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Ultrastructure of meningiomas: autophagy is involved in the pathogenesis of "intranuclear vacuoles"

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Abstract

We report here common ultrastructural findings in a short list of meningiomas. At the lower power magnification, a tumour consisted of elongated or round cells and innumerable cellular processes connected with diverse intercellular junctions. Nuclei presented no specific features, nucleoli were infrequently seen and heterochromatin was clumped beneath the nuclear membranes. In a case of clear cell meningioma, cells were of watery cytoplasm. Occasionally, immobile cilia, completely ensheathed by the cytoplasm and anchored by blepharoplasts were seen; as we did not encounter those rare cilia in cross-sections, no further insight into their inner microtubular-doublet structure was possible. The cytoplasm of the cells and the processes were filled with the intermediate filaments. In the intercellular space, collagen fibrils and electron-dense material was occasionally observed. The majority of the tumour samples were filled with processes. Several types of junctional complexes were observed. The most frequent were desmosomes and in the proper plane of section their whole pentalaminar structure was readily discernible. However, robust tonofilaments, as seen in epithelial neoplasms, were not observed. Those desmosomal junctions were either completely symmetric or asymmetric, but the exact symmetry could not be judged without the assistance of a goniometer. Some junctional complexes were more elaborate, with desmosomal junctions separated by a tight apposition of membranes, which suggests tight junctions. "Intranuclear vacuoles" well-visible even at low power were defined as indentation of the cytoplasm into the nucleus. Within these vacuoles, autophagic vacuoles and lysosomal bodies were seen, suggesting an active macroautophagy process. In 2 cases, severe lipidization of meningioma cell cytoplasm was observed. In a case of anaplastic meningioma, a mitotic figure was found. In another case, empty rectangular spaces in the cytoplasm, suggestive of pre-existing crystalloid structures, were seen.

Key words: *electron microscopy, meningioma, autophagy.*

Electron Microscopy – "the Big Eye of the 20th century in decline" [28] – is a complex, time-consuming technology no longer widely used in the field of surgical neuropathology, as it has been almost totally replaced by immunohistochemistry with ever-growing numbers of more or less specific commercially available antibodies. However, it is still a powerful technique if diligently used, especially if applied to small brain tumour biopsy specimens. Following personal experience, lasting for some quarter of a century, we decid-

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ed to produce a series of papers comprising ultrastructural studies of diverse tumour entities. In the past, we published several reviews [19-21,23] and books [18,24,25]. In this paper, we report the first group of such systematically examined brain tumour specimens, namely meningiomas.

Meningioma [7], formerly "fungus of the dura mater" is one of the most common mostly benign tumour of the central nervous system (CNS), originating from the meningoendothelial tissue [31]. A plethora of different types of meningiomas is known.

Conventional variants, all WHO grade I:

- meningothelial,
- fibrous/fibroblastic,
- transitional,
- psammomatous,
- secretory,
- microcystic,
- metaplastic,
- lymphoplasmocyte-rich.

Aggressive variants:

- atypical (WHO grade II),
- chordoid (WHO grade II),
- clear cell (WHO grade II),
- anaplastic with sarcomatous, carcinomatous or melanoma-like pictures [31] (WHO grade III),
- papillary (WHO grade III),
- rhabdoid (WHO grade III).

Table I. A list of cases used for this study

2418	52	F	Transitional meningioma
2424	48	F	Clear cell meningioma
2462	69	M	Transitional meningioma
2491	57	F	Meningothelial meningioma
2542	54	M	m. haemangiosicyticum
2906	37	F	Fibroblastic meningioma
3750	37	M	Angiomatous meningioma
3751	64	M	Angiomatous meningioma
4023	67	F	Anaplastic meningioma
4174	67	M	Anaplastic meningioma
4262	7	F	Anaplastic meningioma
4267	66	M	Anaplastic meningioma
4353	43	F	Fibroblastic meningioma
4422	30	F	Lymphoplasmacyte rich
4591	8	M	Anaplastic meningioma

Material and methods

We used 15 samples of meningiomas recorded on files from the Department of Molecular Pathology and Neuropathology, Chair of Oncology, Medical University Lodz (Table I). They have been collected for over 25 years, immediately fixed at the operation theatre in 2.5% buffered glutaraldehyde, postfixed in 1% osmium tetroxide and embedded in Epon. The semithin sections were stained with toluidine blue and grids were examined first in Zeiss 109 and then in Jeol 1100 transmission electron microscopes.

Results

Irrespective of the meningioma category, the ultrastructural picture was virtually the same and the findings will be presented here divided into ultrastructural categories.

1. General view. At the lower power magnification, a tumour consisted of elongated or round cells and innumerable cellular processes connected with diverse intercellular junctions (Fig. 1). Nuclei presented no specific features, nucleoli were infrequently seen and heterochromatin was clumped beneath the nuclear membranes. In a case of clear cell meningioma, cells were of "watery" cytoplasm (Fig. 2).

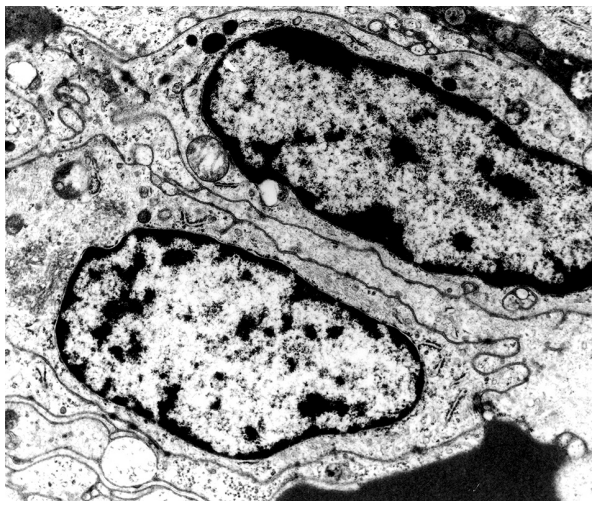

Fig. 1. General view of meningioma. Nuclei are elongated with heterochromatin clumped beneath the nuclear membrane. Original magnification, × 7000.

Occasionally, immobile cilia, completely ensheathed by the cytoplasm and anchored by blepharoplasts were seen; as we did not encounter those rare cilia in cross-sections, no further insight into their inner microtubular-doublet structure was possible. The cytoplasm of the cells and the processes were filled with the intermediate filaments (Fig. 4). In the intercellular space, collagen fibrils and electron-dense material was occasionally observed.

2. Intercellular junctions. The majority of the tumour samples were filled with processes. Several types of junctional complexes were observed (Fig. 5). The most frequent were desmosomes and in the proper plane of section their whole pentalaminar structure was readily discernible (Fig. 6). However, robust tonofilaments, as seen in epithelial neoplasms, were not observed. Those desmosomal junctions were either completely symmetric (Fig. 7) or asymmetric, but the exact symmetry could not be judged without the assistance of a goniometer. Some junctional complexes were more elaborate, with desmosomal junctions separated by a tight

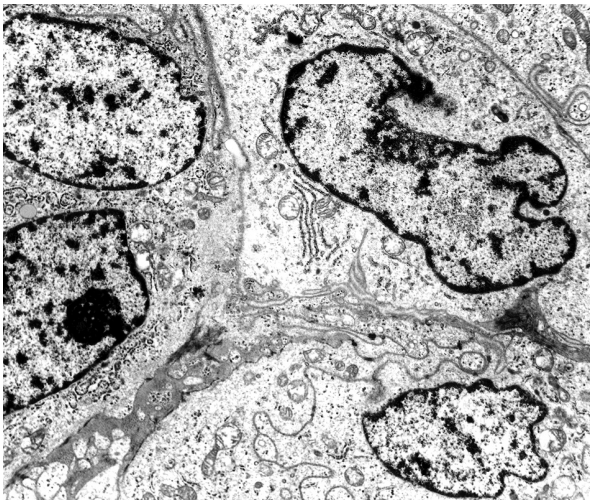

Fig. 2. A clear cell meningioma. Note the "watery" cytoplasm of neoplastic cells. Original magnification, × 4000.

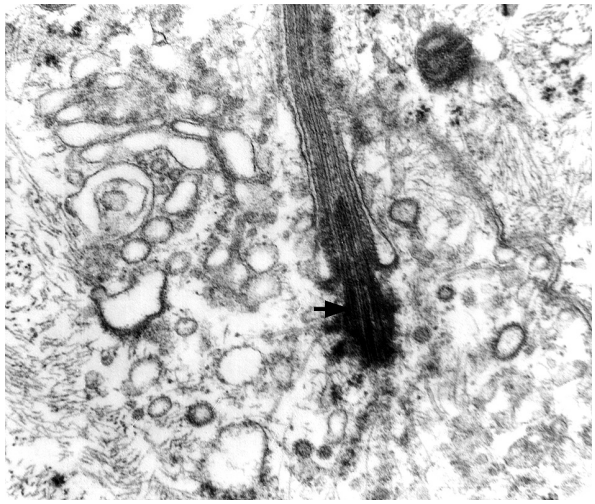

Fig. 3. An immobile cilium is completely ensheathed by cytoplasm and anchored by a blepharoplast (arrow). Original magnification, × 30 000.

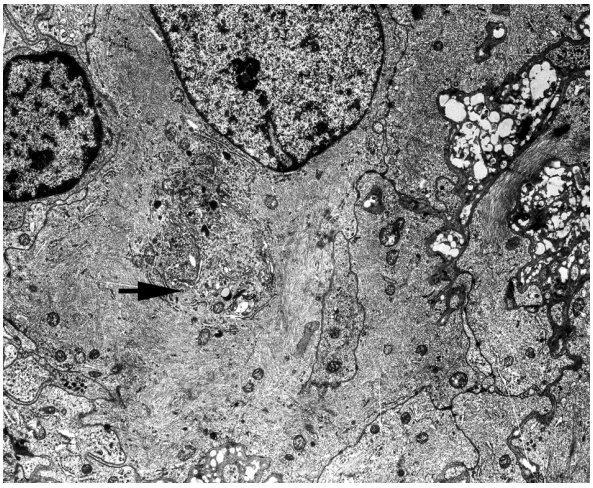

Fig. 4. A case of atypical meningioma. Note innumerable intermediate filaments filling the cytoplasm of cells and processes. Original magnification, × 4400.

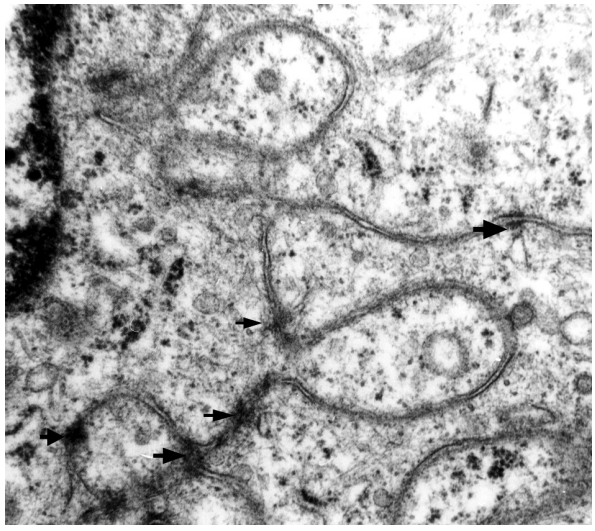

Fig. 5. Processes of tumour cells forming intracellular junctions (arrows). Original magnifications, × 7000.

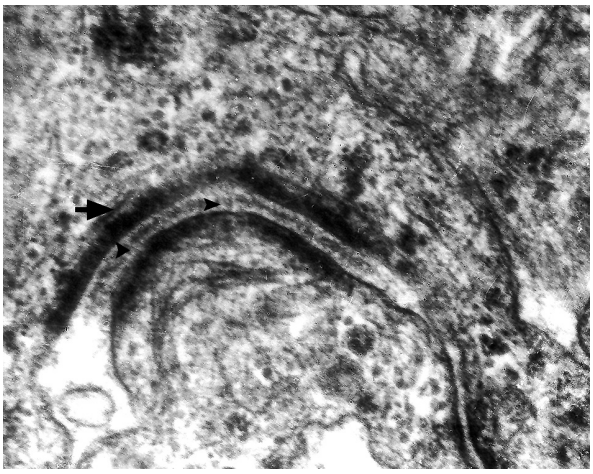

Fig. 6. Desmosomal junction connecting two of neoplastic cells processes. Note electron dense plaque (arrow) and dense line (arrowheads) in the intercellular space. Original magnification, × 30 000.

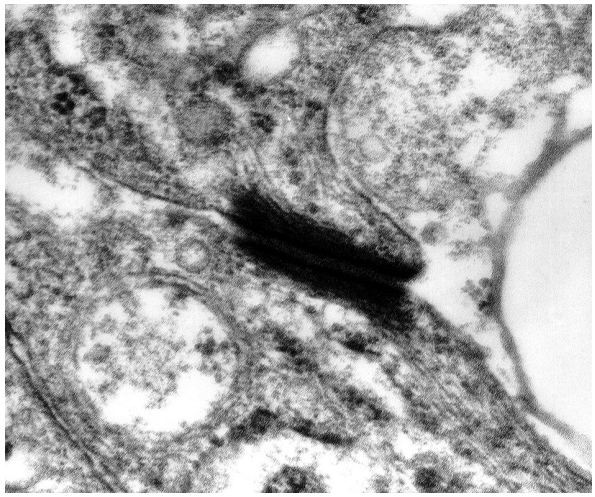

Fig. 7. Symmetric desmosomal junction in a meningioma sample. Original magnification, × 50 000.

apposition of membranes, which suggests tight junctions (Fig. 8).

3. "Intranuclear vacuoles". Those structures, well-visible even at low power (Fig. 9), were defined as indentation of the cytoplasm into the nucleus. Within these vacuoles, autophagic vacuoles and lysosomal bodies were seen, suggesting an active macroautophagy process (Fig. 10A,B).
4. In 2 cases, severe lipidization of meningioma cell cytoplasm was observed (Fig. 11).
5. In a case of anaplastic meningioma, a mitotic figure was found (Fig. 12).
6. In another case, empty rectangular spaces in the cytoplasm, suggestive of pre-existing crystalloid structures, were seen (Fig. 13).

Discussion

The ultrastructural findings as reported here are, in a sense, similar irrespective of the histological type of meningiomas. Numerous cytoplasmic processes

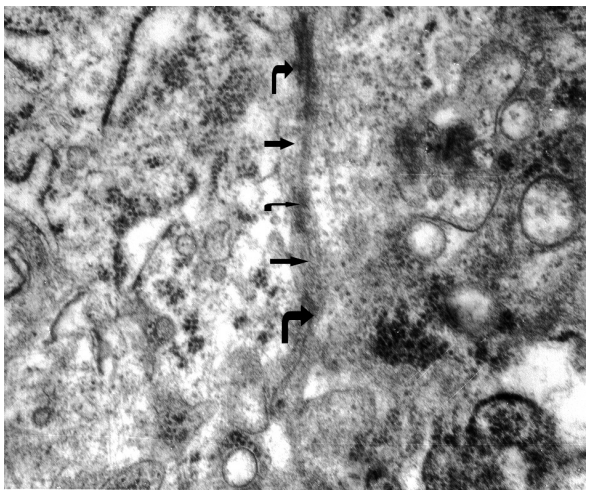

Fig. 8. A long and elaborate junctional complex in which desmosomal junctions (bent arrows) are separated by tight apposition of membranes which suggests the tight junctions. Original magnification, × 30 000.

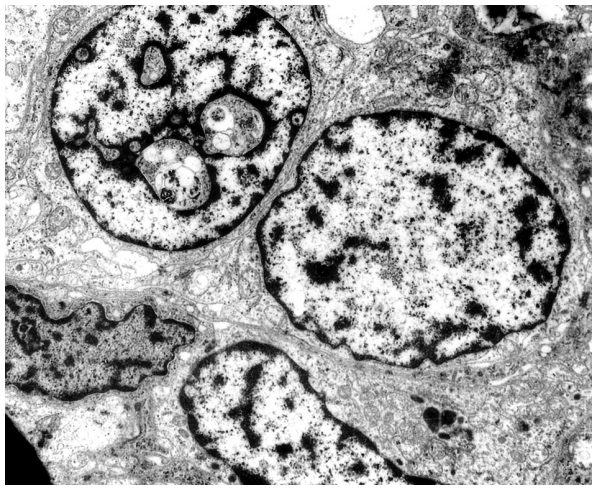

Fig. 9. Low power view showing a meningioma cell with 3 "intranuclear vacuoles". Note numerous membrane-bound autophagic vacuoles within those "intranuclear vacuoles".

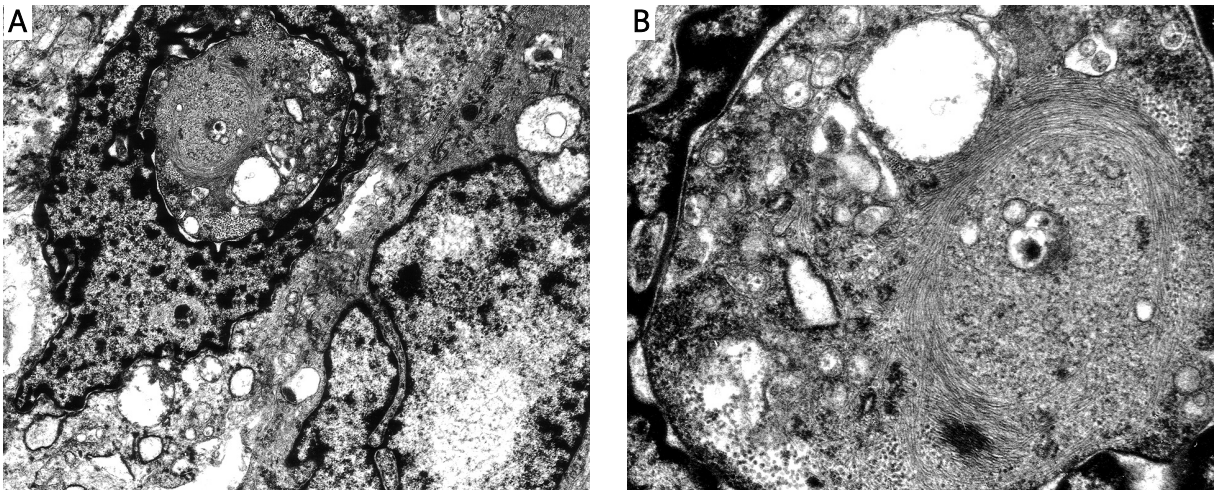

Fig. 10. Low **(A)** and high **(B)** power view of an intranuclear vacuole in a case of anaplastic meningioma, Original magnification, **(A)** × 7000; **(B)** × 50 000.

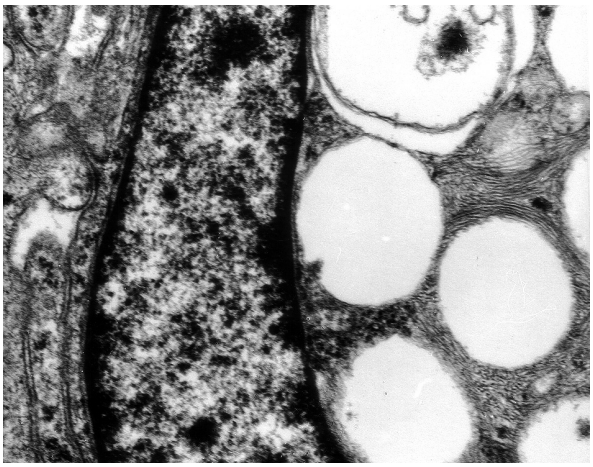

Fig. 11. Lipid-laden vacuoles in a case of angiomatous meningioma. Original magnification, × 20 000.

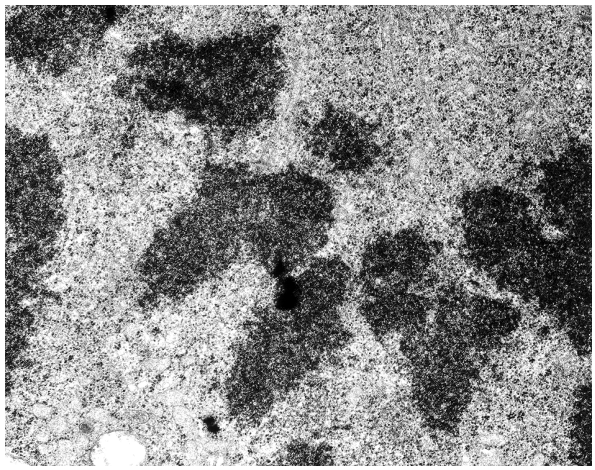

Fig. 12. A mitotic figure in a case of anaplastic meningioma. Original magnification, × 20 000.

connected by long and tortuous zipper-like adhesive plaque junctions are common. Other typical findings include "intranuclear inclusions" (vacuoles) containing autophagic vacuoles and other subcellular organelles. Intracytoplasmic intermediate filaments composed of vimentin are also typical.

The fine structural studies of meningiomas are almost as old as the whole field of neurosurgical electron microscopy [14]. The first to present the fine structure of meningiomas was Lewenthal in 1961 followed by Luse [26], Kepes [13], Gusek [10], Napolitano *et al.* [29], Cervós-Navarro *et al.* [3,4], Castaigne *et al.* [2], Robertson [32], Woyke *et al.* [37,38], and Szymaś *et al.* [33]. In meningothelial meningioma, amianthoid fibers, i.e. disorderly patterns of collagen fibres, were reported [5]. In secretory meningiomas, numerous microvilli are seen. In rhabdoid meningiomas, round to oval rhabdoid cells filled with whorls of intermediate filaments are, as in other rhabdoid tumours, typical [1]. In chordoid meningioma, chordoma-like cells are encountered; by electron microscopy, these cells contain abundant mitochondria and intracytoplasmic vacuoles [11]. The reports of these earlier findings were elegantly summarized by Kepes [14].

Analogously to Kepes [14], our data support the notion that the fine structure of hoard of meningioma

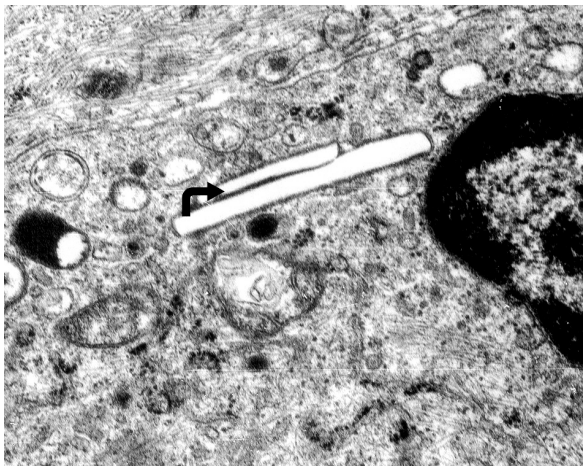

Fig. 13. Empty spaces following washing-off crystals in a case of meningioma. Original magnification, × 30 000.

subtypes are reduced at the ultrastructural level to the same basic pattern. The exceptions are, and also to a certain degree only, clear cell meningioma, chordoid meningioma and rhabdoid meningioma. In the anaplastic meningiomas, mitotic figures may be found as expected, and as illustrated in Fig. 12.

Several features may need additional comments. The intercellular junctions are either well-developed desmosomal junctions, albeit without robust tonofilaments, or desmosomal junctions interspaced with adhesive plaque (tight) junctions as shown by Tani *et al.* [34]. The latter investigators supplemented transmission electron microscopy by freeze-fracturing technique to demonstrate particles at the fractured sites of the junctions. The tight junction on freeze-fractured faces revealed stretches of complex ridges and furrows. Copeland *et al.* [6] reported on another type of junctions composed of cisternal dilatation of intercellular space filled with electron-dense structureless material. Xanthochromic changes were already reported by Matyja *et al.* and Taraszewska *et al.* [27,35] in cases of anaplastic meningiomas and we observed lipid-laden vacuoles in a case of angiomatous meningioma. Thus, those vacuoles may not be specific to any particular type of meningioma.

The most interesting ultrastructural finding in meningiomas is the presence of "intranuclear vacuoles", well-visible at the light microscopy level and described for the first time by Wolf and Orton in 1932 [36]. Those structures were first recognized by Gusek *et al.* [10] followed by Robertson [32]. The latter investigator noticed "dense and granular membrane-bound bodies" within those vacuoles, but did not relate those structures to the autophagy process, as the latter was first discovered a year later [30]; Kepes [15] was the first to demonstrate autophagic vacuoles in meningiomas. The autophagy or "eaten alive" process [16,40] is a complex mechanism involved in bulk removal of intracellular organelles and its role in diverse cellular pathologies from prion diseases to cancer is recently well recognized [22]. According to the recommendations of the Nomenclature Committee on Cell Death [8,17], three major types of programmed cell death (PCD) can be discriminated. The first type is "apoptosis" and this is unrelated to the observations we report here. The second type – involving macroautophagy (called "autophagy" in abbreviation) – is characterized by the presence of numerous autophagosomes that subsequently fuse with lysosomes to form autolysosomes. The molecular mechanism of this process differs from that of apoptosis and consists of a complex interplay of numerous proteins including the mTOR (mammalian target of rapamycin) kinase [39]. The third type is similar to the second type, except for the negligible or absent involvement of lysosomes. Electron-microscopically, type 3 cell death is characterized by swelling of intracellular organelles resulting in the formation of large empty spaces within the cytoplasm and this leads to necrosis. The electron microscopic features of the contents of "intranuclear vacuoles" in meningiomas resemble those of macroautophagy. Thus, unequivocally, autophagy is taking place there, but it is doubtful if it exerts any significant role in meningioma pathogenesis. Of note, intranuclear inclusions are diversely labelled by anti-p62 antibodies [12], a scaffolding protein involved in the ubiquitination process [for review, 9]. However, the exact role of the p62 in meningioma pathogenesis and its putative linkage to autophagy is lacking.

It is hard to explain why the autophagic vacuoles and autophagosomes are evident only within the "intranuclear vacuoles" in meningiomas, but it is entirely possible that the cytoplasm that is entrapped within this cytoplasmic indentation is "strangulated" by the nuclear mass and dies by autophagy.

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