Arch Dermatol Res (2015) 307:151–157
DOI 10.1007/s00403-014-1505-3

ORIGINAL PAPER

The immunohistochemical profile of granular cell (Abrikossoff) tumor suggests an endomesenchymal origin

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Received: 12 March 2014 / Revised: 15 September 2014 / Accepted: 15 September 2014 / Published online: 28 September 2014

Abstract Granular cell tumor (GCT) is an uncommon soft tissue neoplasm which has an unclear histogenesis. The aim of this study was to analyze its immunophenotype and hypothesize on the histogenesis of GCT. A database of 2,250 soft tissue tumors was examined to identify and characterize the particularities of GCTs. A large panel of antibodies was used. Of the 2,250 tumors, only 15 were GCTs (0.66 %); these were diagnosed in patients whose average age was 37 years. Among them, 5 had malignant potential, the remaining 10 were benign. One of these benign tumors was associated with a metachronous chondrosarcoma with metastases in the lungs. No recurrences were reported in these cases. The benign tumors displayed positivity for S-100, neuron-specific enolase (NSE), CD56, epithelial membrane antigen (EMA), and inhibin. In the atypical GCTs, NSE, S-100 protein, c-KIT, RET and EMA were positive, while inhibin and CD56 were negative; rare osteoclastic-like histiocytes, marked by CD68, were seen. All cases were negative for CD31, CD34, smooth muscle actin, desmin, maspin, and calretinin. Ovoid bodies expressed CD105, synaptophysin, and HER-2. All the cases were microsatellite-stable tumors. The immunoprofile suggests that the GCT seems to have an endomesenchymal origin. The c-KIT and RET positivity, associated with microsatellite stability, and the immunoprofile of the ovoid bodies have never reported before in GCTs.

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Keywords Granular cell tumor · Abrikosoff tumor · Histogenesis · c-KIT · CD105 · RET oncoprotein · Microsatellite · Maspin

Introduction

Granular cell tumor was first described in 1854 by Weber and Virchow [18] as a cluster of large cells with granular eosinophilic cytoplasm. Believing that it originates from skeletal muscle fibers, Abrikosoff named it granular myoblastoma; he diagnosed the tumor in the tongue [1]. Refuting the myogenic origin, it was later named first as Abrikossoff's tumor, and subsequently as granular cell tumor (GCT), because of its granular cytoplasm, a consequence of lysosomal accumulation. GCT is a rare, slow-growing mostly benign neoplasm, representing about 0.5 % of all soft tissue tumors that can occur anywhere in the subcutaneous tissues of the body; the most common locations (about 40–50 % of cases) being the head and neck areas. There were also mentioned, as case reports, other locations such as vulva, breast, lung, appendix, and pituitary gland [13, 16, 17].

Although several studies were carried out for a thorough understanding of the tumor, its histogenesis continues to be controversial. While most of the researchers propose a neural/schwanian origin [17], some propose myofibroblastic, muscular or even neuroendocrine origin [8, 13]. Moreover, some authors consider that GCT is a neoplasm, whereas others classify it as a manifestation of reactive changes of the neural/schwanian cells, not a true neoplasm [15, 17].

The immunohistochemical expressions of benign and potentially malignant GCTs could be helpful in elucidating the histogenesis of these tumors. With this in view, the

Table 1 The main characteristics of the antibodies used for immunostains

Antibody (company)	Clone	Dilution	Antigen retrieval	Positive control
Maspin (Novocastra, Newcastle-upon-Tyne, UK)	EAW24	1:50	Incubation with a 10 mM sodium citrate buffer (pH 6.0) for 30 min at 100 °C	External: prostate and myoepithelial cells (breast) Internal: NS
p53 (LabVision, Fremont, CA, USA)	DO-7	1:50	Incubation with a 10 mM sodium citrate buffer (pH 6.0) for 30 min at 100 °C	External: normal colon mucosa, basal cells Internal: NS
Ki67 (LabVision)	SP6	1:200	Incubation with a 10 mM sodium citrate buffer (pH 6.0) for 30 min at 100 °C	External: tonsils Internal: NS
CD31/PECAM-1 (LabVision)	JC/70A	1:50	Incubation with Tris–EDTA solution (pH 10.0) for 30 min at 100 °C	External: placenta Internal: blood vessels, endothelial cells
CD34 (Dako Glostrup, Denmark)	QBEnd 10	1:100	Incubation with a 10 mM sodium citrate buffer (pH 6.0) for 30 min at 100 °C	External: portal vessels, endothelial cells Internal: blood vessels, endothelial cells
CD105 or endoglin (LabVision)	SN6H	1:50	No antigen retrieval—incubation for 60 min at room temperature	External: tonsils Internal: NS
SMA (LabVision)	1A4	1:600	Incubation with a 10 mM sodium citrate buffer (pH 6.0) for 30 min at 100 °C	External: appendix, blood vessels, smooth muscle cells Internal: blood vessels, endothelial cells
EMA (Dako)	E29	1:50	Incubation with a 10 mM sodium citrate buffer (pH 6.0) for 30 min at 100 °C	External: breast, epithelial cells Internal: epithelial cells
c-KIT (Dako)	Polyclonal rabbit	1:500	Incubation with Tris–EDTA solution (pH 10.0) for 30 min at 100 °C	External: GIST Internal: NS
RET oncoprotein (LabVision)	3F8	Ready to use	Incubation with a 10 mM sodium citrate buffer (pH 6.0) for 30 min at 100 °C	External: small intestine, enteric ganglion cells Internal: NS
Chromogranin A (Novocastra)	5H7	1:100	Incubation with a 10 mM sodium citrate buffer (pH 6.0) for 30 min at 100 °C	External: pancreatic islets Internal: NS
Synaptophysin (Dako)	SY38	1:100	Incubation with a 10 mM sodium citrate buffer (pH 6.0) for 30 min at 100 °C	External: neuroendocrine cells, appendix Internal: NS
S-100 protein	IR504	1:100	No antigen retrieval—incubation for 60 min at room temperature	External: peripheral nerves, Schwann cells Internal: nerves and melanocytes
NSE (Dako)	BBS/NC/VI-H14	1:50	Incubation with a 10 mM sodium citrate buffer (pH 6.0) for 30 min at 100 °C	External: pancreatic islets Internal: NS
GFAP (Novocastra)	GA5	1:40	Incubation with a 10 mM sodium citrate buffer (pH 6.0) for 30 min at 100 °C	External: brain, glial cells Internal: NS
HER-2 (Novocastra)	5A2	1:100	Incubation with a 10 mM sodium citrate buffer (pH 6.0) for 30 min at 100 °C	External: ductal epithelial cells of the breast Internal: NS
Calretinin (Dako)	DAK-Calret 1	1:40	Incubation with a 10 mM sodium citrate buffer (pH 6.0) for 30 min at 100 °C	External: appendix, ganglion cells Internal: NS
Inhibin-α (Dako)	R1	1:100	Incubation with a 10 mM sodium citrate buffer (pH 6.0) for 30 min at 100 °C	External: placenta, trophoblasts Internal: NS
MLH-1 (Novocastra)	ES05	1:100	Incubation with a 10 mM sodium citrate buffer (pH 6.0) for 30 min at 100 °C	External: colon, normal mucosa Internal: lymphocytes

Table 1 continued

Antibody (company)	Clone	Dilution	Antigen retrieval	Positive control
MSH-2 (Novocastra)	25D12	1:100	Incubation with a 10 mM sodium citrate buffer (pH 6.0) for 30 min at 100 °C	External: lymph node, germinal centers Internal: lymphocytes
CD56 (Dako)	123C3	1:100	Incubation with a 10 mM sodium citrate buffer (pH 6.0) for 30 min at 100 °C	External: colon, ganglion cells Internal: nerves
CD68 (Dako)	KP1	1:50	Incubation with Tris–EDTA solution (pH 10.0) for 30 min at 100 °C	External: lymph node, germinal centers, macrophages Internal: granulocytes and macrophages
HMB45 (Novocastra)	HMB45	1:100	No antigen retrieval—incubation for 30 min at room temperature	External: melanoma cells Internal: NS

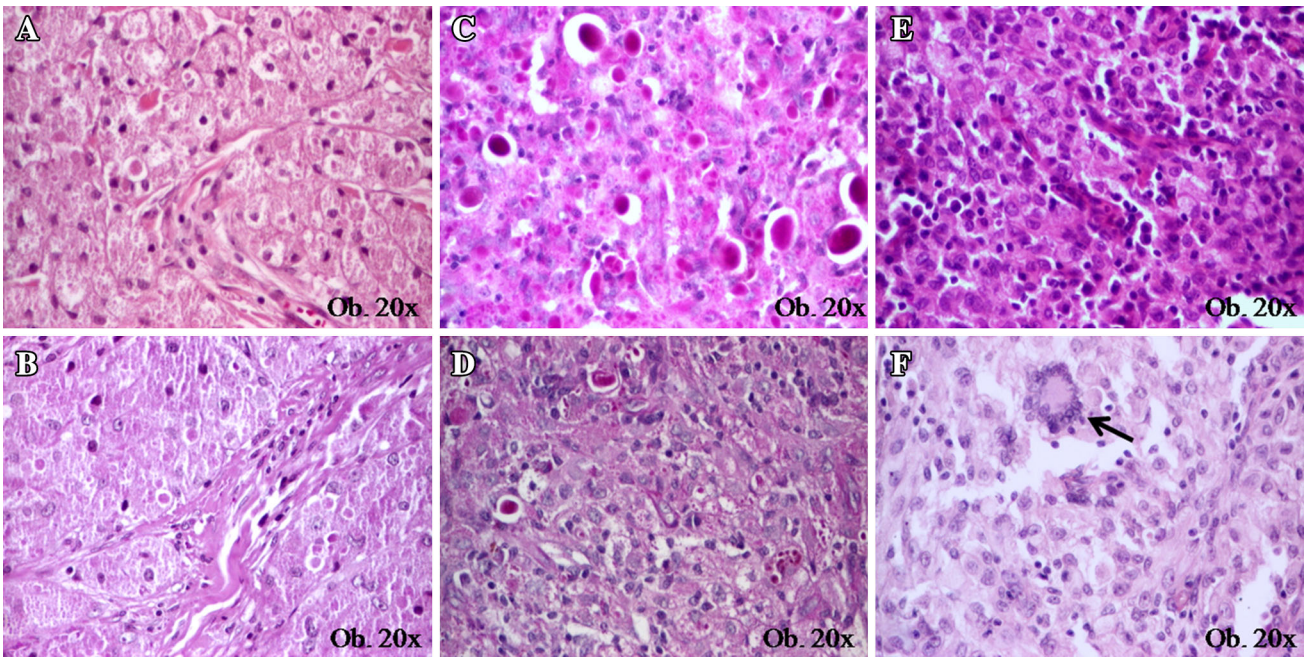

Fig. 1 Histopathological findings of granular cell tumor, in hematoxylin–eosin (**a**, **c**, **e**, **f**) and PAS–hematoxylin stain (**b**, **d**). The benign type is characterized by monomorphic small nuclei, granular cytoplasm and ovoid bodies surrounded by clear halo (**a**, **b**). In the atypical variant, nuclear pleomorphism, increased number of ovoid bodies (**c**–**e**), and one multinucleated giant cell (**f**) can be seen

immunohistochemical expressions of a large panel of antibodies in GCT cells were analyzed. Based on our results and a review of literature on this subject, the histogenetic hypothesis of GCT was restated.

Materials and methods

The authors retrospectively searched the pathology department's database for soft tissue tumors, diagnosed over a period of 14 years (1999–2013), to identify the incidence of GCTs and their histological and immunohistochemical particularities. Processing of the cases was approved by the head of the Department of Pathology at the University of Medicine and Pharmacy of Tirgu-Mures, Romania.

The methods used for antigen retrieval, dilution of the antibodies clones, and the positive controls used to check the quality of the immunostains are shown in Table 1.

Microsatellite status was determined following melting point analysis method, and using the real-time PCR (Roche) and the nucleotides BAT25 and BAT26. To confirm the molecular results, markers MLH-1 and MSH-2 were used.

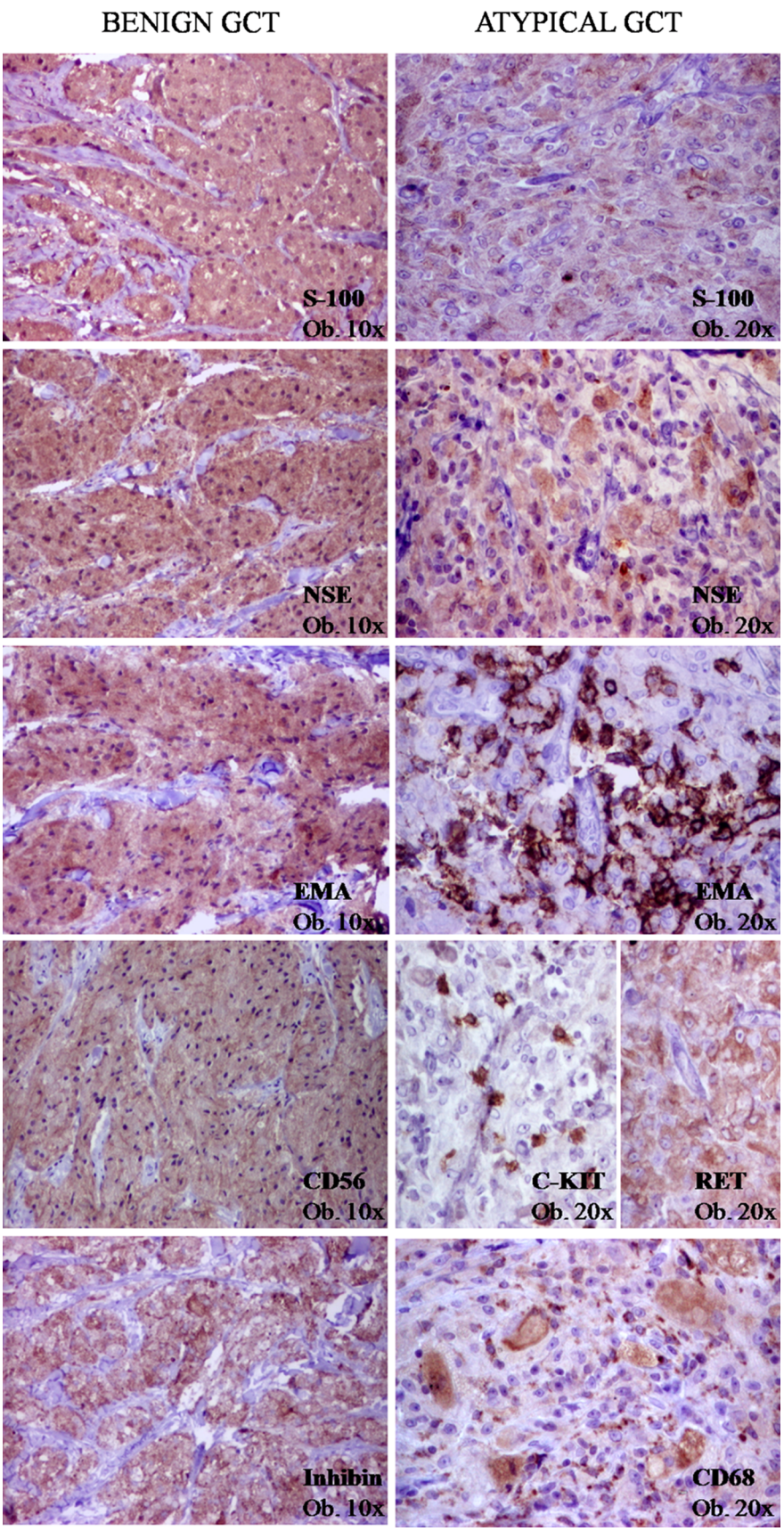

Fig. 2 Immunohistochemical findings of granular cell tumors (GCTs). Both benign and atypical variants are positive for S-100, neuron-specific enolase (NSE) and epithelial membrane antigen (EMA). The benign type also display positivity for *CD56* and inhibin, while the atypical type is immunoreactive to c-KIT and RET. *CD68* marks the cytoplasmic lysosomes and the giant histiocyte-like cells

Table 2 Immunoprofile of benign granular cell tumor (GCT) vs. atypical variant

IHC marker	Benign GCT ($n = 10$)	Atypical GCT ($n = 5$)
S-100	Diffuse positive	Diffuse positive
NSE	Diffuse positive	Diffuse positive
MLH-1	Diffuse positive	Diffuse positive
MSH-2	Diffuse positive	Diffuse positive
EMA	Diffuse positive	Focal positive
RET kinase	Negative	Diffuse positive
c-KIT	Negative	Focal positive
CD56	Diffuse positive	Negative
Inhibin	Diffuse positive	Negative
Ki67	Positive in 5 % of the nuclei	Positive in 5 % of the nuclei
P53	Negative	Positive in >50 % of the nuclei
CD31	Negative	Negative
CD34	Negative	Negative
CD105	Negative	Negative
SMA	Negative	Negative
GFAP	Negative	Negative
Chromogranin A	Negative	Negative
Synaptophysin	Negative	Negative
HER-2	Negative	Negative
Calretinin	Negative	Negative
Maspin	Negative	Negative
HMB45	Negative	Negative
CD68	Negative	Negative

IHC immunohistochemical, *NSE* neuron-specific enolase, *EMA* epithelial membrane antigen, *SMA* smooth muscle actin, *GFAP* glial fibrillary acidic protein

Results

Clinicopathological features

Out of a total of 2,250 cases of soft tissue tumors diagnosed over a period of 14 years, only 15 cases of GCTs (0.66 %) were identified. The median age of the patients was 36.31 ± 16.32 years, ranging from 10 to 66 years, with a male to female ratio of 1.1:1. Macroscopically, in all of the 15 cases, single encapsulated nodules were identified throughout the skin, the mean tumor diameter being of 18 ± 2.87 mm. One of the benign GCTs, diagnosed in a 64-year-old female, was associated with metachronous chondrosarcoma of the prepubic region that occurred 3 years after surgical resection of the GCT, ultimately leading to lung metastases (from chondrosarcoma) after another 8 months.

Based on their microscopic features, which will be described in the next paragraph, five cases were considered 'atypical GCT', the other 10 being benign tumors. No past medical history was declared in any of the cases.

Microscopically, in all the benign cases, the architecture was predominantly solid, and the tumor clusters were separated by connective bands. The tumor cells were large, polygonal or oval-shaped, presenting abundant granular eosinophilic cytoplasm with Pas-positive intracytoplasmic inclusions of lysosomes, but with no Alcian blue positivity. Pas-positive 'pustulo-ovoid bodies of Milian', surrounded by a clear halo, were present in all the cases (Fig. 1), and their incidence was highest in the atypical GCTs. Centrally located small nuclei and rare mitotic figures were also noted. The atypical GCTs were characterized by medium-sized granular cells containing large pleomorphic or vesicular nuclei with prominent nucleoli; the mitotic activity was low-grade. Among the atypical cells, a few giant uninucleated histiocytes and multinucleated osteoclast-like structures were also seen (Figs. 1, 2). Based on the presence of nuclear pleomorphism and large vesicular nuclei with well-defined nucleoli, and also the immunoprofile (p53, Ki67), these cases were considered 'atypical GCTs'. Besides, the absence of necrosis and low mitotic activity did not support their inclusion in the category of malignant GCTs.

Immunohistochemical and molecular aspects

From the 23 antibodies used to explore the immunoprofile of benign GCT versus the atypical variant, 16 presented similar aspect, being even diffuse positive or negative, independently of the tumor microscopic type (Table 2). For example, in all of the 10 benign cases, the IHC immunoreactivity of the epithelial membrane antigen (EMA) was diffusely observed in the tumor cells; it became focally expressed in the atypical GCTs. RET kinase and c-KIT positivity was seen in only atypical tumors while CD56 and inhibin marked only the benign GCTs (Table 2; Fig. 2). The c-KIT-positive cells were irregular in shape with dendrite-like extensions. No c-KIT expression was seen in the vascular endothelium.

All of the 15 cases proved to have a microsatellite-stable status with both BAT25 and BAT26 nucleotides; it was confirmed by the nuclear positivity of the antibodies MLH-1 and MSH-2.

CD68 was negative in the tumor cells, in both benign and atypical GCTs; its immunoexpression was limited to the intracytoplasmic lysosomal granules. The giant osteoclastic-like cells, identified in the atypical GCTs, were immunoreactive to CD68, NSE, and S-100, and did not express EMA and c-KIT (Fig. 2).

In both benign and atypical GCTs, the 'pustulo-ovoid bodies of Milian' showed positivity for CD105, HER-2, and synaptophysin (Fig. 3).

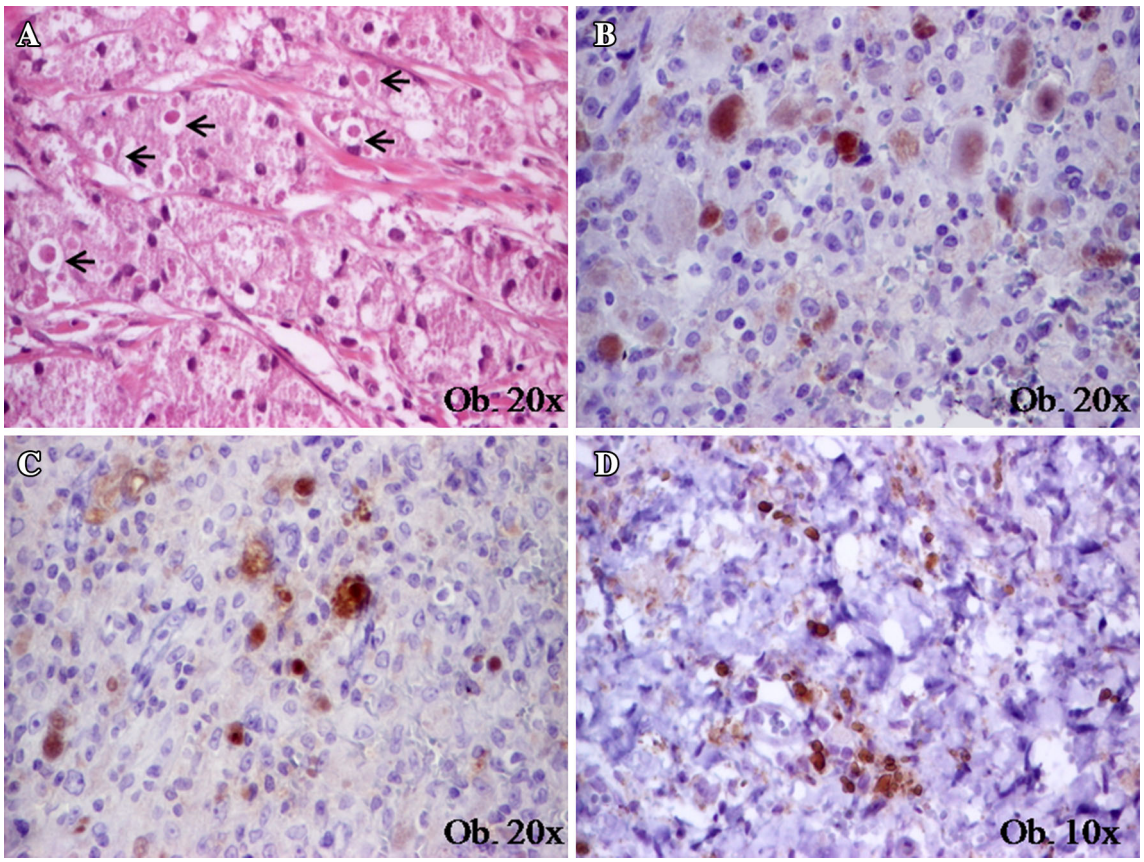

Fig. 3 The 'pustulo-ovoid bodies of Milian', surrounded by a clear halo, indicated by *arrows* in hematoxylin–eosin (**a**), are immunoreactive to CD105 (**b**), synaptophysin (**c**), and HER-2 (**d**)

Discussion

GCT is described in literature as a benign lesion with multicentric aspect in about 5–7 % of cases, and malignant behavior in 2 % of cases, respectively; the malignancy occurs especially in the thigh and internal organs [3, 15–17]. GCT is usually a painless, slowly growing encapsulated nodule of indefinite margins, with the highest incidence in middle-aged women of black ethnicity [3, 16, 17]. The recurrence rate depends on the quality of the resection margins; incomplete excision with positive margins may cause recurrences in more than 20 % of cases, as compared with 2–8 % of cases with margins free of tumor [16]. No recurrences occurred in any patients of this study, even in the two cases with positive margins. Differentiation between benign and malignant variants is based mainly on necrosis, p53 negativity and a low Ki67 index [3, 17]. However, invasive growth, perineural spread and invasion in subendothelial vascular layers were also detected in benign GCTs, without intraluminal emboli [3].

Although GCT was studied by many researchers, its histogenesis remains controversial. Moreover, malignant variants are very aggressive and do not respond to chemotherapy and even radiotherapy [16]. Molecular aspects are also not fully known, as they are not discussed much in literature, except in one paper that deals with a case wherein none of the GCTs had BRAF gene alterations [13]. The present study proved, for the first time, the microsatellite-stable status of both benign and atypical GCTs.

Immunohistochemical staining results suggest that GCT cannot be considered a purely neural tumor. Although most authors believe, on the basis of S-100 protein and NSE positivity, in neural/schwann-cell origin [8], the data of this study, which is partly in conformity with the literature data, suggest an endomesenchymal origin.

First, CD68 positivity is useful for differential diagnosis, its positivity being infrequently reported from intracytoplasmic granules [17]. SMA negativity rules out the myofibroblastic differentiation, and lack of synaptophysin and chromogranin expression [13] goes against the neuroendocrine origin, postulated in literature [8].

On the other hand, acquiring c-KIT and RET positivity and losing CD56 and inhibin expression in the atypical form, compared with benign GCTs, support a mixed origin, because it is known that tumors of the

peripheral nerves are negative for c-KIT [9] and inhibin-α [5]. Although the triple positivity for S-100, RET and c-KIT indicates a possible ectomesenchymal origin, similarly to the schwannoma, EMA positivity negates the ectodermis origin and advocates for a possible relationship with the endodermal structures [4, 7]. Moreover, ultrastructural differences were also noted between schwannomas and GCTs [15]. The origin of GCT from pluripotential mesenchymal stem cells of the connective tissue, that express CD105, CD34 and SMA, though negative for c-KIT [6, 10, 11], also goes against by the tumor immunoprofile.

Based on c-KIT/S-100 co-expression in the atypical tumor cells and considering that both nuclei and cytoplasm were S-100 positive, it can be assumed that these cells are glial-like cells, because similar co-expression was reported in the perivascular dendritic cells of the intestinal tract of the Chinese soft-shelled turtle, *Pelodiscus sinensis* [2]. However, negativity of the tumor cells for GFAP negates the origin of GC in the neurofibrils [12–14, 17].

Based on the immunohistochemical profile of GCT and the data from literature, the authors suggest an endomesenchymal origin, without associated microsatellite instability, of this unusual tumor.

Further studies are necessary to confirm c-KIT and RET kinase positivity and fusion gene in the malignant variant, and to eventually suggest a possible new target for individualized treatment of soft tissue tumors subgroups, including malignant GCTs, with multi-tyrosine-kinase inhibitors.

Acknowledgements The English-language manuscript was polished by SPI Global Professional Editing Service.

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