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Original Article

Reactive hyperplasia of vascular pericytes in meningioma: immunohistochemical and ultrastructural studies of two cases

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Abstract: An isolated proliferation of pericytes is a unique vascular reaction seen almost exclusively in the stroma of secretory meningioma. We report the results of immunohistochemical and ultrastructural studies of a pericytic proliferation that was found in two cases of meningioma (a secretory meningioma of the sphenoid ridge and a parasagittal atypical meningioma showing predominantly fibroblastic features). Pericytes had hyperchromatic nuclei and scant cytoplasm, and showed stratification or formed small clusters within the walls of small blood vessels. They occasionally showed close contact with pseudopsammoma bodies in secretory meningioma. Pericytes showed immunoreactivity for α-smooth muscle actin but were not immunoreactive for desmin. They also exhibited characteristic ultrastructural features of pericytes, including the presence of microfilaments and abundant pinocytotic vesicles, and investment by the basal lamina. This isolated pericytic proliferation is likely a peculiar response of the vascular wall, probably induced by some cytokines secreted from neoplastic meningothelial cells. The close contact of proliferating pericytes with pseudopsammoma bodies suggests a close pathogenetic association between them. The occurrence of pericytic proliferation that was found in our second case (atypical meningioma with predominantly fibroblastic features) is exceptional and has not been documented to date.

Keywords: Atypical meningioma, pericyte, pseudopsammoma body, reactive hyperplasia, secretory meningioma

Introduction

An isolated proliferation of vascular pericytes not associated with endothelial hyperplasia is an extremely uncommon phenomenon that is observed in the walls of stromal small blood vessels in meningioma [1-7]. It is intriguing that its occurrence is almost restricted to one subtype of meningioma, that is, secretory meningioma [3], but its pathogenesis or pathological significance remain obscure. We observed pericytic proliferation in a case of secretory meningioma and close contact of pericytes with pseudopsammoma bodies [8], suggesting a close association. In addition, we also noted pericytic proliferation in a parasagittal atypical meningioma.

Clinical history

Case 1

The patient was a 73-year-old man, who developed progressive gait instability and suffered several falls over the last 10 months. On examination, he showed clear consciousness and no other neurological abnormalities. Radiological study demonstrated an intensely enhanced, extra-axial mass of 3 cm in diameter at the tip of the right temporal lobe (**Figure 1A**). The tumor was fed by the middle meningeal artery and accompanied by marked edema of the adjacent area of the brain. The serum level of carcinoembryonic antigen (CEA) was normal. Total resection of the tumor was performed under the diagnosis of sphenoid ridge meningioma. A soft tumor with a gray color was attached to the dura mater. The postoperative course was uneventful, and the patient has remained free from tumor recurrence.

Case 2

The patient was a 74-year-old woman with a past history of Basedow disease and breast cancer (stage I), which had been operated on at the age of 62 years. At the time of the breast

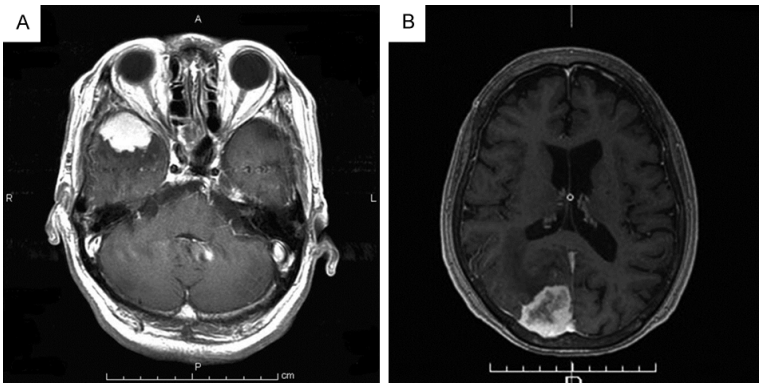

Figure 1. Neuroradiologic findings. A. Case 1. T1-weighted magnetic resonance image (MRI) with contrast enhancement showed an intensely enhanced mass at the tip of the right temporal lobe. The adjacent brain tissue showed edema. B. Case 2. T1-weighted MRI with contrast enhancement demonstrated an intensely enhanced, dura-attached mass in the parasagittal region of the right occipital lobe. Edema of the adjacent cerebral white matter is evident.

surgery, she was incidentally found to have a small tumor in the parasagittal region of the right occipital lobe. Because she did not have any subjective symptoms possibly related to the tumor, she was placed under close observation for three years. At 74 years of age, she noticed visual disturbance, and an ophthalmologic examination demonstrated left lower homonymous quadrantanopsia. Radiological examination showed an increase in size of the parasagittal tumor and marked vasogenic edema of the surrounding brain tissue (**Figure 1B**). Subtotal removal of the tumor (Simpson grade IV) was performed. The tumor was found to be firmly attached to both the superior sagittal sinus and cerebral cortical surface. The postoperative course was uneventful. This is a recent case, and the follow-up duration is still short.

Materials and methods

Immunohistochemical study was performed after formalin fixation and paraffin embedding, and monoclonal antibodies against the following substances were employed: pan-cytokeratin (clone AE1/AE3, Dako, Glostrup, Denmark, 1:400), epithelial membrane antigen (EMA) (clone M0613, Dako, 1:400), CEA (clone II-7, Dako, 1:200), CD34 (clone QBEnd/10, Leica Biosystems, Wetzler, Germany, 1:400), vimentin (clone V9, Novocastra Vector Labs, Burlingame, CA, USA, 1:600), α-smooth muscle actin (α-SMA) (clone 1A4, Dako, 1:1,000), desmin (clone D33, Dako, 1:200), and Ki-67 antigen (clone MIB-1, Dako, 1:100). Immunostains were performed using an automated immunostainer, Leica Bond-Max (Leica Biosystems). In Case 1, ultrastructural examination was done according to the routine method after the fixation of fresh tissue by glutaraldehyde and osmium tetroxide.

Histopathologic findings

Case 1: The tumor showed a typical appearance of secretory meningioma and consisted of a sheet-like, diffuse proliferation of polygonal cells with round nuclei and relatively abundant, eosinophilic cytoplasm (**Figure 2A**). Some nuclei showed a clear, vacuolated appearance or the formation of intranuclear cytoplasmic invagination. A tendency toward whorl formation was focally noted, but no psammomatous calcification was seen. Atypical features, such as nuclear hyperchromatism, pleomorphism, an increase of mitotic activity, or necrosis, were absent. Many pseudopsammoma bodies (hyaline inclusions) of a globular shape were mainly distributed in the areas surrounding blood vessels as a single body or multiple aggregates. They were densely eosinophilic, positive for periodic acid-Schiff (PAS) reaction, and stained black with periodic acid-methenamine silver (PAM) stain.

The tumor had a scant fibrous stroma, but small blood vessels were well-developed. In the walls of some small vessels, a proliferation of pericytes with hyperchromatic, small elliptical nuclei, and scant cytoplasm was noted (**Figure 2B**). The pericytes showed stratification and occasionally formed small clusters, but the vascular lumens were preserved intact. In many places, the pericytic proliferation showed a close topographic association with pseudopsammoma bodies, and they formed large aggregates together (**Figure 2C**). In some areas, this pericytic proliferation formed large, confluent cellular aggregates containing several vascular lumens within them (**Figure 2D**). No mitotic figures were observed in pericytes. Each

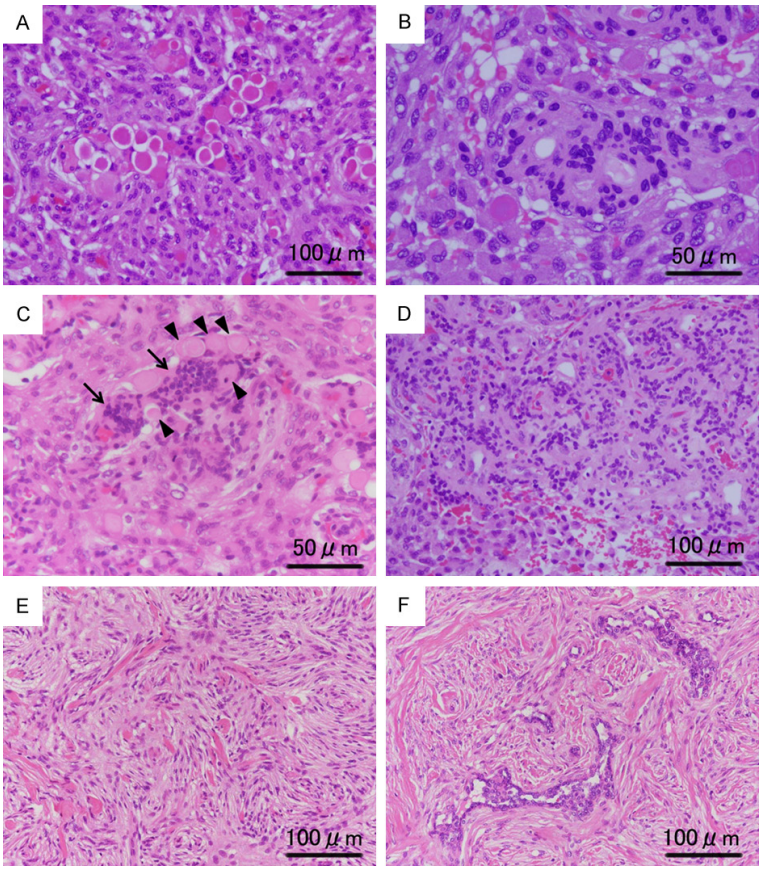

Figure 2. Histopathologic findings. A. The tumor consisted of a sheet-like proliferation of polygonal cells, and many deeply eosinophilic, pseudopsammoma bodies were seen. (Case 1, Hematoxylin-Eosin stain, ×200, scale bar: 100 μm). B. A proliferation of pericytes with hyperchromatic nuclei and scant cytoplasm was seen within the walls of some small blood vessels (center of the figure). (Case 1, Hematoxylin-Eosin stain, ×400, scale bar: 50 μm). C. Proliferating pericytes (arrows) occasionally showed close contact with pseduopsammoma bodies (arrowheads) and together formed cellular masses. (Case 1, Hematoxylin-Eosin stain, ×400, scale bar: 50 μm). D. Proliferating pericytes occasionally formed large cellular aggregates containing multiple lumens (upper two-thirds of the figure). (Case 1, Hematoxylin-Eosin stain, ×200, scale bar: 100 μm). E. The tumor consisted of a fascicular or diffuse proliferation of spindle cells with varying amounts of collagen. (Case 2, Hematoxylin-Eosin stain, ×200, scale bar: 100 μm). F. A proliferation of pericytes with hyperchromatic nuclei and scant cytoplasm was seen in some blood vessels in the peripheral region of the tumor. (Case 2, Hematoxylin-Eosin stain, ×200, scale bar: 100 μm).

pericyte was closely surrounded by PAM-positive basal lamina.

Case 2: The tumor consisted of a fascicular or haphazardly arranged proliferation of spindle cells admixed with varying amounts of collagen fibers (**Figure 2E**). This histologic feature was consistent with fibrous (fibroblastic) meningioma, but small, scattered areas of a sheet-like proliferation of small cells and direct tumor invasion of the superficial cerebral cortical layers, findings consistent with atypical meningioma [9], were observed. A proliferation and stratification of pericytes was focally noted in the walls of small blood vessels (**Figure 2F**), especially in the peripheral areas of the neoplasm near the brain surface, but its intensity and extent was less than in Case 1. Nuclei of the pericytes were more hyperchromatic than those of neoplastic meningothelial cells, and the cytoplasm was scant. No pseudopsammoma bodies were observed throughout the tumor.

Immunohistochemical and ultrastructural findings

In both cases, tumor cells showed membranous or finely granular immunoreactivity for EMA. In Case 1, pseudopsammoma bodies were immunoreactive for CEA, and tumor cells surrounding them showed strong immunoreactivity for pan-cytokeratin. Whereas pericytes in Case 1 were only partly and weakly immunoreactive for α-SMA (**Figure 3A**), those in Case 2 were diffusely and moderately immunoreactive (**Figure 3B**). Pericytes in both cases were immunoreactive for vimentin, but not desmin. No proliferation of endothelial cells accompanied pericytic proliferation in either case (**Figure 3C**). Also, proliferating pericytes showed no labeling for Ki-67 antigen in either case. The labeling indices of neoplastic meningothelial cells in both cases were also very low (Case 1: 1.4%, Case 2: 1.2%).

On ultrastructural examination of Case 1, tumor cells contained nuclei occasionally showing a deep intranuclear cytoplasmic invagination and abundant cytoplasm with well-developed mi-

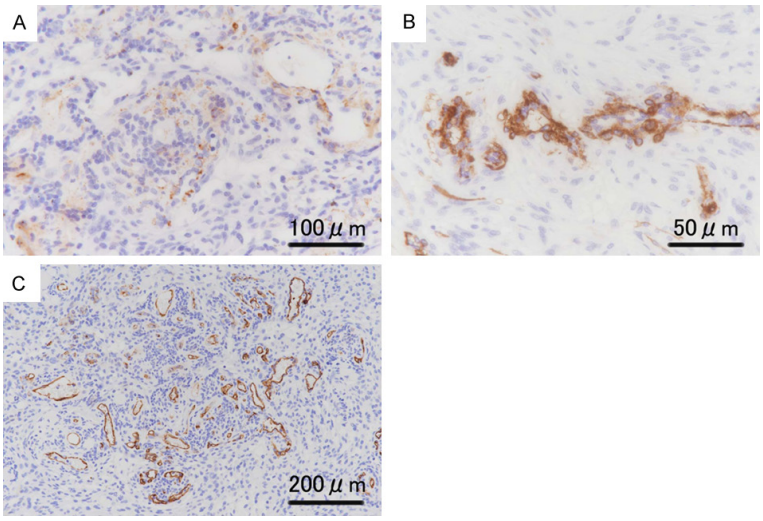

Figure 3. Immunohistochemical findings. A. Pericytes proliferating within the vascular walls were focally and weakly immunostained by α-SMA. (Case 1, Immunostain for α-SMA, ×200, scale bar: 100 μm). B. Proliferating pericytes showed moderate immunoreactivity for α-SMA. (Case 2, Immunostain for α-SMA, ×400, scale bar: 50 μm). C. Endothelial cells did not show proliferative change. (Case 1, Immunostain for CD34, ×100, scale bar: 200 μm).

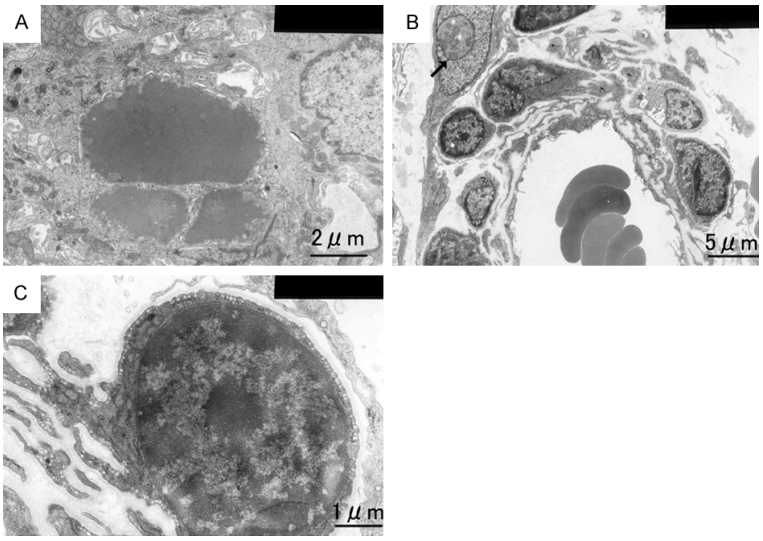

Figure 4. Ultrastructural findings of Case 1. A. Large intracellular lumens containing electron-dense, amorphous material corresponding to pseudopsammoma bodies were found. (×6,000, scale bar: 2 μm). B. Pericytes had small, round or elliptical nuclei with dense heterochromatin and scant cytoplasm, and were invested by the basal lamina. A neoplastic meningothelial cell showing intranuclear cytoplasmic invagination was seen (arrow). (×2,500, scale bar: 5 μm). C. Abundant pinocytotic vesicles arranged in a single row beneath the cytoplasmic surface of a pericyte. (×10,000, scale bar: 1 μm).

tochondria, endoplasmic reticulum, and Golgi apparatus. Tumor cells were connected to each other by many junctional complexes, and formed numerous, filopodia-like cytoplasmic processes. Large intracellular lumens containing electron-dense, amorphous material corresponding to pseudopsammoma bodies were found (**Figure 4A**), and the cytoplasmic membrane lining these lumens formed many microvilli. Pericytes formed multiple cellular layers surrounding endothelial cells of capillaries, and each pericyte was completely invested by the basal lamina (**Figure 4B**). In comparison with neoplastic meningothelial cells, the nuclei of pericytes were small, of uniformly round shape, and contained abundant heterochromatin. The scant cytoplasm contained microfilaments, mitochondria, and free ribosomes. The presence of many pinocytotic vesicles showing a linear arrangement beneath the cytoplasmic membrane was a prominent finding (**Figure 4C**).

Discussion

Pericytes are a variant of vascular smooth muscle cells that were first described by Rouget in 1873 [10]. Their functions are multifaceted and include mechanical support of small vessels, regulation of the vascular caliber, regulation of vascular permeability, modulation of endothelial cell growth, “pruning” of capillaries produced in excess, modulation of inflammatory events within vascular walls, and potential phagocytotic activity [10-15]. Furthermore, pericytes have the ability to produce collagen fibers or proteoglycans and play an important role in the biosynthesis of the basal lamina and angiogenesis (“sprouting”) or remodeling [10, 11, 15, 16]. In the brain, peri-

cytes are important for maintenance of the blood-brain barrier [17].

In the reactive microvascular proliferation seen in glioblastoma, pericytes or vascular smooth muscle cells actively participate in the formation of "glomeruloid" structures in association with endothelial cells [18, 19]. On the other hand, a proliferation of pericytes not accompanied by endothelial proliferation has not documented to date in ordinary meningiomas, nor even in the field of tumor pathology of the systemic organs [2]. This isolated pericytic proliferation was first described in a case of meningioma by Mirra et al., who reported its ultrastructural features [2]. Although they diagnosed their case as meningothelial meningioma, in retrospect, it belongs to secretory meningioma, judging from the presence of pseudopsammoma bodies [8]. (The term "secretory meningioma" was proposed by Alguacil-Garcia et al. four years after their report [3]).

A pericytic proliferation is seen in the majority of cases of secretory meningioma and considered a characteristic finding of this subtype of meningioma [1-7], which shows prominent epithelial differentiation and is currently defined by specific genetic abnormalities (combined *KLF4* K409Q and *TRAF7* mutations) [20]. Alguacil-Garcia et al. examined 58 surgical cases of meningioma of other subtypes and did not find any examples showing pericytic proliferation [3]. Our case 2 is therefore exceptional in that it demonstrated pericytic proliferation in atypical meningioma (mostly featuring fibroblastic meningioma but showing brain invasion).

Immunohistochemical studies on pericytes proliferating in secretory meningioma are scant. Robinson et al. reported that these cells were immuoreactive for vimentin and α-SMA but not desmin [4]. In the two present cases, pericytes showed the same immunohistochemical phenotype. Nehls and Drenckhahn [10] and Hellström et al. [21] subdivided pericytes into two types according to their localization and immunohistochemical properties: smooth muscle-like, transitional pericytes and non-muscle-like, mid-capillary pericytes. In their studies, while the former type was immunoreactive for both desmin and α-SMA, the latter type was immunoreactive for only desmin and not α-SMA. The pericytic proliferation in meningioma in our cases showed an immunohistochemical phenotype (α-SMA-positive but desmin-negative) different from that of ordinary pericytes [10, 13, 21]. A large series of meningiomas should be investigated to further assess this point.

The pathogenesis of an isolated pericytic proliferation in secretory meningioma remains obscure. Some investigators regard the pericytic proliferation as an early stage of divergent differentiation or transition of neoplastic meningothelial cells to hemangiopericytoma [1]. However, hemangiopericytoma is considered to be an entity distinct from meningothelial neoplasms in the current WHO classification of brain tumors [9]. Other investigators regard the pericytic proliferation as a peculiar response of the stromal vasculature, that is probably induced by cytokines secreted from tumor cells [4]. We observed a close contact of pericytes and pseudopsammoma bodies, a finding that has not been emphasized to date. This finding may suggest that pseudopsammoma bodies contain some trophic substance to promote pericytic proliferation and exert a paracrine influence on pericytes. Pseudopsammoma bodies have been reported to contain various substances, such as secretory components, immunoglobulins A and M [5, 22], and it is possible that they also contain some growth factors for pericytes.

Some studies demonstrated that pericytes had a receptor for platelet-derived growth factor-β (PDGF-β). Both PDGF-β and its receptor were critically involved in the recruitment of pericytes in the perivascular immature mesenchyme to vascular walls and also in the proliferation of pericytes during the process of angiogenic sprouting of pre-existing blood vessels [13-15, 21, 23, 24]. PDGF-β is expressed in most cases of meningioma [25], and neoplastic meningothelial cells in secretory meningioma may secrete PDGF-β and stimulate the mobilization and proliferation of pericytes with its receptor.

Pericytic proliferation in secretory meningioma has also been considered in connection with the marked vasogenic cerebral edema around the tumor that characterizes this subtype of meningioma [1, 4-6]. Akdemir et al. suggested that reactive "pericytosis" may act to open the blood-brain-barrier and disrupt the tight junctions by a contractive action of pericytes, leading to diffuse vasogenic edema [6]. However,

since edematous changes are usually not found in areas directly surrounding the foci of pericytic proliferation within meningioma tissue, this hypothesis needs to be further scrutinized.

In conclusion, the proliferation of pericytes in meningiomas most likely represents a peculiar response of the stromal vasculature. We demonstrated that: (1) close contact of pericytes with pseudopsammoma bodies exists in secretory meningioma, (2) the immunophenotype of pericytes proliferating in meningiomas might differ from that of ordinary pericytes, and (3) pericytic proliferation can exceptionally occur in other subtypes of meningioma.

Disclosure of conflict of interest

None.

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