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ORIGINAL ARTICLE

Ultrastructure of pheochromocytoma: undescribed morphologic features

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Abstract We examined samples of human pheochromocytoma from 11 patients aged 30–70 years including one case of malignant pheochromocytoma with a view to identifying previously unreported ultrastructural details.

We identified two types of nuclear inclusions consisting of irregularly shaped singular or multiple granulofibrillar formations with a typical concentric halo, on the one hand, and accumulations of egg-shaped structures consisting of granules and microfilaments, on the other. In some of the tumor cells, membrane-covered inclusions containing parallel laminar elements arranged in a paracrystalline, periodic fashion, or megamitrochondriae characterized by increased electrodensity of their matrix, and fibrillary material in the spaces between the cristae were present. A frequent finding consisted of typical ciliary formations, while rough/smooth tubular aggregates of different size occurred less frequently. Finally, we were able to demonstrate the uptake of norepinephrine by smooth muscle fibers in the periphery of arterial vessels as evidenced by linear accumulations of membrane-covered granules separating bands of contractile smooth muscle components in the peripheral layers of arterial vessels close to norepinephrine producing neoplastic cells.

These findings represent ultrastructural features that contribute to further elucidating the ultrastructural characteristics of the human pheochromocytoma.

Keywords Pheochromocytoma · Nuclear inclusions · Paracristaline membrane bound bodies · Intracytoplasmic oligocilia · Rough/smooth tubules aggregates · Noradrenaline uptake

Introduction

Reports on the ultrastructural diagnostics of pheochromocytomas mostly analyze the morphology of the neuroendocrine granules within the cytoplasm [1–6]. As additional points, commonly found phenomena such as nuclear pseudoinclusions [7], mitochondrial abnormalities [8], striated rough tubular aggregates [9], intracytoplasmic desmosomes [10], lipid degeneration [11], eosinophilic globules [12], and cytoplasmic melanosomes [13] have been reported.

The purpose of this study consists in relating previously unreported ultrastructural details which further contribute to the ultrastructural description of the adrenal pheochromocytoma.

Materials and methods

Samples of human pheochromocytoma were obtained from 11 patients ranging in age from 30 to 72 years including one case that was classified as a malignant pheochromocytoma. Small fresh pieces were fixed and postfixed in 2.5% glutaraldehyde and osmium tetroxide in sodium cacodylate buffer, respectively, and embedded in Spurr's epoxy resin; ultrathin sections were stained with uranyl acetate-lead citrate. Of each tissue

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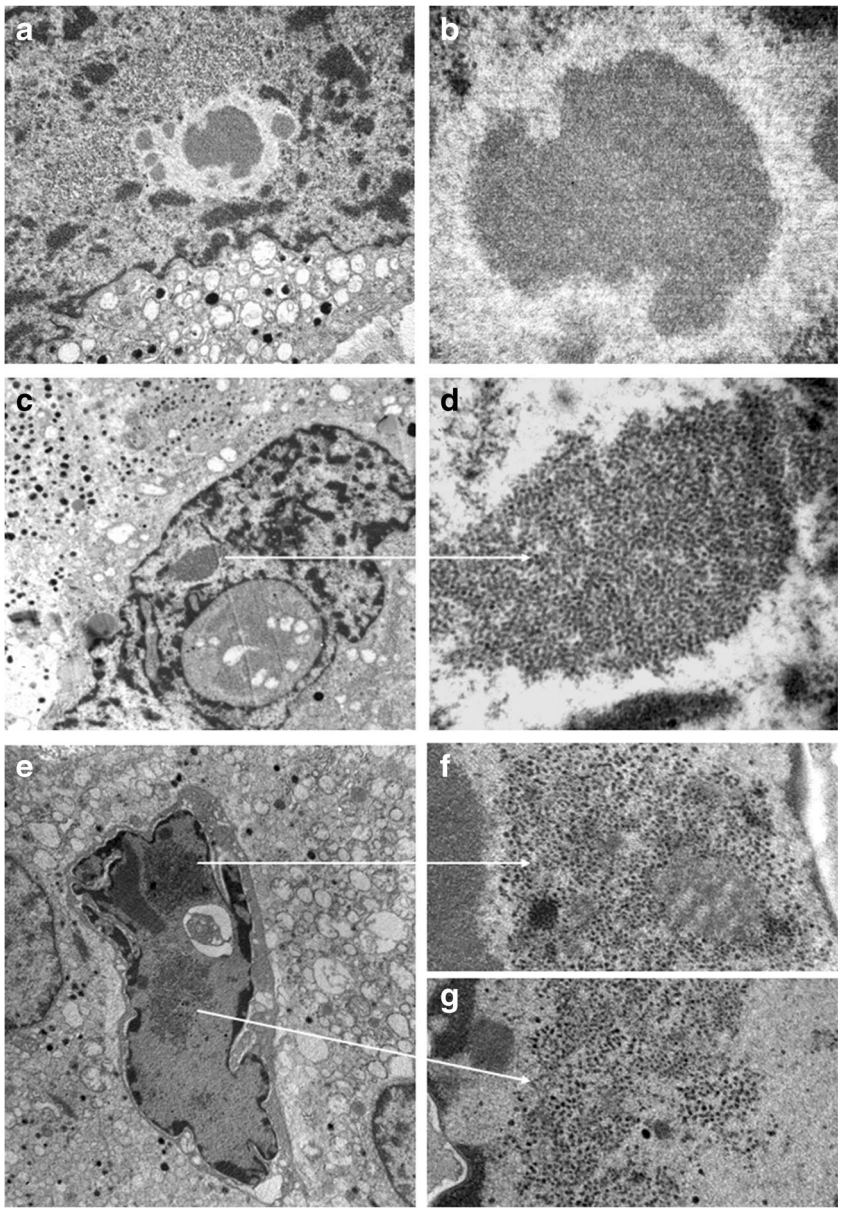

Fig. 1 **a** Central main inclusion with irregular contour and bright halo, surrounded by several other inclusions of smaller diameter. **b** Granulofibrillar structure of the inclusion viewed at greater magnification. **c** Ovoid inclusion located next to a cytoplasmic pseudoinclusion. **d** Closely spaced granulofilamentous elements. **e** Two inclusions with loosely arranged granulofilamentous elements in a nucleus with peripheral condensed chromatin. **f** Pseudoviral aspect seen at greater magnification. (**a** ×20000, **b** ×80000, **c** ×10000, d ×80000, **e** ×10000, **f**, **g** ×80000)

block, at least five serial sections cut with an interval of 15 μm were examined.

Results

We observed singular or multiple granulofibrillar formations with irregular contours (Fig. 1a) that are surrounded by a concentric bright halo separating the inclusions from the nuclear chromatin. The diameter of these nuclear bodies, which are finely granulated (Fig. 1b) and contain microfibrils at their edges, varies between 0.2 and 2 μm. In each ultrafine section, several singular inclusions were present, while multiple inclusions were found less frequently. The multiple inclusions are characterized by the presence of an element with a larger diameter in the center that is surrounded by usually five or six smaller inclusions of an identical structure.

Additionally, we identified a second type of nuclear body consisting of structures with an irregular egg-shaped contour (Fig. 1c) and a longer diameter of approximately 1.5 μm. Viewed at appropriate magnification, these structures were found to consist of granules and microfilaments each measuring between 22 and 25 nm (Fig. 1d) in diameter. Located very close to each other, these elements form larger accumulations. These formations were identified in several nuclei within each of the ultrafine sections of pheochromocytoma. In the nuclei of apoptotic cells (Fig. 1e), granular accumulations were occasionally seen with their constituent elements dispersed and

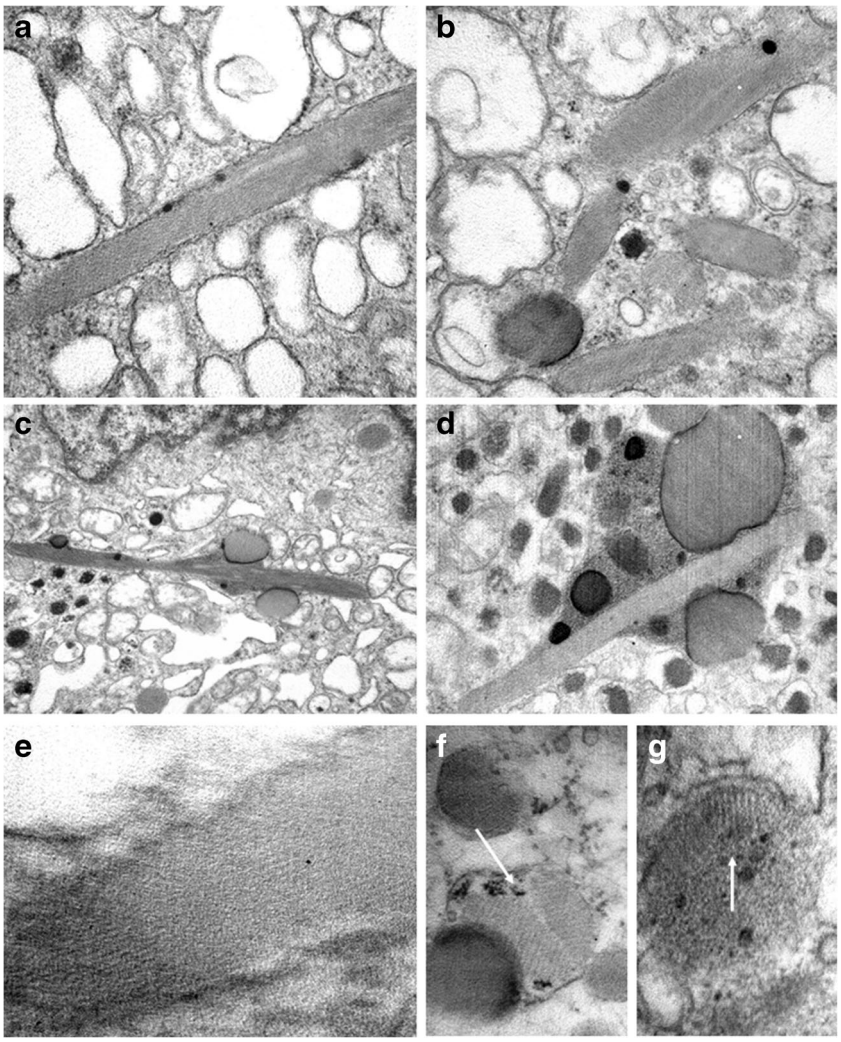

Fig. 2 **a** Singular lamellar complex with individual ribosomes adhering to the external membrane, located between dilated cisternae of the rough endoplasmic reticulum. **b** Complex consisting of five individual units. **c** Singular inclusion associated with lipid droplets. **d** Complex associated with a lysosomal formation containing lipids and lipofuscin pigments. **e** Paracrystalline aspect of the inclusion visible in some areas of the image, with periodic structure. **f** Paracrystalline alignment in two directions with individual elements forming an angle between them (*arrow*) **g** Peripheral paracrystalline aspect (*arrow*). (**a** ×60000, **b** ×60000, **c** ×30000, d ×50000, **e** ×250000, **f**, **g** ×300000)

with a pseudoviral appearance but still of the aforementioned characteristic diameter (Fig. 1e, f).

In some of the tumor cells, we found large structures delimited by membranes which contain fine and regularly aligned parallel laminar elements arranged in a paracrystalline, periodic configuration. The periodic interval is variable and ranges from 8 nm (Fig. 2d) to 20 nm (Fig. 2f) depending on the respective structure studied. The maximum length of these formations is 5 μm, and their thickness varies between 0.2 and 0.4 μm. Some segments of the membrane containing the periodic content are studded with ribosomes (Fig. 2a) while other segments appear bare. These elements occur individually (Fig. 2a) or in groups of units arranged in different spatial orientation (Fig. 2b). Some of them are associated with several lipid vacuoles (Fig. 2c), and others with secondary lysosomes containing lipids, neuroendocrine granules, and fine granular material (Fig. 2d). The paracrystalline inclusions may be aligned longitudinally (Fig. 2e) or at an angle (Fig. 2f) to one another.

In a small number of cells that varies from case to case, there is a relevant increase in the number of mitochondria per cell. If this is the case (Fig. 3a), the quantity of neuroendocrine granules decreases in proportion, and the cytoplasm may gain a truly oncocytic appearance. Frequently, this alteration coincides with the presence of significantly enlarged mitochondria exceeding 3 μm in length but without changes of their cristae or matrix (Fig. 3g). Mega-mitochondriae of considerable volume, in particular, may be present, with increased diameter and electrodensity of their matrix and fibrillary material in the spaces between the cristae (Fig. 3c).

In the cytoplasm of individual cells, we found accumulations of fine bundles of filaments located at a small distance from each other and oriented in different directions. The thickness of these bundles varies between 0.2 and 0.4 μm. In the periphery of these complexes, the secretory granules of the cell are located (Fig. 3d). The filamentous units have a diameter of approximately 10 nm. Occasionally, such aggregates of filamentous structures may be found incorporated within the interior of secondary lysosomes (Fig. 3e).

Rather frequently, we found ciliary formations with an axoneme emerging from a basal body and located within a

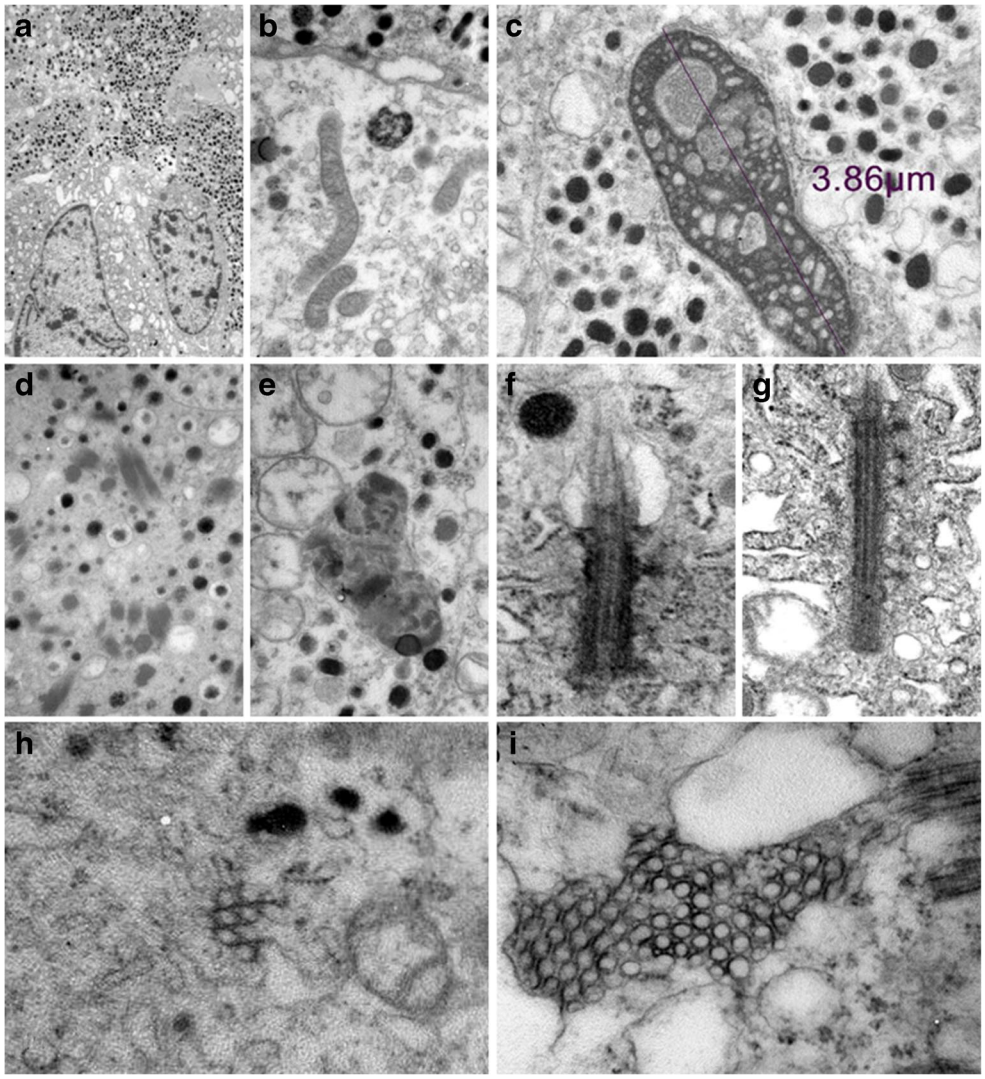

Fig. 3 **a** Two cells in the lower half of the image with a small number of granules. **b** Image showing the cytoplasm of a cell with few granules but containing enlarged mitochondriae, with the length of the mitochondriae by far exceeding normal dimensions. **c** Individual mitochondrium with dense matrix, with the spaces between the cristae filled with filaments of low electrodensity. **d** Bundles of filaments cut in different directions, intermixed with neuroendocrine granules. **e** Bundles of filaments within a secondary lysosome, delimited by a membrane and containing lipids and waste pigments. **f** Ciliary structure with the tallo within a cytoplasmic vacuole. **g** Basal body of atypical length. **h** Tubular aggregate with few elements in the vicinity of cisternae of the endoplasmic reticulum and mitochondriae. **i** Aggregate with a considerable number of tubules cut in two directions. Ribosomes can be seen in the transversal sections. (**a** ×4000, **b** ×30000, **c** ×40000, **d** ×20000, **e** ×30000, **f** ×80000, **g** ×30000, **h** × 80,000, **i** × 80,000)

vacuole (Fig. 3f). Less frequently, basal bodies reaching a length of 2.25 nm with a typical thickness of 2.35 nm were present (Fig. 3g).

In some cases, we found in the cytoplasm rough/smooth tubular aggregates consisting of a variable number of elements arranged all in the same direction or in an irregular fashion. The smaller aggregates with fewer tubules (Fig. 3h) are located close to the cisternae of the rough endoplasmic reticulum. Other, larger aggregates (Fig. 3i) are characterized by a honeycomb-like appearance of the cross-section through the parallel tubules. The average diameter of the tubules is approximately 80 nm. Ribosomes are present on the surface of the membranes of some of the tubules and absent on others.

The smooth muscle cells of the peripheral layers of arterial vessels close to norepinephrine producing cells within pheochromocytomas (Fig. 4a) contain linear accumulations of different thickness of membrane-covered granules within their cytoplasm. These are characterized by a bright halo between the granule and the membrane as well as by an eccentric appearance (Fig. 4b). These accumulations separate bands of contractile smooth muscle components (Fig. 4c) which adequately maintain their character (dense cytoplasmic and membrane plaques, caveolae) (Fig. 4d). This phenomenon occurs only within some arterial vessels in areas in which the neoplastic cells in the periphery of the vessel contain predominantly norepinephrine within their cytoplasm. In our cases,

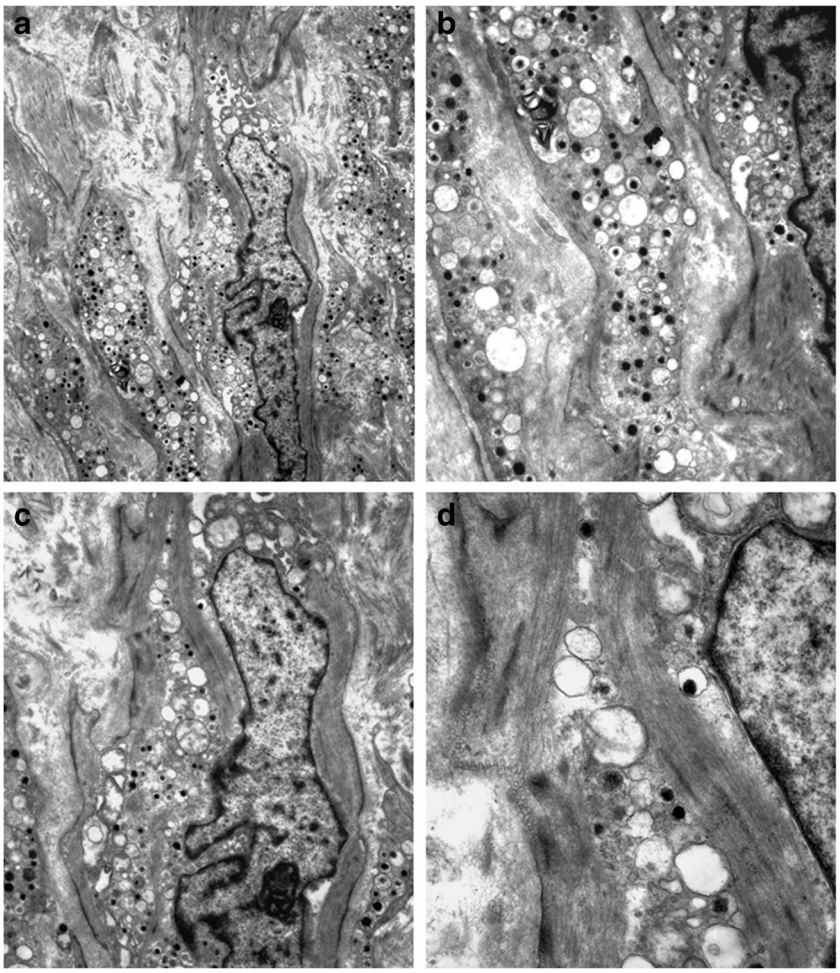

Fig. 4 **a** Central smooth muscle cells with prolongations adjacent to norepinephrine producing cells . **b** Magnification of a segment of image (**a**), with contractile elements and a considerable number of granules with bright halo. **c** Detail of smooth muscle fiber. The neuroendocrine granules separate bundles of fibers with dense plaques. **d** Membrane of a contractile cell with caveoles, dense membrane plaques, contractile filamentous components and a part of the nucleus. Typical aspect of granules with dense core and eccentric halo. (**a** ×3000, **b** × 7000, **c** ×7000, **d** ×30000)

this observation required multiple serial sections from each tissue block at different levels.

Discussion

We are reporting, as complementary ultrastructural features, two types of nuclear inclusions and, within the cytoplasm of the cells, paracrystalline complexes, mega-mitochondria, bundles of intermediary filaments, intracytoplasmic cilia, aggregates of mixed smooth and rough cisternae, as well as the uptake of norepinephrine by smooth muscle fibers in the periphery of arterial vessels.

The most frequent and most characteristic nuclear inclusions within pheochromocytoma cells are, in fact, pseudoinclusions which are always delimited by the two membranes derived from an invagination of the nuclear envelope [7]. The solitary or multiple granulofibrillar structures described in the first place, and surrounded by a bright halo, probably consist of histons. Exact understanding of these will require the use of multiple marker proteins for their investigation [14]. The granulofilamentous bodies, in contrast, consist of chromatin fibers characterized by their individual diameter of 25 nm as well as by their characteristic electrodensity [15]. Due to this high electrodensity, these fibers appear separated and gain a pseudoviral aspect within apoptotic cells. In all cases examined, we have found both types of inclusions.

The paracrystalline inclusions in the cisternae of the endoplasmic reticulum are not a frequent feature, and we in fact only identified them in two of the cases we investigated where they occurred within pheochromocytoma cells in the form of isolated groups. Similar complexes of ribosomes and lamellae were described in parathyroid adenomas [16–18] as well as in normal plasmatic cells present in the infiltrate of a case of mycosis fungoides of the skin and a fibrosarcoma of a lower extremity [19].

Isolated ciliae with their axoneme in a cytoplasmic vacuole are seen with great frequency, and more than one may be present per cell. These formations were referred to as oligociliae [20]. In fact, they are so frequent that the problem now is to find out why certain cell types never seem to possess them [21].

In contrast, atypical basal bodies of the kind we are describing are rare, given that the centrioles and basal bodies in

human cells are about 0.4 mm long and 0.25 mm in external diameter [22].

The rough/smooth tubular aggregates are derived from the endoplasmic reticulum. There are, however, problems of nomenclature when it comes to referring to tubules and microtubules. The feature that distinguishes tubules from microtubules is their diameter, with the separating line drawn at 30 nm. Our description comprises tubules (not microtubules) with a diameter of approximately 80 nm. In the parallel sections, ribosomes are attached to their walls, while the remaining tubules are bare. Continuity may exist between smooth and rough tubules [9]. Rough tubular aggregates were described (unpublished observation) in neuroendocrine tumors. [9] Similar formations were reported to exist in different other tumors such as a malignant stromal tumor [23], an acinar cell carcinoma [24], or in a solid and cystic acinar cell tumor of the pancreas [25].

Previous authors described mitochondrial abnormalities such as swelling and scant cristae, intramitochondrial dense bodies, septate-like junktions, intercristal fusion plus spheroidal bodies, and intramitochondrial rodlets. [8]. Swelling of mitochondrial components was reported to occur occasionally [5] and to reach dimensions of up to 2 μm. Isolated cells with a number of swollen mitrochondria are intermixed with other, typically secretory cells. In none of our cases, however, we have come upon an oncocytic differentiation of cell groups, it being recognized that pheochromocytomas with oncocytic differentiation are extremely rare [26].

The bundles of intermediary filaments we are describing probably correspond to vimentin bundles. Vimentin was found to be positive in some cases of pheochromocytomas [27, 28]. In earlier immunohistochemical observations [29], we have in fact demonstrated pheochromocytomas to be positive for vimentin.

Norepinephrine uptake by smooth muscle cells was examined by means of pharmacologic studies in different species with the fluorescence histochemical technique [30, 31]. While the uptake of norepinephrine by non-innervated as well as by innervated smooth muscle cells exposed to concentrations of norepinephrine has previously been reported, we have not encountered any reference to ultrastructural studies whatsoever. By studying multiple serial sections, it is, however, possible to observe this phenomenon within the peripheral muscle cells of some arterial vessels located in the neighborhood of extensions of pheochromocytoma cells even though we have not identified the cause of the different response, in terms of uptake, by different vessels.

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Compliance with ethical standards The manuscript conforms to the principles of the Declaration of Helsinki and complies with the Ethical Standards accordingly.

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Conflict of interest The authors declare that they have no conflicts of interest.

References

1. Eyden B (2013) The diagnostic electron microscopy of tumors. In: Stirling JW, Curry A, Eyden B (eds) Diagnostic electron microscopy: a practical guide to interpretation and technique, 1st edn. Wiley, Chichester, p 171 174
2. Gómez RR, Osborne BM, Ordoñez NG, Mackay B (1991) Pheochromocytoma. Ultrastruct Pathol 15:557–562
3. Medeiros LJ, Wolf BC, Balogh K, Federman M (1985) Adrenal pheochromocytoma: a clinicopathologic review of 60 cases. Hum Pathol 16:580–589
4. Cervós-Navarro J, Bayer JM, Käser H (1973) Ultrastrukturelle Differenzierung der Phäochromocytome. Virchows Archiv A 361: 51–69
5. Brown WJ, Barajas L, Waisman J et al (1972) Ultrastructural and biochemical correlates of adrenal and extra-adrenal pheochromocytoma. Cancer 29:744–759
6. Tannenbaum M (1970) Ultrastructural pathology of adrenal medullary tumors. Pathol Annu 5:145–171
7. DeLellis RA, Suchow E, Wolfe HJ (1980) Ultrastructure of nuclear "inclusions" in pheochromocytoma and paraganglioma. Hum Pathol 11:205–207
8. Watanabe H, Burnstock G, Jarrot B, Louis WJ (1976) Mitochondrial abnormalities in human phaeochromocytoma. Cell Tissue Res 172:281–288
9. Ghadially FN (1997) Ultrastructural pathology of the cell and matrix, 4th edn. Butterworths, London, p 506
10. Ghadially FN (1997) Ultrastructural pathology of the cell and matrix, 4th edn. Butterworths, London, p 1070
11. Ramsay JA, Asa SL, van Nostrand AWP, Hassaram ST, de Harven EP (1987) Lipid degeneration in pheochromocytomas mimicking adrenal cortical tumors. Am J Surg Pathol 11:480–486
12. Kawai K, Senba M, Tsuchiyama H (1986) Eosinophilic globules in pheochromocytoma of the adrenal medulla. A histochemical, immunohistochemical and ultrastructural study. APMIS 96:911–916
13. Landas SK, Leigh C, Bonsib SM, Layne K (1993) Occurrence of melanin in pheochromocytoma. Mod Pathol 6:175–178
14. Matera AG, Izaguire-Sierra M, Praveen K, Rajendra TK (2009) Nuclear bodies: ramdon aggregates of sticky proteins or crucibles of macromolecular assembly? Dev Cell 17:639–647
15. Ghadially FN (1997) Ultrastructural pathology of the cell and matrix, 4th edn. Butterworths, London, p 144
16. Szakacs JE, Bryant M (1980) Ultrastructure of parathyroid adenomas. Ann Clin Lab Sci 10:13–25
17. Cinti S, Osculati F, Parravicini C (1982) RER associated structure in parathyroid glands removed because of tertiary hyperparathyroidism. Ultrastruct Pathol 3:263
18. Cinti S, Osculati F (1982) Ribosome-lamellae complex in the adenoma cells of the human parathyroid gland. J Submicrosc Cytol 14:521
19. Zimmerman KG, Payne CM, Nagle RB (1984) Ribosome-lamellae complexes in benign plasma cells accompanying neoplastic infiltrates. Am J Clin Path 81:364–367

20. El-Shoura SM, Herrera GA, Ghadially FN (1992) Detection of oligocilia arising from smooth muscle cells of leiomyosarcoma and bilharzial ureters. Ultrastruct Pathol 16:679–686
21. Wheatley DN (1993) Incidence and significance of oligocilia in normal and pathologic tissues. Ultrastruct Pathol 17:565–566
22. Ghadially FN (1997) Ultrastructural pathology of the cell and matrix, 4th edn. Butterworths, London, p 1284
23. Tokue A, Yonese Y, Mato M, Ookawara S (1985) Unusual intracytoplasmic lamellar bodies in a malignant gonadal stromal tumor. Virchows Arch B 40:261–267
24. Bockus D, Remington F, Friedman S, Hammar S (1985) Electron microscopy what izzist. Ultrastruct Pathol 9:1–30
25. Arai T, Kino L, Nakamura SI, Koda K (1986) Solid and cystic acinar cell tumors of the pancreas. Acta Pathol Jpn 36:188
26. Shibamori K, Kobayashi K, Itoh N, Minase T, Satoh M (2013) Oncocytic pheochromocytoma of the adrenal gland with preoperative endocrine examination. Int Cancer Conf J 2:165–168
27. Kimura N, Nakazato Y, Nagura H, Sasano N (1990) Expression of intermediate filaments in neuroendocrine tumors. Arch Pathol Lab Med 114:506–510
28. Li M, Wenig BM (2000) Adrenal oncocytic pheochromocytoma. Am J Surg Pathol 24:1552–1557
29. Fraga M, García-Caballero T, Antunez J, Couce M, Beiras A, Forteza J (1993) A comparative immunohistochemical stufy of pheochromocytomas and paragangliomas. Histol Histopathol 8: 429–436
30. Burnstock B, McLean JR, Wright M (1971) Noradrenaline uptake by non-innervated smooth muscle. Br J Pharmac 43:180–189
31. Bell C, Gillespie JS (1981) Dopamine and noradrenaline levels in peripheral tissues of several mammalian species. J Neurochem 36: 703–706