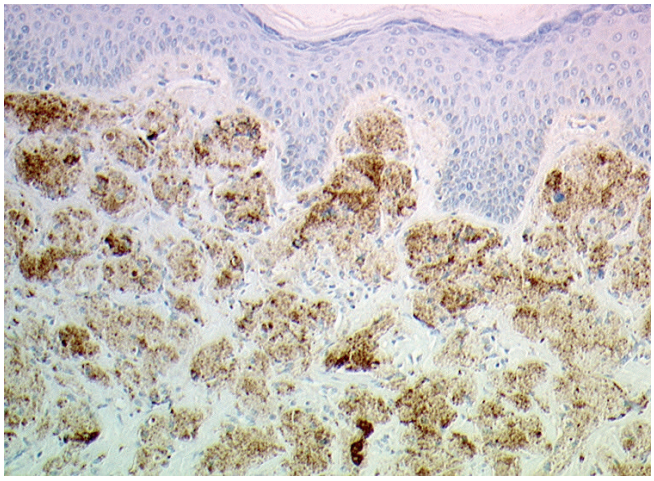

Fig. 3. Cytoplasmatic immunoreactivity for HBME-1. x 200.

Table 3. Immunohistochemical results.

Markers	Immunohistochemical score				Positive
	0	1	2	3	cases (%)
S-100	0	0	1	21	100
CD68	0	1	2	19	100
Galectin-3	0	0	0	22	100
HBME-1	1	4	4	13	95
Calretinin	13	1	5	3	41
Inhibin-a	14	2	4	2	36

increased mitotic activity or other features suggestive of malignancy were observed.

Immunohistochemical results

The immunohistochemical findings are reported in Table 3. All cases showed diffuse and strong expression of S-100 and CD68. Immunohistochemistry for Galectin-3 (Fig. 2) gave a nuclear and cytoplasmic stain in all cases. HBME-1 was expressed in 95% of cases, but in 9 tumors less than 50% of tumor cells were positive (Fig. 3). No immunohistochemical reaction was noted for Calretinin and Inhibin-α in 13 and 14 cases respectively.

Discussion

In this series we present 22 cases of granular cell tumors. These lesions are rare, typically benign, soft tissue neoplasms which arise principally in dermal and subcutaneous tissues, submucosal sites and, less often, in skeletal muscle and viscera (Cavaliere et al., 1994; Le et al., 2004). Any part of the body may be a tumor site but in more than a half of the cases GCT are located in the head and neck region or on the tongue.

Currently, most investigators favour a Schwann cell derivation based on immunohistochemical and electron microscopic findings, which show a continuous basal layer around the tumor cells, reminiscent of perineurium (Carvalho et al., 1994). On the other hand, some authors retain that granular cell tumors are not a specific entity, but rather an expression of degenerative changes which can occur in many different types of cells, including Schwann cells (Alidina et al., 1994).

In the present study, we confirm the well-known immunoreactivity of GCT, from all sites, for S-100, which supports the hypothesis of peripheral nerve sheath origin.

In a recent paper describing a case of paratracheal granular cell tumor occurring simultaneously with a papillary thyroid carcinoma, we found a unexpected positivity for Galectin-3 (Colella et al., 2007). This finding drove us to test this protein in a large series of GCT. Our results demonstrate that all cases show a diffuse and intense cytoplasmic and less strongly nuclear immunoreactivity for Galectin-3. This feature has not been previously reported in literature. We think that pathologists must keep this aspect in mind when they observe abundant granular cells, for example, in material obtained by fine-needle aspiration cytology or small biopsies. Furthermore, it is still more important to consider that positive staining for Galectin-3 in a thyroid nodule aspirate or in a tissue situated near the thyroid does not necessarily indicate a thyroid neoplasm.

Galectin-3 is a member of a growing family of beta-galactoside binding animal lectins. It is widely distributed in normal human tissue and cell types (Barondes et al., 1994) and has a different distribution within the cell (Le et al., 2004). It has several functions, such as cell-cell and cell-matrix interactions (Matarrese et al., 2000), cell growth, cell-cycle regulation and apoptosis (Yang et al., 1996), cell damage and repair processes. Besides this relatively wide spectrum in physiological conditions, Galectin-3 has been identified in different human tumors, including thyroid carcinoma,

breast carcinoma, colorectal carcinoma, melanoma and hepatocellular carcinoma (Volante et al., 2004a). In a large series of GCT, immunoreactivity for Galectin-3 has not been previously observed and could be related with a neural origin. Indeed, Galectin-3 is also expressed by Schwann cells under traumatic conditions (Reichert et al., 1994) and in some neoplastic neural cells (Kuklinski et al., 2000). Some authors have recently demonstrated by cytogenetic analysis that malignant GCT express some chromosomal abnormalities that had been previously reported for malignant peripheral nerve sheath tumors (Di Tommaso et al., 2002). Furthermore, immunoreactivity for Galectin-3 has just been reported for indubitably neural tumors, such as schwannoma, neurofibroma, ganglioneuroma and malignat peripheral nerve sheath tumor (Bigotti et al., 2003). A contribution to the neural histogenetic hypothesis is also the positivity of some cases in the present series for Calretinin, a calcium-binding protein related structurally to S-100 (Fine and Li, 2003) and also expressed in the nervous system and thymus. In GCT, immunolabelling for Calretinin is inconstant, varying from 41% (present series) to 93% of cases (Fine and Li, 2003).

HBME-1 recognizes an unknown antigen located on the microvilli of mesothelial cells. It is employed in the differential diagnosis of mesothelial lesions and thyroid tumors. Interestingly, in our series, we observed a strong and diffuse positivity for HBME-1. Its immunoreactivity for GCT and relationship with pathogenesis remains unclear. Some authors have reported a positive stain for HBME-1 in oncocytic cell tumors (Volante et al., 2004b) and adenocarcinomas (Oates and Edwards, 2000). These observations, together with our results, enlarge the spectrum of reactivity for HBME-1 limiting its diagnostic utility.

In our series we also tested immunoreactivity for Inhibin-α. However, despite previous reported data (Le et al., 2004), we were able to demonstrate only a low expression for this antibody.

In conclusion, our study expands the GCT immunophenotype, demonstrating the classic immunoreactivity for S-100 and CD68 as well as the not previously reported positivity for Galectin-3 and HBME-1. This panel of markers could help the pathologist in differential diagnosis of GCT, especially when they arise in an atypical site.

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