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CASE REPORT

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Melanotic paraganglioma of the posterior mediastinum

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Abstract A melanotic paraganglioma occurred in a 57-year-old woman, located in the left paravertebral space of the upper mediastinum. It was totally resected. During a 5 year follow up period neither tumour reccurrence nor metastasis were observed. Histological examination of the tumour revealed a paraganglioma with monomorphous chief cell like elements which were arranged in a "zellballen" pattern. Immunohistochemical results also were in accordance with the diagnosis since neuron-specific enolase, chromogranin and synaptophysin were found in tumour cells whereas keratin was not. Additionally, neurosecretory granules were found in tumour cells during electron microscopy. A peculiar feature of the tumour was its strong pigmentation due to melanin located within the tumour cells and tumour associated melanophages. The simultaneous expression of functional properties of two different neural crest derived cells in one tumour stresses the close relationship between all neural crest elements and is in accordance with the observation of other melanotic, non-melanomatous tumours.

Key words Paraganglioma · Melanin · Neurosecretory granules · Mediastinum

Dedicated to Prof. Dr. Dr. mult. h.c. W. Doerr on the occasion of his 80th birthday

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Introduction

The widespread system of paraganglions, the cells of which derive from the neural crest, can be subdivided into the different families of branchiomeric, intravagal, aortico-sympathetic and visceral-autonomic paraganglia on the basis of anatomical distribution. They give rise to the paragangliomas which are similarly classified into different families [6, 13]. Mediastinal paragangliomas are rare and either belong to the family of branchiomeric paragangliomas if located in the anterior mediastinum or the family of aortico-sympathetic paragangliomas located in the posterior mediastinum. Tumours of the former group are less frequent than the latter [17]. We report on a case of a paraganglioma of the posterior mediastinum which exhibited melanin production as an additional feature. Only three cases of melanotic paragangliomas have been reported in the literature [19, 20] and our case is the first occurring in the posterior mediastinum. The simultaneous expression of functional properties of two different neural crest derived cells in one tumour stresses the close relationship between all neural crest elements and is in accordance with the observation of other melanotic, non-melanomatous tumours.

Case report

A 57-year-old woman presented with discomfort and fatigue. A chest radiograph showed a solid tumour in the left upper posterior mediastinum. In computed tomography and magnetic resonance imaging it was seen to be adjacent to the dorsal pleura and spine and to displace the aortic arch. Infiltration of the descending aorta by the tumour was suspected. Bronchoscopically the back wall of the left main bronchus seemed to be elevated by the tumour. Mucosal irregularities were not found bronchoscopically. No clinical or biochemical signs of a hormonally active tumour were found.

The preoperative diagnosis was that of a tumour of probable neurogenic origin. A left antero-lateral thoracotomy via the fourth intercostal space was performed. Intraoperatively a solid extrapulmonary tumour was found located in the left paravertebral space covered by the parietal pleura. Only some adhesions to segments 2 and 6 of the left lung as well as to the descending aorta were found and dissected. The tumour was totally resected.

The postoperative course of the patient was uneventfull. She was discharged from the hospital at the 14th postoperative day.

About 5 years after surgery there are no signs of tumour recurrence or metastasis.

Materials and methods

Immediately after removal the tumour was fixed in 4% buffered formalin; small formalin-fixed pieces were retrieved and refixed in 3% glutaraldehyd for electron microscopic examination. Formalin-fixed material was embedded in paraffin and 4–5 µ thick sections were cut for light microscopic and immunohistological examination. Glutaraldehyde-fixed tissue was postfixed in 2% buffered osmium tetroxide, embedded in epon araldite and semithin and ultra-

Fig. 1a Normal histology of the tumour shows monomorphous tumour cells with indistinct cytoplasmic outlines arranged in a "zellballen" pattern (PAS, ×150). **b** Densly packed, pigment laden macrophages are situated around intratumoural blood vessels (HE, ×193). **c** Capillary blood vessels surrounding tumour cells and grouping them to small nests (semithin section, toluidine blue, ×380). **d** Pigment granules are visible within the cytoplasm of tumour cells (semithin section, toluidine blue, ×609)

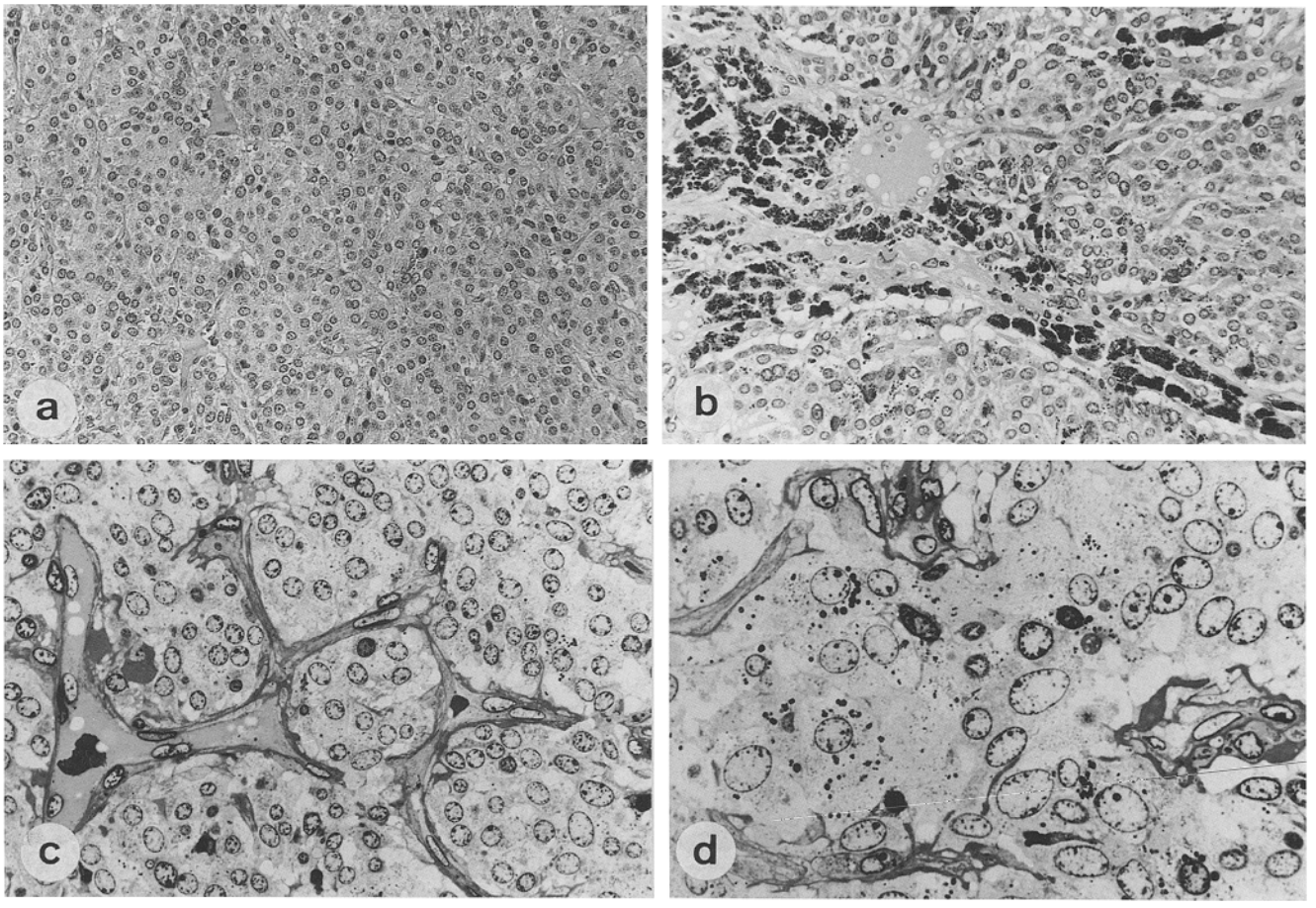

Table 1 Primary antibodies used, source, dilution and immunoreactivity with tumour cells in immunohistochemical examination

Antibody against	Clone	Source	Dilution	Reactivity with tumour cells
Keratin	KL1	Dianova	1:100	Negative
Keratin	AE1+3	Camon	Ready to use	Negative
Vimentin	Vim3B4	Camon	Ready to use	Positive
Glial fibrillary acidic protein (GFAP)	6F-2	Dako	1:100	Negative
Neurofilament	DP 5.43.12	Camon	Ready to use	Negative
S-100 Protein	Polyclonal	Dako	1:100	Negative
Melanoma associated antigen	HMB45	Camon	Ready to use	Negative
Neuron-specific enolase (NSE)	MIG-N3	Camon	Ready to use	Positive
Chromogranin	LK2H10	Camon	Ready to use	Positive
Synaptophysin	Sy38	Progen	Ready to use	Positive
Calcitonin	Polyclonal	Dako	1:100	Negative
Glucagon	Polyclonal	BioGenex	Ready to use	Negative
Insulin	Polyclonal	BioGenex	Ready to use	Negative
Serotonin	5HT-H209	Dako	1:10	Negative
Somatostatin	Polyclonal	BioGenex	Ready to use	Negative

thin sections were cut for light microscopic and electron microscopic examination, respectively.

Paraffin sections were stained with haematoxylin and eosin, PAS, Prussian blue, Masson Fontana reaction with and without bleaching. Semithin sections were stained with toluidine blue, ultrathin sections were contrasted with uranyl acetate and lead citrate. Immunohistological investigation was carried out with the streptavidin-biotin peroxidase method using the Vectastain elite kit from Vector laboratories and amino ethylcarbazole as chromogen. The monoclonal and polyclonal antibodies used are listed in Table 1.

Results

Macroscopically the tumour measured 11×8×4.5 cm, was encapsulated and the formalin-fixed material showed a dark-grey to black cut surface.

Histologically, the tumour was composed of relatively uniform large cells with round to oval nuclei and with inconspicuous cell borders. Cell pleomorphism was minimal and only few mitotic figures were found. The cells were arranged in small nests surrounded by capillary blood vessels (Fig. 1a, c, 2a). A histological hallmark of the tumour was its strong pigmentation: small and round, dark-brown to black granules were visible in the cytoplasm of tumour cells (Fig. 1d). The number of granules varied from cell to cell and within the tumour exhibiting heavy and less pigmented areas. The heaviest pigmented cells, however, did not belong to the tumour cell population but were macrophages which accumulated around blood vessels (Fig. 1b). The pigment was iron negative and gave a positive reaction with the Masson Fontana stain (Fig. 2b).

Immunohistological examination of the tumour with a battery of monoclonal and polyclonal antibodies revealed the following results (Table 1): all tumour cells including those with pigment granules exhibited a positive reaction with antibodies against neuroendocrine marker-proteins (neuron specific enolase, chromogranin A, synaptophysin) (Fig. 2c, d). The tumour cells were positive for vimentin but negative for keratin intermediate filaments. None of the tumour cells were positive for glial fibrillary acidic proteins (GFAP), neurofilament and S-100 protein. None of the five hormones tested could be found in tumour cells.

Electron microscopic examination revealed typical dense core, neuroendocrine granules with diameters from 100 to 200 nm in the cytoplasm of tumour cells (Fig. 3b). Numbers of granules, however, varied from cell to cell, with some cells having only few or none of them. Additionally, membrane limited pigment granules 750 to 1000 nm in diameter were found (Fig. 3a). The

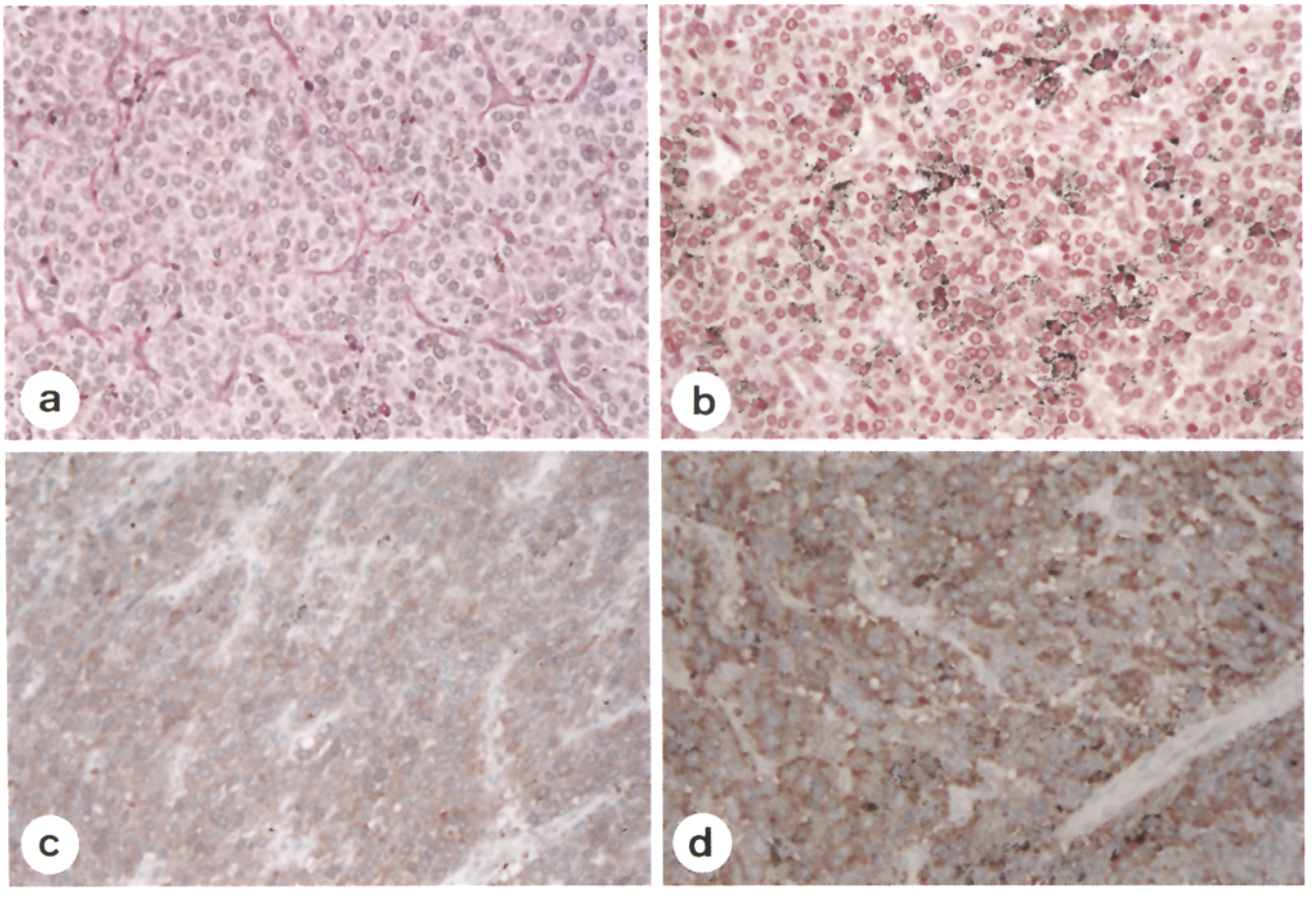

Fig. 2a The tumour cells are PAS negative (PAS, ×200). **b** Positive reaction of the pigment granules with the Masson Fontana stain (×200). **c** Homogeneous positivity of tumour cells for NSE (anti-NSE, ×156). **d** Positivity of the tumour cells for chromogranin A. Note the pigment in chromogranin A positive tumour cells (anti-chromogranin, ×200)

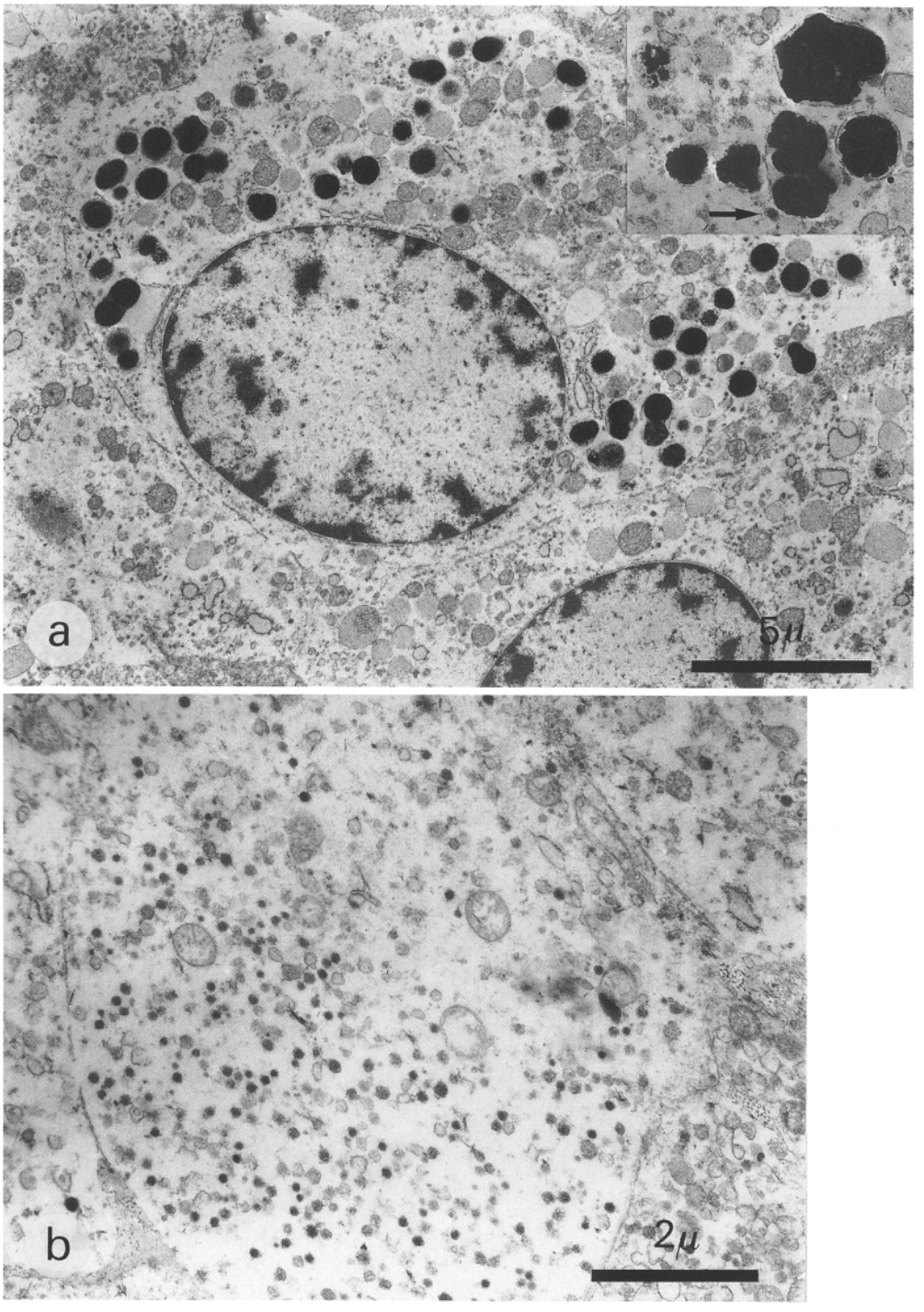

Fig. 3a Membrane bound pigment granules within the cytoplasm of a tumour cell, which also contains much smaller neurosecretory granules (*arrows*). Inset: polycyclic pigment granules (additional magnification factor of inset 2.3). **b** Numerous neurosecretory granules in a tumour cell without pigment granules (glutaraldehyde-refixed after retrieval from formalin-fixed material)

overall shape of the pigment was round to oval but polycyclic forms were also observed. Most of the pigment was electron dense. The pigment of some smaller granules, however, also showed an inner striated structure. Neuroendocrine granules and pigment granules sometimes were found in the same cell.

Discussion

A variety of possibilities had to be considered in the differential diagnosis of this tumour of the posterior mediastinum. A tumour of neurogenic or Schwann cell origin [1, 12] was suspected from its location and intense contact to structures of the paravertebral region. The melanin pigmentation of the tumour would also speak in favour for this diagnosis since melanotic Schwannomas are well known. Neither the histomorphology, however, nor the negativity for S-100 protein of the tumour cells are in accordance with this diagnosis. The melanin pigmentation of the tumour raised the question of a malignant melanoma, primary or metastatic. The relative uniformity of the tumour cells with only some mitotic figures on the one hand and the lack of S-100 protein positivity and

the negative immunohistochemical result with mAb HMB45 on the other strongly argues against this suggestion. This is supported clinically, since no primary malignant melanoma could be found and, retrospectively, no tumour reccurrence or metastasis has occurred up to 5 years after resection.

The relative uniformity of the tumour cells and their neuroendocrine nature demonstrated by positivity for NSE, chromogranin A and synaptophysin and by the presence of neuroendocrine granules suggested the possibility of a neuroendocrine tumour in the sense of a carcinoid. Melanotic carcinoids of the mediastinum have been reported in the literature [10, 14], however, they occurred in the thymic region of the anterior mediastinum and their melanin was synthesized by melanocytes or melanocyte-like dendritic cells associated with the neuroendocrine tumour cells. Additionally, carcinoids are, unlike the tumour under discussion, keratin positive, a feature which was repeatedly used to discriminate them from tumours such as paragangliomas [9, 16], although few examples of paragangliomas of the head and neck have been reported to be positive for keratin [11].

The diagnosis which fits best with the different features of the tumour is paraganglioma with melanin production. The location of the tumour in the paravertebral space in close connection to the sympathetic trunk is typical for the paragangliomas of the aortico-sympathetic family [6]. Histomorphology also is in accordance with this diagnosis, since the monomorphic tumour cells resembling chief cells of normal paraganglia were arranged in small groups surrounded by capillary blood vessels revealing the aspect of the so called "zellballen" pattern [13]. Furthermore, the neuroendocrine nature of the tumour and the negativity of the tumour cells for keratin intermediate filaments is a typical constellation for this tumour type [9, 16, 17]. The lack of immunoreactivity for S-100 protein and GFAP might be due to the absence of sustentacular cells in the tumour portion examined. Sustentacular cells of paraganglia may be rare in their tumours [6, 13] and lack of [17] or focal positivity for S-100 protein and GFAP in only a small portion of the tumour was reported [16, 17, 20].

The pigmentation of this non-melanomatous tumour is a peculiar feature. The pigment was identified as melanin histochemically as well as by electron microscopy. Its localization within the tumour cells could be proved by the simultaneous positivity of the neuroendocrine marker proteins and the simultaneous presence of neurosecretory granules. Since no typical melanocytes were identified within the tumour and since such cells are not known to occur in this region of the body it is assumed that the melanin was synthesized rather than phagocytized by the tumour cells.

Non-melanomatous tumours with melanin pigmentation are described and many of them belong to the family of neuroendocrine or neural crest cell derived tumours [3, 5, 7, 10, 14, 15, 19, 20]. They may be subgrouped into those, with melanin production by melanocyte-like cells, which either are believed to be only tumour associated [10] or a second tumour cell population [7, 14, 15], and into those where the pigment is produced by a non-melanocytic tumour cell population [3, 5, 19, 20]. Our tumour belongs to the last group, since melanin and neurosecretory granules, the morphologic equivalents of two different functional properties, were found in one and the same cell. Apart from the observation of melanin production by phaeochromocytomas [8] only three melanotic paragangliomas with the same functional properties are described in the literature, one occurrng in the orbit [19] the other two in the uterus [20].

The occurrence of cells within one tumour with different functional properties may serve as evidence for their interrelationship and close histogenetic origin. In the case of two tumour cell populations, however, the possibility of tumour collision has to be considered [7]. This can be eliminated if the two properties occur in one and the same cell population.

An explanation for the feature of simultaneous production of paraganglionic neurosecretory granules and melanosomes comes from the close connection of the two biochemical pathways producing either melanin or epinephrines. In both pathways the primary substrate is L-tyrosine, which is hydroxylated to L-Dopa either by tyrosinase giving rise to melanin and melanosome differentiation in the melanocyte or by tyrosine hydroxylase with subsequent development of epinephrines and neurosecretory granules in the paraganglionic cell [2]. Bagnara, in his concept of the common origin of pigment cells [2] pointed out that the content of a Golgi derived fusion vesicle with an endoplasmatic reticulum derived primordial vesicle is responsible for the development of the pigment granule. He further suggested to extend this concept to non-pigment cell differentiation of neural crest since melanophore differentiation in the cultured neural crest elements which normally form ganglia were already reported [4, 18]. Thus, the availability or unavailability of one enzyme might be responsible for the development of an epinephrine containing neurosecretory granule or a melanosome. The observation of a simultaneous presence of both organelles in the cells of the tumour under discussion indicates activity of both enzymes and argues in favour of a genetic rather than an enzymatic [20] regulation of the process.

Thus, this tumour and the three others [19, 20] reported in the literature together with melanin-producing phaeochromocytomas [8] underline in vitro observations [5, 18] and support the concept of the close relationship of all neural crest derivates. Finally, the features of this tumour first assumed to be contradictory, support the diagnosis of a melanotic paraganglioma of the posterior mediastinum.

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