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Case Report

Extremely intricate labyrinthine structures: an electron microscopic observation of peculiar invaginations of the plasma membrane found in secretory meningioma

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Abstract: Large, extremely intricate labyrinthine structures were observed during an electron microscopic examination of a secretory meningioma that originated in the sphenoid ridge of a 73-year-old man. In many neoplastic cells, these labyrinthine structures formed round-shaped regions within the cytoplasm, and profiles indicated their internal structure consisted of numerous thin fragments of cytoplasm and intervening flocculent material. These labyrinthine structures did not exhibit apparent topographic association with pseudopsammoma bodies, and direct communication with the extracellular space by very thin channels was rarely observed. The structures resembled the "intracytoplasmic lumina" commonly seen in epithelial neoplasms; but they did not appear to be true "intracytoplasmic" closed structures but rather deep and elaborate invaginations of the plasma membrane into the cytoplasm. These structures are distinct from pseudopsammoma bodies and might represent another expression of the epithelial properties of neoplastic cells in this subtype of meningioma. Although their pathogenesis or significance remain unknown, these structures may significantly increase the surface area of neoplastic cells and facilitate the uptake of extracellular material.

Keywords: Invagination, labyrinthine structure, secretory meningioma, ultrastructure

Introduction

Secretory meningioma is a rare subtype of meningioma characterized by distinct epithelial differentiation of neoplastic meningothelial cells and the formation of many pseudopsammoma bodies (hyaline inclusions) containing glycoproteins [1-11]. Genetic mutations specific to this subtype of meningioma (combined *KLF4* K409Q and *TRAF7* mutations) were recently reported [12]. To date, many articles have been published concerning the ultrastructure of neoplastic cells in secretory meningioma, especially regarding the formation of pseudopsammoma bodies [1-4, 6-10]. In this report, we describe extremely intricate, labyrinthine structures observed during ultrastructural examination of a case of secretory meningioma. To the best of our knowledge, similar structures have not been documented in meningioma.

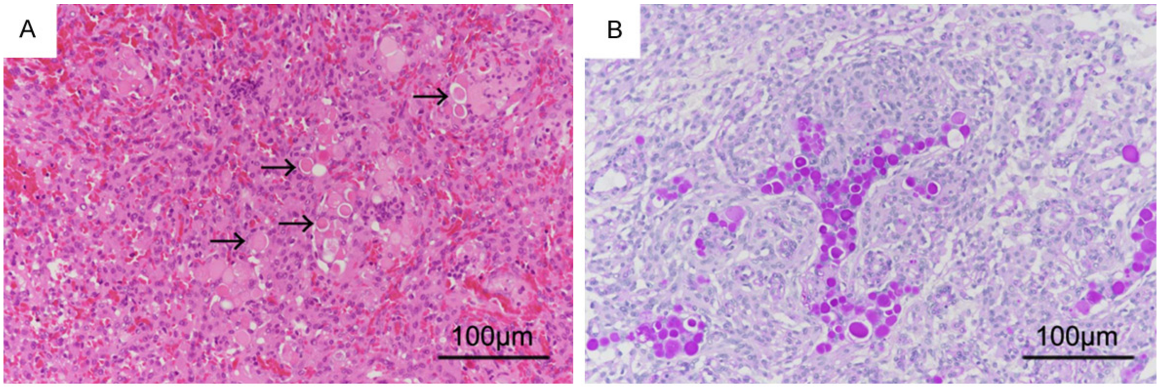
Case history

The patient was a 73-year-old man who presented with progressive gait instability over the last 10 months and was found to have an extra-axial tumor, measuring 3 cm in diameter, on the right sphenoid ridge. Marked cerebral edema was noted around the tumor. The tumor was completely excised, and the pathological diagnosis was secretory meningioma. (This patient was included in a recent pathological study of reactive pericytic proliferation in meningioma [13]).

Pathological findings

Histopathological and immunohistochemical findings

The tumor exhibited typical histopathological findings of secretory meningioma. It consisted of a diffuse proliferation of polygonal cells with round nuclei and a moderate amount of eosinophilic cytoplasm, and numerous, strongly periodic acid-Schiff (PAS)-positive, pseudopsammoma bodies (hyaline inclusions) were observed within the tumor tissue (**Figure 1A**, **1B**). In many areas, neoplastic cells appeared



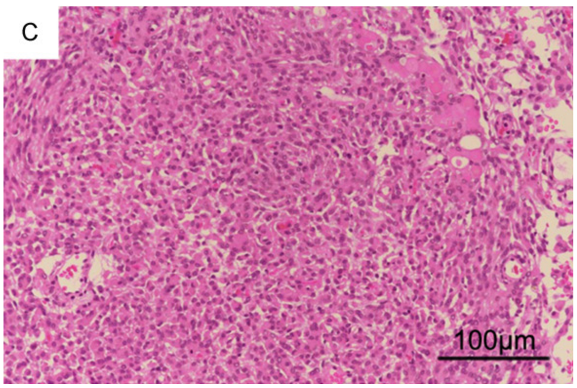


Figure 1. Histopathology of the meningeal tumor. A. The tumor consisted of a diffuse proliferation of polygonal cells, and many deeply eosinophilic, pseudopsammoma bodies were observed (arrows). (Hematoxylin-Eosin stain, ×200, scale bar: 100 μm). B. Pseudopsammoma bodies were strongly reactive to PAS reaction. (PAS reaction, ×200, scale bar: 100 μm). C. In many areas, the extracellular space was widened, and neoplastic cells were less cohesive (Hematoxylin-Eosin stain, ×200, scale bar: 100 μm).

to be less cohesive in comparison with the ordinary meningioma, and the extracellular space was widened (**Figure 1C**).

Immunohistochemical study was performed on formalin-fixed and paraffin-embedded specimens, and monoclonal antibodies against pan-cytokeratin (clone AE1/AE3, Dako, Glostrup, 1:400) and epithelial membrane antigen (EMA) (clone M0613, Dako, 1:400) were employed. Immunostains were performed using an automated immunostainer, Leica Bond-Max (Leica Biosystems, Wetzler, Germany). Tumor cells exhibited membranous or finely granular cytoplasmic immunoreactivity for EMA, and cells surrounding the pseudopsammoma bodies were immunoreactive for pan-cytokeratin.

Ultrastructural findings

The ultrastructural study was performed using fresh pieces of excised tumor tissue, which were immediately fixed in 5% glutaraldehyde and post-fixed in 1% osmium tetroxide. After the routine processing involving dehydration, embedding, and sectioning, ultrathin sections were stained with uranyl acetate and lead citrate and then observed under an electron microscope.

The nuclei of neoplastic meningothelial cells were round in shape and contained a moderate amount of diffusely distributed euchromatin. Intranuclear cytoplasmic invaginations (nuclear pseudoinclusions) were observed in many cells (**Figure 2A**). The cytoplasm contained a moderate number of mitochondria, rough-surfaced endoplasmic reticulum, and Golgi apparatus. Pseudopsammoma bodies appeared as intra- or extracellular spherical bodies exhibiting variable electron density and occasionally contained granular or flocculent material (**Figure 2A**). These bodies were surrounded by the plasma membrane bearing numerous microvilli.

Large, extremely intricate labyrinthine structures were observed in many neoplastic cells (**Figure 2B-D**). These structures formed ill-defined, intracytoplasmic regions, and their profiles revealed numerous thin septa or fragments of cytoplasm within the structures. Flocculent material exhibiting the same electron density as that of the extracellular space filled the space between these cytoplasmic fragments. On closer examination, some of these labyrinthine structures were found to communicate with the extracellular space by very thin

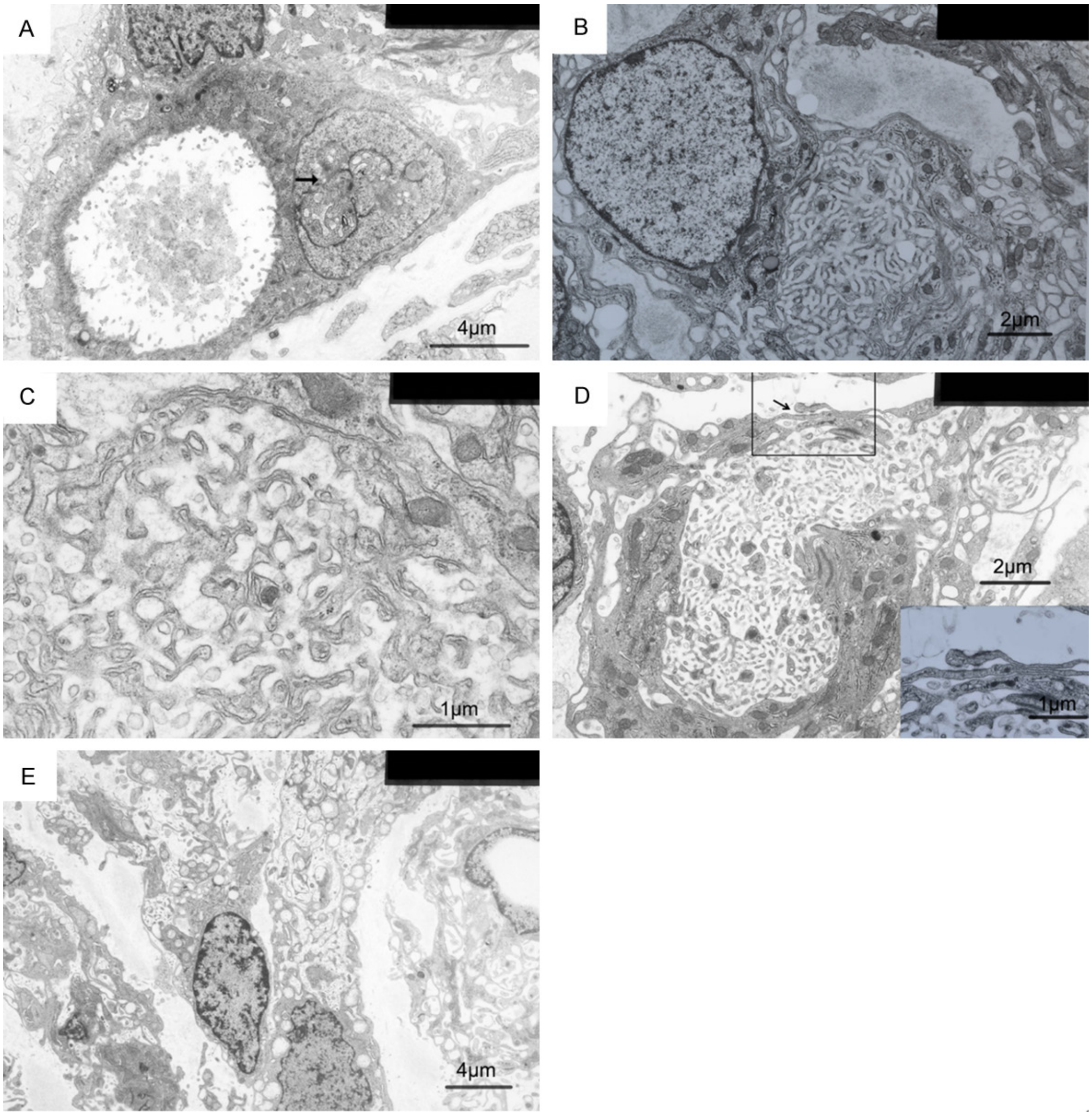


Figure 2. Ultrastructure of neoplastic meningothelial cells. A. Nuclei of neoplastic cells were round and exhibited sporadic intranuclear cytoplasmic invaginations (arrow). A large pseudopsammoma body containing moderately electron dense, granular material was observed in the cytoplasm (×4,000, scale bar: 4 μm). B. A large, intricate labyrinthine structure containing many cytoplasmic profiles or fragments was observed (×5,000, scale bar: 2 μm). C. Higher magnification of a large, intricate labyrinthine structure. The spaces between the cytoplasmic fragments or profiles were filled with flocculent material (×15.000, scale bar: 1 μm). D. A large, intricate, labyrinthine structure. Note that this structure communicates with the extracellular space through a very thin channel (arrow) (×5,000, scale bar: 2 μm). *Inset* is a high-magnification image of the area denoted by the rectangle (×16,000, scale bar: 1 μm). E. In some neoplastic cells, a large proportion of the cytoplasm was occupied by large labyrinthine structures. The extracellular space was relatively wide, and the plasma membranes of adjacent cells did not exhibit close contact or interdigitation (×2,500, scale bar: 4 μm).

channels (**Figure 2D**). In some neoplastic cells, a large proportion of the cytoplasm was occupied by these large labyrinthine structures (**Figure 2E**).

It was noteworthy that these labyrinthine structures did not contain any electron-dense secretory material, and no topographic association was observed between the structures and pseudopsammoma bodies. To what light-microscopic structures these labyrinthine structures might correspond remains unclear, but from the above findings we surmised that most of these structures were probably not truly intracytoplasmic closed structures but rather deep and elaborate "invaginations" of the plasma membrane into the cytoplasm.

The neoplastic meningothelial cells in the present case differed from those of ordinary meningioma in that they were not compactly apposed, the extracellular space was relatively wide, and contained flocculent material of medium electron density (**Figure 2A**, **2D**, **2E**). The cytoplasmic surface was decorated with many filopodia-like, fine cytoplasmic projections. Although sporadic junctional complexes connecting these projections were observed, close apposition of the adjacent plasma membrane and interdigitation of the plasma membrane were not observed. A few short fragments of the basal lamina and hemidesmosomes were observed on the cytoplasmic surface.

Discussion

The large, extremely intricate labyrinthine structures we described here resembled "intracytoplasmic lumina" commonly observed in many types of malignant epithelial neoplasm [14, 15] and initially appeared to be intracytoplasmic structures. However, on closer examination, some of the structures were found to communicate with the extracellular space by very thin channels. In addition, the electron density and nature of the flocculent material within these labyrinthine structures were similar to those of extracellular material. From these findings, we consider that most of these labyrinthine structures were probably not purely intracytoplasmic closed structures but rather deep and elaborate invaginations of the plasma membrane.

The pathogenetic role of these labyrinthine structures remains unknown, but we suspect that their formation is closely related to the properties of the cytoplasmic surface of tumor cells in this secretory meningioma. In ordinary meningioma, adjacent neoplastic cells are closely apposed over a long distance, and the extracellular space is usually very narrow [16, 17]. The plasma membranes of adjacent cells form conspicuous interdigitations, and the junctional complexes are well developed between the plasma membranes [16, 17]. In contrast, in the present case, neoplastic cells were not compactly apposed in part, and the extracellular space was relatively wide and contained flocculent material. The cytoplasmic surface was decorated with numerous filopodia-like, fine cytoplasmic projections. Although many junctional complexes were found, neither close apposition nor interdigitation of the adjacent plasma membrane was observed. We hypothesize that these characteristics of the cytoplasmic surface allowed the formation of these deep and elaborate invaginations of the plasma membrane.

A widened extracellular space and the presence of numerous filopodia-like cytoplasmic projections on the cell surface were previously observed in cases of secretory meningioma reported by Yagishita et al. [4] and Alguacil-Garcia et al. [8] and considered characteristics of this subtype of meningioma. Yagishita et al. [4] also documented deep indentations (invaginations) of the plasma membrane that formed small "intracytoplasmic ductules". The large, intricate labyrinthine structures we observed may be an exaggerated or extreme form of these "intracytoplasmic ductules" [4].

It is noteworthy that these labyrinthine structures had no direct topographic association with pseudopsammoma bodies. No accumulation of the electron-dense amorphous or granular material that characterizes pseudopsammoma bodies was observed within or around the labyrinthine structures. Many ultrastructural studies have been performed on the pathogenesis of pseudopsammoma bodies, which are generally considered to be products of active secretion by neoplastic cells [1-4, 6, 9, 10]. Yagishita et al. [4] reported continuity between pseudopsammoma bodies and the "intracytoplasmic ductules" formed by deep invagination of the plasma membrane. Kubota et al. [7] classified pseudopsammoma bodies and the related structures into three types based on morphology. The inclusions they classified as type I appear to resemble the labyrinthine structures we describe here [7]. These type I inclusions were surrounded by microvilli-like, fine cytoplasmic projections, and the authors regarded them as representing an early stage in the formation of pseudopsammoma bodies [7]. However, these investigators did not report such complex labyrinthine structures as we observed in the present case.

The labyrinthine structures we present here might represent the expression of an epithelial phenotype of neoplastic meningothelial cells in secretory meningioma [5, 10, 11]. They closely resemble "intracytoplasmic lumina" commonly observed in adenocarcinoma of the breast [14] or stomach (e.g., signet ring cell carcinoma) [15], and some of the structures communicate with the extracellular space. We speculate that they are distinct from pseudopsammoma bodies and may represent another morphologic aspect of secretory meningioma.

The pathological significance of these labyrinthine structures is unknown, but we propose a hypothesis. Secretory meningioma is known to contain various serum protein components, such as immunoglobulin (Ig) A, IgM, and IgG [5, 8, 9, 11]. Tumor cells are thought to take up these materials from the blood and accumulate them within pseudopsammoma bodies. We hypothesize that the labyrinthine structures open to the extracellular space significantly increase the surface area of tumor cells, thereby enhancing the uptake of serum protein components into the cytoplasm.

The clinical significance of large, extremely intricate labyrinthine structures in secretory meningioma is also obscure. However, widening of the extracellular space and the formation of these structures may be related to marked perilesional edema, which is characteristically observed on radiological examination in many cases of this subtype of meningioma [8, 11].

Disclosure of conflict of interest

None.

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