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-
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 matter” 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).

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).

<table>
<thead>
<tr>
<th>Table I</th>
<th>A list of cases used for this study</th>
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<tbody>
<tr>
<td>2418</td>
<td>52 F Transitional meningioma</td>
</tr>
<tr>
<td>2424</td>
<td>48 F Clear cell meningioma</td>
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<tr>
<td>2462</td>
<td>69 M Transitional meningioma</td>
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<tr>
<td>2491</td>
<td>57 F Meningothelial meningioma</td>
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<tr>
<td>2542</td>
<td>54 M m. haemangiosicyticum</td>
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<tr>
<td>2906</td>
<td>37 F Fibroblastic meningioma</td>
</tr>
<tr>
<td>3750</td>
<td>37 M Angiomatous meningioma</td>
</tr>
<tr>
<td>3751</td>
<td>64 M Angiomatous meningioma</td>
</tr>
<tr>
<td>4023</td>
<td>67 F Anaplastic meningioma</td>
</tr>
<tr>
<td>4174</td>
<td>67 M Anaplastic meningioma</td>
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<tr>
<td>4262</td>
<td>7 F Anaplastic meningioma</td>
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<td>4267</td>
<td>66 M Anaplastic meningioma</td>
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<tr>
<td>4422</td>
<td>30 F Lymphoplasmocyte rich</td>
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<tr>
<td>4591</td>
<td>8 M Anaplastic meningioma</td>
</tr>
</tbody>
</table>

![Fig. 1. General view of meningioma. Nuclei are elongated with heterochromatin clumped beneath the nuclear membrane. Original magnification, × 7000.](image-url)
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
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
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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 fibers, 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. 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.
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, choroid 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 bod-
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