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CASE REPORT

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GANT-like gastrointestinal pacemaker cell tumours with oncocytic features

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Abstract We describe two cases of gastrointestinal stromal tumours with prominent oncocytic features. Both had features consistent with differentiation towards the interstitial cells of Cajal (CC). They were composed of nests and bundles of cells with abundant, deeply granular, eosinophilic cytoplasm. Immunohistochemical investigations revealed positivity with c-*kit*, vimentin and CD34 antibodies in both neoplasms. Ultrastructurally the neoplastic cells showed characteristic features of CC; they had synapse-like structures and dense core cytoplasmic granules. Oncocytic features were confirmed by immunohistochemistry using anti-mitochondrion antibody in both cases and by electron microscopy in one case (case 1). Although the CC are frequently described as mitochondrion-rich cells, oncocytic changes have not previously been reported as a feature of gastrointestinal autonomic nerve tumour (GANT)-like stromal tumours.

Keywords Oncocytoma · Gastrointestinal autonomic nerve tumour · Mitochondria · Oncocytic changes

Introduction

Gastrointestinal pacemaker cell tumours (GIPACT) is the designation recently proposed [12] for stromal tumours arising in the gastrointestinal tract. These tumours are derived from neoplastic elements that share ultrastructural and immunohistochemical features with the cells of Cajal (CC), a complex network of interstitial cells involved in the regulation of intestinal motility [28].

In this paper, two cases of gastrointestinal stromal tumour with evidence of differentiation toward the CC are presented. Both tumours showed prominent oncocytic changes documented by immunohistochemistry and ultrastructure. Oncocytic changes in nonepithelial tumours are rare, and only occasional examples have been reported [8, 10, 12, 17, 19, 25].

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Case reports

Case 1

A 50-year-old woman presented with nausea, vomiting and abdominal discomfort. CT scan revealed a large intra-abdominal mass measuring 10 cm in its major axis, located in the anterior side of the gastric body. At surgery, the tumour appeared well circumscribed with expanding margins and was closely adherent to the muscular wall of gastric body; a partial gastrectomy was performed. One year after surgery the patient is alive with liver metastases.

Case 2

The patient, a 60-year-old man, presented with abdominal pain. CT scan revealed the presence of a mass of 8.5×5×3 cm on the lesser curvature wall of the stomach. At surgery, the tumour was found to be well circumscribed, without infiltration of the gastric wall and was easily resected. The patient is in good health 6 months after surgical treatment.

Materials and methods

Tissues were fixed in 10% buffered formalin, embedded in paraffin and routinely stained with haematoxylin-eosin (HE). For immunohistochemistry, the avidin-biotin-peroxidase complex method was used. The antisera are listed in Table 1. For electron microscopy, formalin fixed tumour samples from case 1 were postfixed in osmium tetroxide, dehydrated in graded ethanol solutions and embedded in Araldite. Thin sections were stained with uranyl

Table 1 Antisera employed for immunohistochemistry

Antiserum (clone)	Source	Dilution
c-*kit* (polyclonal)	Santa Cruz Biotechnology (Calif.)	1:1000
Vimentin (V9)	Dako, Milan, Italy	1:100
Mitochondrion (Mito 113)	Biogenex,SanRamon, Calif.	1:50
Neuron specific enolase (NH3)	Biogenex	1:50
Neurofilaments (2F11)	Dako	1:60
S100 Protein (Polyclonal)	Dako	1:1000
CD68 (PGM1)	Dako	1:100
Cytokeratin (MNF116)	Dako	1:100
Smooth muscle actin (1A4)	Dako	1:100
CD34 (Qb-end 10)	Novocastra, Newcastle, UK	1:50
CD21 (1F8)	Dako	1:20
Synaptophysin (SY38)	Dako	1:40
Chromogranin (LK2H10)	Biogenex	1:400
Desmin (D33)	Dako	1:200

acetate and lead citrate and examined in a Philips 400T transmission electron microscope.

In order to evaluate the number and distribution of mitochondria in "ordinary" GIPACT, we used anti-mitochondrial antibody to stain 9 consecutively observed tumours with ultrastructural and/or immunohistochemical (c-*kit* positivity) differentiation toward CC but without apparent oncocytic features.

Pathological findings

Both tumours appeared well circumscribed and were surrounded by a fibrous pseudocapsule. They were composed of whitish grey soft tissue with a pseudocystic appearance and large areas of necrosis and haemorrhage.

Histologically, the tumours were mainly composed of cellular proliferations of cells arranged in small nests and fascicles (Fig. 1). Most of the neoplastic cells had abundant eosinophilic and finely granular cytoplasm (Fig. 2). In occasional cells, the nucleus was located peripherally and the cytoplasm showed large empty, clear vacuoles, giving an appearance reminiscent of that of signet ring cells. In these cells, the nuclei varied in shape from round to oval and sometimes showed prominent nucleoli. In case 2, some areas of the tumour were composed of spindle cells arranged in short bundles with a storiform architecture, and a syncytial growth pattern focally. In these latter areas, the neoplastic cells had irregular, pleomorphic nuclei, with clumped chromatin and prominent nucleoli. Multinucleated neoplastic cells were also occasionally present. Irregular mitoses were numerous in both cases, as were areas of necrosis and haemorrhage. Numerous lymphocytes, plasma cells and eosinophils were evident throughout the tumours, and were particularly prominent in case 2.

A network of small, delicate capillaries surrounded the cellular nests throughout the lesions.

In case 1, metastases were found in two out seven lymph nodes of the greater gastric curvature. The metastatic tumour showed the same histological features as the primary.

Most cells in both cases were diffusely stained by anti-vimentin and anti-c-*kit* antisera (Fig. 3a, b). In case 2 anti-neuron-specific enolase (NSE) stained numerous neoplastic elements, while in case 1 only rare cells were stained with this antiserum. CD34 antibody stained about 25% of the neoplastic cells in both cases. Anti-S100 protein antibody highlighted the presence of numerous dendritic cells throughout the tumour in both cases. The same cells were also CD68 positive. Neurofilaments, wide-spectrum cytokeratin, chromogranin, synaptophisin, smooth muscle actin, desmin, S100 protein, CD68 and CD21 antibodies were all consistently negative in the neoplastic cells in both cases.

When stained with anti-mitochondrion antiserum, most of the neoplastic cells in both cases showed a diffuse and intense granular positivity throughout the cytoplasm (Fig. 4a, b). The positive granules varied from coarse to very minute, and were irregularly distributed throughout the cytoplasm, occupying more than 60% of it. In the areas showing clear cell changes at the H-E level the positive granules were seen packed together in large aggregates, located sometimes at the periphery of the cytoplasm (Fig. 4b).

In eight out of nine cases of "ordinary" GIPACT (Fig. 5a) only a few cytoplasmic granules were stained with anti-mitochondrial antibody in 10–30% of the neoplastic cells (Fig. 5b), while the positivity with this antibody was not detectable in a large majority of the cells. In the last case about 30% of the neoplastic elements showed numerous positive granules throughout the cytoplasm.

Ultrastructural examination in case 1 confirmed that in most of the neoplastic cells the cytoplasm was entirely filled with numerous enlarged mitochondria (Fig. 6). Other cytoplasmic organelles were scarce. The cells displayed prominent bulbous cytoplasmic projections, containing empty vesicles and rare electron-dense granules, ranging in diameter from 180 to 290 nm. Focally, the cytoplasmic projections were joined by asymmetrical junctions, resembling postsynaptic membranes (Fig. 6, inset). Nuclei were irregularly shaped and showed clumped chromatin and prominent nucleoli.

Discussion

The cases here described show morphological features of gastrointestinal stromal tumours with evidence of differentiation toward the interstitial cells of Cajal. They stained with c-*kit*, vimentin, CD34 and NSE antisera, and in case 1 electron-dense granules and synapse-like structures were observed at the ultrastructural level.

Stromal tumours arising in the gastrointestinal tract have been under discussion for many years, particularly with regard to their origin and differentiation. The existence of a wide spectrum of tumours with variable ultrastructural and immunohistochemical findings led to the identification of different subgroups, including tumours with smooth muscle differentiation, neural differentiation (previously named "plexosarcomas" or "gastrointes-

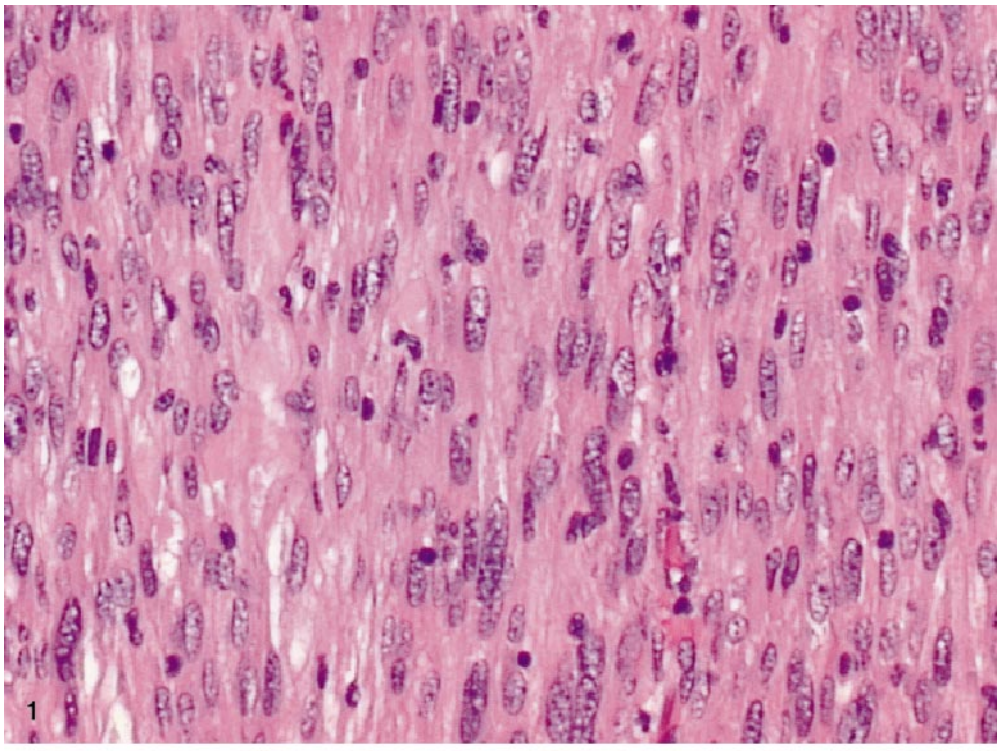

Fig. 1 Case 2: in this tumour, the neoplastic cells are spindle shaped and are arranged in parallel fascicles

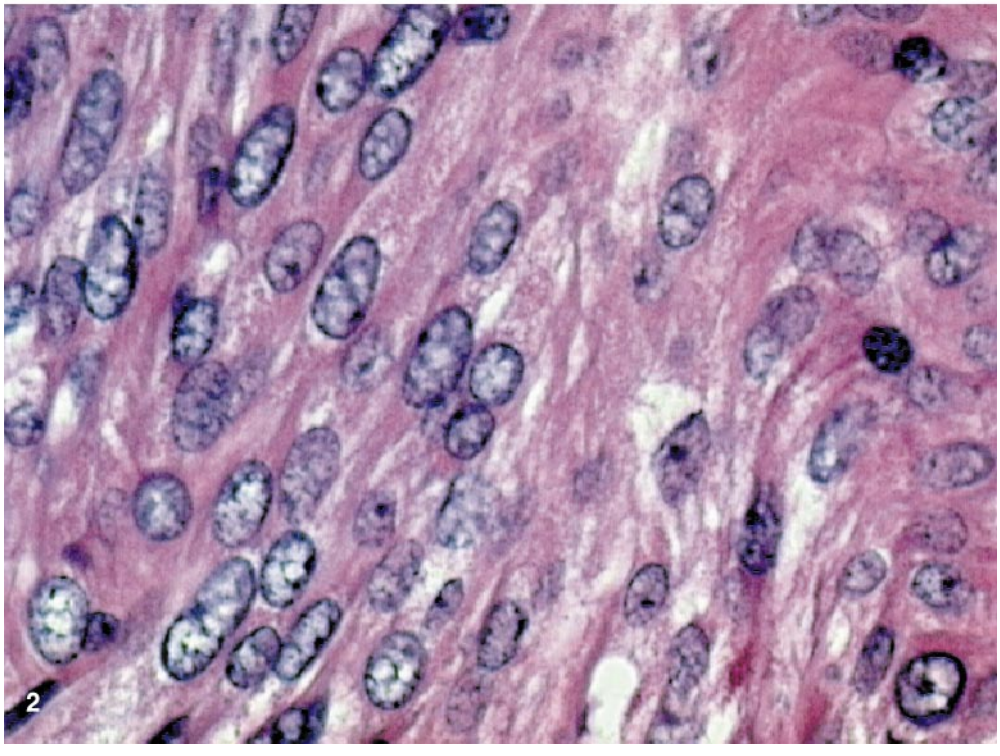

Fig. 2 Case 2: the cells have granular, deeply eosinophilic cytoplasm and pleomorphic nuclei with coarse chromatin

tinal autonomic nerve tumours and dual muscle and neuronal differentiation" [2, 5, 6, 10, 13, 16, 29].

The interstitial cells of Cajal are stromal cells present in the myenteric and submucosal plexuses. They are intercalated between the nerve fibres and muscle cells and are now considered as pacemakers in the regulation of the intestinal motility [1]. Recently, the identification of the c-*kit* tyrosine kinase receptor as a specific marker for the interstitial cells of Cajal [15] prompted investigators to strive for better definition of the morphological and functional features of these cells.

Using anti-c-*kit* antibodies to identify the interstitial cells of Cajal in the intestinal wall, it has been demonstrated that the morphological features reported to be di-

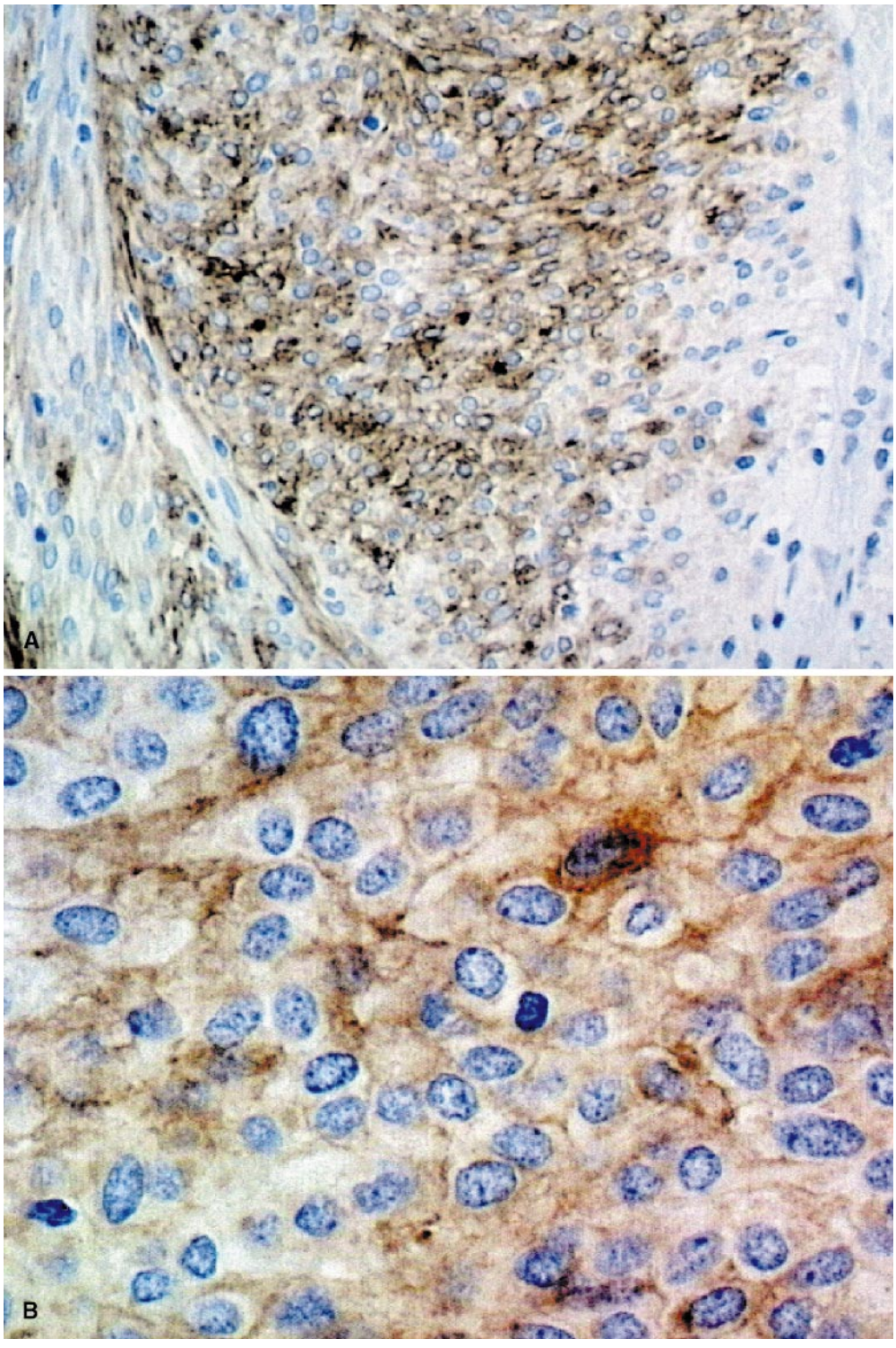

Fig. 3A, **B.** Immunostaining for anti-c-*kit* antibody: most of the neoplastic cells in case 1 (**A**) and in case 2 (**B**) are diffusely positive for this antibody. In case 1 (**A**) the tumour is composed of round to polygonal cells with regular ovoid nuclei and granular eosinophilic cytoplasms and well-defined borders. C-*kit* positivity is mainly localized at the cell membrane

agnostic of neuronal differentiation in the so-called gastrointestinal autonomic nerve tumours can be superimposed on the cell of Cajal [11] and that these same cells can also exhibit myoid and fibroblast-like features [26–28].

Consequently, Kindblom et al. [12] introduced the unifying concept of gastrointestinal pacemaker cell tumours (GIPACT) to describe neoplasms showing c-*kit* immunoreactivity and variable evidence of differentiation toward the cells of Cajal.

Focal positivity with CD34 antiserum was seen in both the present cases. The significance of CD34 positivity in GIPACT is uncertain, but it has been described in most GIST/GIPACT [3, 6, 12, 16, 20] as well as in non-

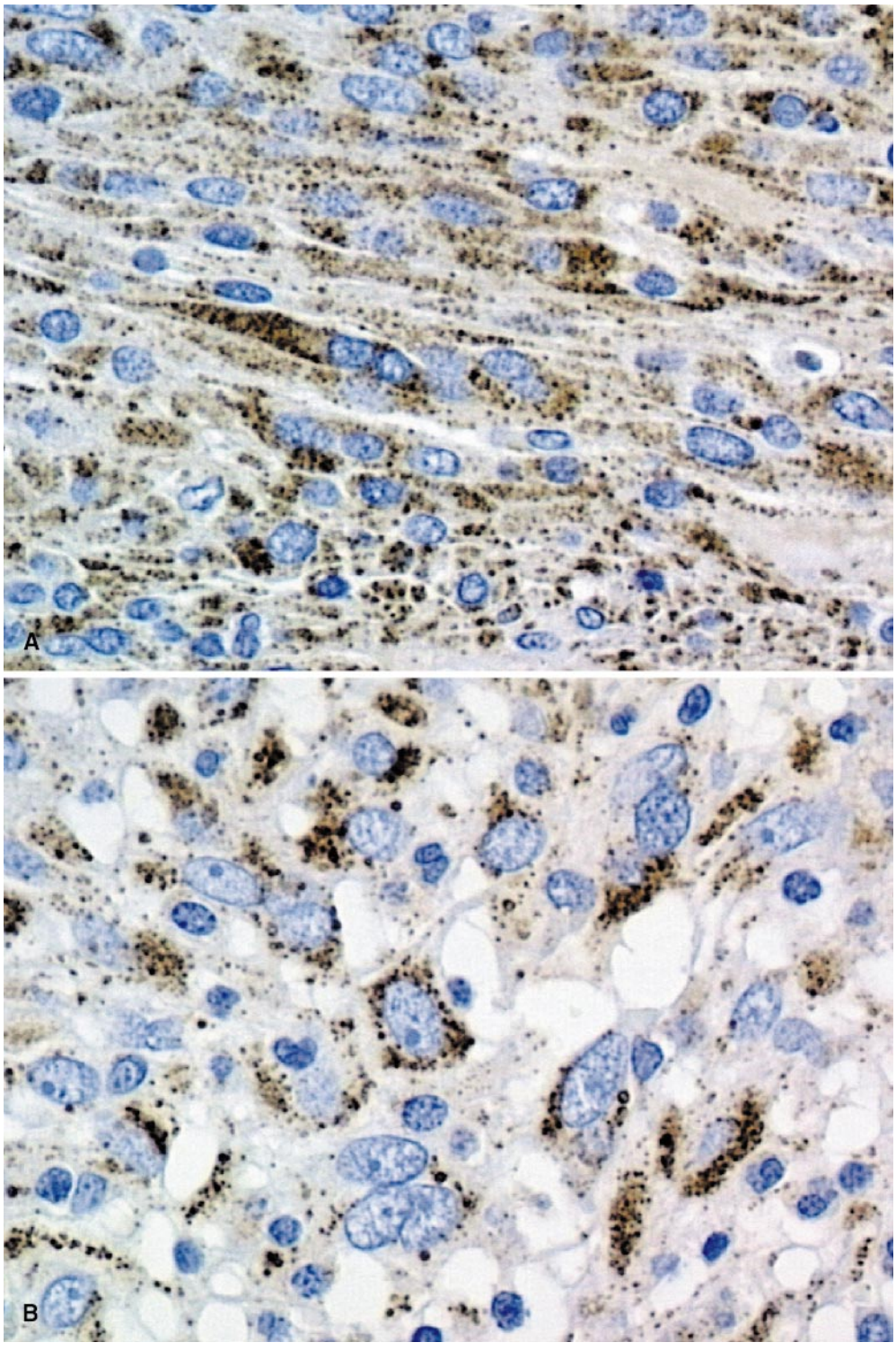

Fig. 4A, **B.** Immunostaining for anti-mitochondrial antibody. **A** Case 2: almost all the neoplastic elements are positive with mitochondrion antiserum. The positive granules are irregularly dispersed throughout the cytoplasm. **B** Case 1: anti-mitochondrial antibody stains the cytoplasmic granules heavily. In some areas the nuclei are peripherally located and the cytoplasm show large empty vacuoles, somewhat reminiscent of signet ring cells. In these areas, mitochondria are packed together, some at the periphery of the cytoplasm

neoplastic interstitial cells of Cajal [12]. Gastrointestinal leyomiomas and schwannomas are consistently CD34 negative [12, 16, 32].

At light microscopic level, the cytological features of the present cases of GIPACT were indicative of oncocytic changes. The neoplastic cells were characterized by abundant deeply eosinophilic granular cytoplasm. The granules were demonstrated to be mitochondria by immunohistochemistry and, in case 1, by the ultrastructural study. Most of the neoplastic cells displayed 60% of their cytoplasm occupied by mitochondria.

The specificity of the anti-mitochondrion antiserum was previously assessed in routinely processed tissues by Papotti et al. [21] and by Western-blot analysis by

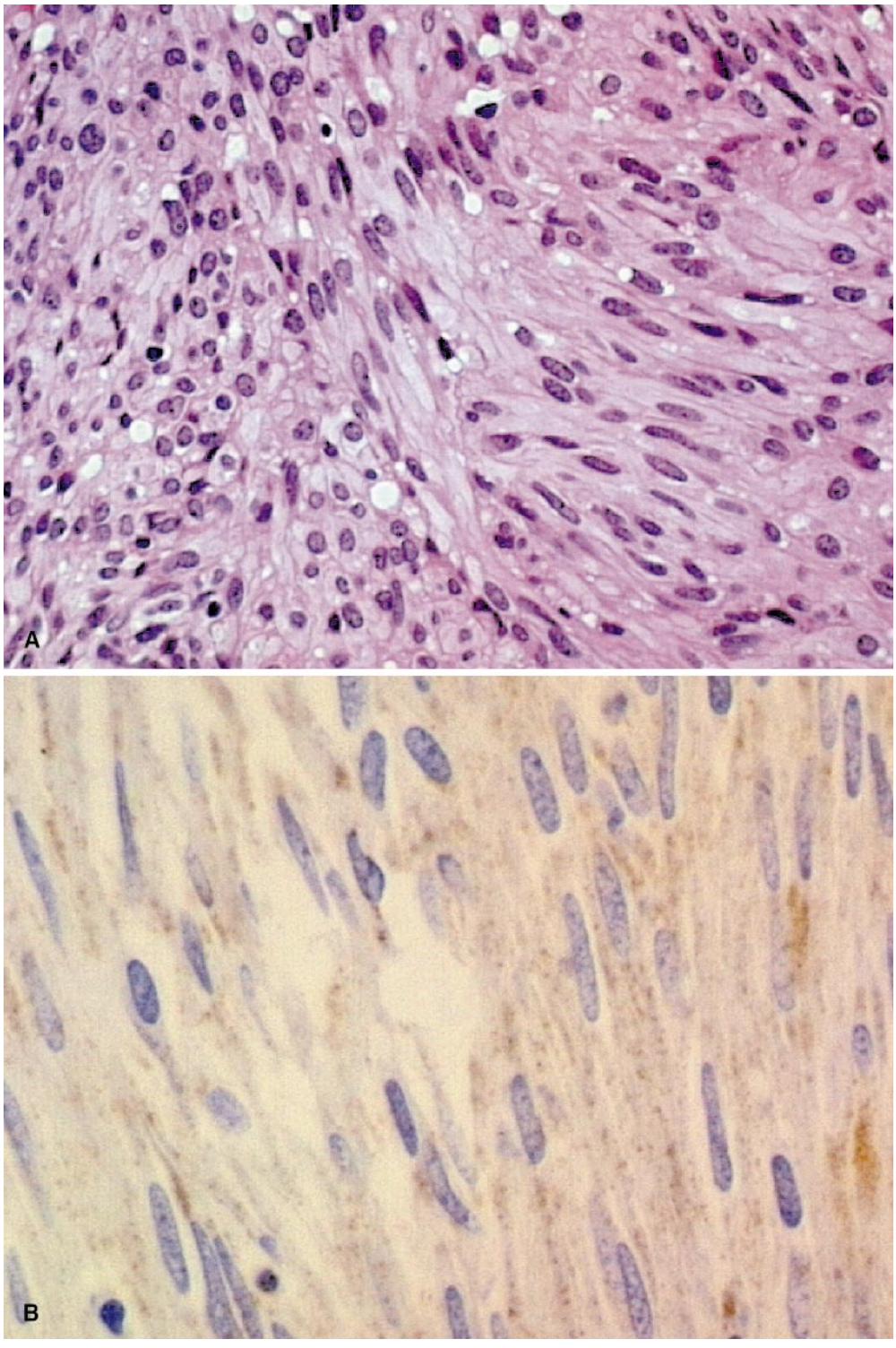

Fig. 5A, **B.** Ordinary (nononcocytic) gastrointestinal pacemaker cell tumour. **A** The tumour is composed of spindle-shaped cells with well-defined borders. The cytoplasm of the neoplastic cells is pale, eosinophilic, and homogeneous. **B** Anti-mitochondrial antibody stains only few cytoplasmic granules

Roncaroli et al. [25]. In addition, the same antibody stained no more than 30% of the cells in the control cases of GIPACT without apparent oncocytic features.

It appears that the present tumours should be included in the definition of oncocytomas originally proposed by Hamperl in 1962 [9]. The neoplastic cells appeared ”swollen”, with an epithelioid appearance. Tremblay and Pearse [31] requested that this should be specified as a criterion to distinguish between mitochondrion-rich and oncocytic elements. Most of the cells in both our cases had their cytoplasm entirely occupied by mitochondria, exceeding the 60% minimum of the total cytoplasmic area that was the cut-off point requested by Ghadially to qualify a cell as oncocytic [8].

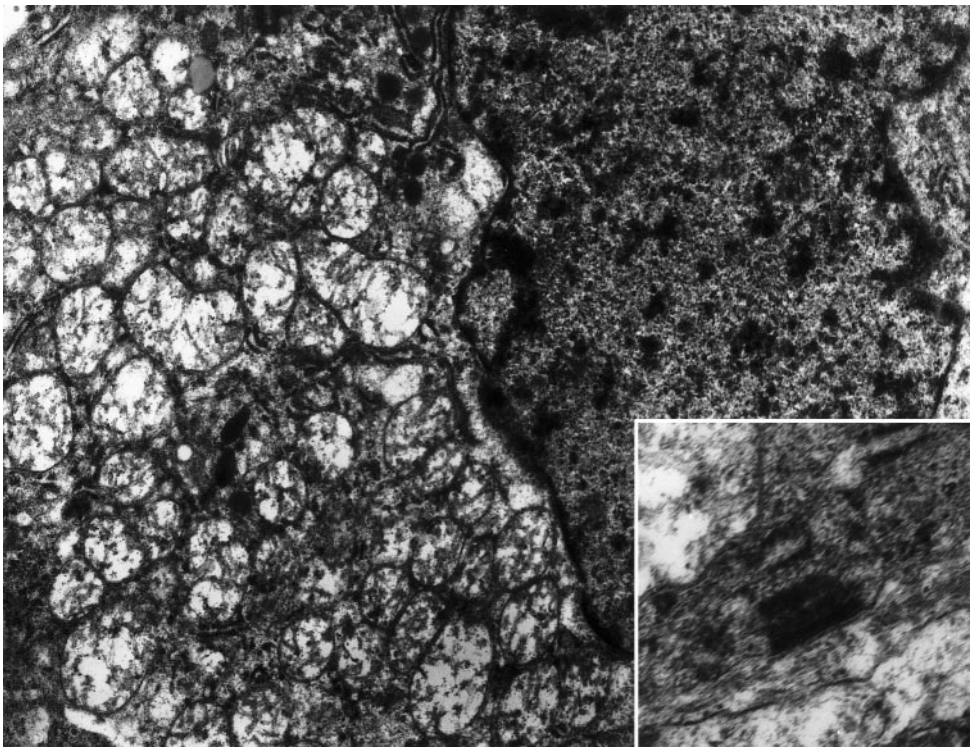

Fig. 6 On ultrastructural examination, the cytoplasm of the tumour cells appears to be entirely occupied by large mitochondria. Clusters of electron-dense granules are also evident. (×16,000). In set: Cytoplasmic projection with a solitary junction of the postsynaptic type. ×50,400

Nonepithelial oncocytomas are rare. Apart from a series of oncocytic meningiomas [25], only single cases of oncocytic mesenchymal tumours have been reported [14, 23, 30]. Oncocytic changes have been described in a case of intestinal leiomyosarcoma [18]. Although the presence of numerous large mitochondria is described as a characteristic feature of CC [28], the occurrence of oncocytic changes has not previously been reported as a feature of GIPACT. Millard and Bishop, in 1984 [17], reported a 5-cm tumour of the lesser curvature of the stomach. Although the illustrations provided are very suggestive of a GANT-like GIPACT, no attempt was made to establish the nature of the tumour, apart from the ultrastructural demonstration of the presence of abundant mitochondria in the cytoplasm of the neoplastic cells.

The significance of oncocytic changes in GANT-like GIPACT is not known, and whether they represent a merely morphological finding or a biologically meaningful change cannot be determined here. Nevertheless, oncocytic GIPACT may occur, and the presence of "swollen" cells with abundant, deeply eosinophilic, granular cytoplasm can lead to problems in the differential diagnosis against other neoplasms sharing similar features, such as neuroendocrine carcinomas, granular cell tumours and lysosome-rich smooth muscle cell neoplasms.

In the present cases, the lack of anti-cytokeratin and chromogranin antisera ruled out the possibility of carcinomas with neuroendocrine differentiation, while the negative results obtained with muscular markers together with CD68 antibody allowed confident exclusion of granular cell smooth muscle tumours [22, 25]. Granular cell tumours of peripheral nerve origin may express NSE [19], as in the present cases, but are also diffusely S100 protein positive, and moreover, the granular quality of the cytoplasm is due to the presence of membrane-bound vesicles filled with flocculent amorphous material, while only rare mitochondria are usually evident [4]. Extranodal follicular and interdigitating dendritic cell sarcomas are other lesions that can lead to problems in differential diagnosis. These neoplasms frequently arise in intra-abdominal locations. The neoplastic cells have slightly eosinophilic cytoplasm and often exhibit a syncytial growth pattern and prominent lymphocytic infiltrate [7], as in our case 2. However, the absence of CD21, CD68 and S100 protein immunoreactivity ruled out this possibility.

We have presented an hitherto undescribed feature of GIPACT, which must be recognized to avoid erroneous histological diagnoses. The biological significance must be established in a larger series.

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