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An ultrastructural and immunohistochemical study of olfactory neuroepithelioma with rhabdomyoblasts

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Abstract A rare case of olfactory neuroepithelioma with rhabdomyoblasts in a 61-year-old man was investigated using electron microscopic and immunohistochemical methods. A large tumor enhanced by gadolinium-diethylenetriamine pentaacetic acid (DTPA) was demonstrated on magnetic resonance imaging (MRI), located within the anterior cranial fossa without bone destruction. The tumor mostly consisted of small cells with scant cytoplasm. Tubular rosettes were often found. Immunoreactivity for cytokeratin and epithelial membrane antigen (EMA) was strongly positive. Most of the tumor cells were shown to be positive for neuron-specific enolase (NSE) and vimentin and weakly positive for synaptophysin and S-100. Rhabdomyoblasts, which showed oval cells with abundant eosinophilic cytoplasm and a nucleus sometimes displaced toward the periphery of the cell body, were frequently intermingled with the tumor cells. The immunoreactivity for myoglobin was frequently positive in these oval cells. The MIB-1 index showed high values, of 20%–40%. About 10% of the tumor cells revealed positivity for p53 protein and vascular endothelial growth factor (VEGF). Ultrastructurally, numerous junctional complexes were observed between cell bodies and processes. The cell processes frequently contained numerous microtubules. There were sometimes numerous filaments with small aggregates of Z-band material and thick filament-ribosomal complexes in the oval cells. They were concluded to be consistent with rhabdomyoblasts on light microscopic and immunohistochemical findings.

Key words Olfactory neuroepithelioma · Olfactory neuroblastoma · Rhabdomyoblast · Ultrastructure · Immunohistochemistry

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Introduction

Olfactory neuroepithelioma is classified as a rare variant of olfactory neuroblastoma arising in the nasopharynx.[1] In 1924, Berger et al.[2] originally reported a case of an olfactory neuroepithelioma, which was characterized to have an epithelial cell component with tubular arrangements (olfactory rosettes) and poorly differentiated cells with fibrous processes resembling neurites. Hassoun et al.[3] also reported a case of esthesioneuroepithelioma and distinguished this tumor from the usual olfactory neuroblastomas. Skolnik et al.[4] presented the clinicopathological features of two patients with olfactory neuroepithelioma and a review of the world literature on this condition. Myogenic differentiation in olfactory neuroepithelioma has, to the best of our knowledge, not been reported previously, in particular in ultrastructural studies. There has been only a brief report about histological and immunohistochemical studies in a case that included rhabdomyoblasts in olfactory neuroblastoma, by Slootweg and Lubsen (1991).[5]

We have recently had the opportunity of ultrastructurally and immunohistochemically examining an uncommon tumor of olfactory neuroepithelioma with the presence of rhabdomyoblasts arising as an intracranial mass.

Case report

History and examination

The patient was a 61-year-old man who had been in good health until he presented to another clinic with frequent

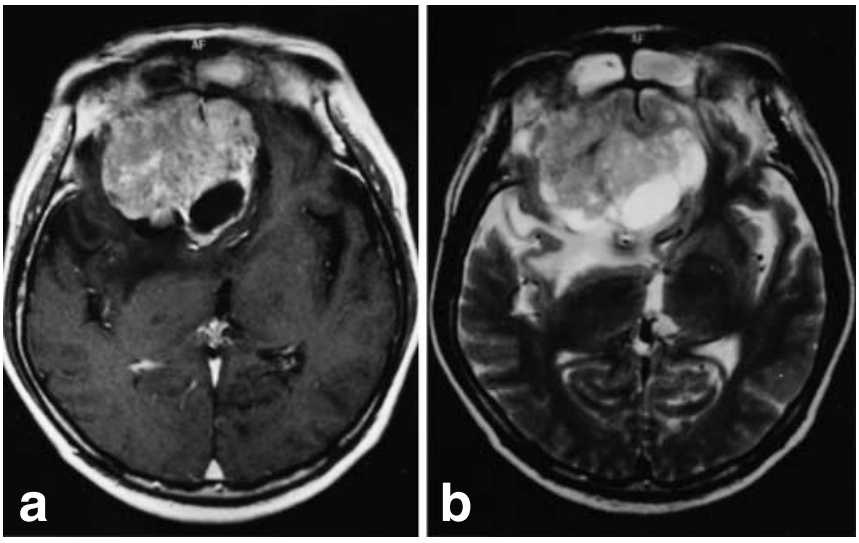

Fig. 1a,b. Magnetic resonance (MR) images. **a** Gadolinium-diethylenetriamine pentaacetic acid (DTPA) T1-weighted image. **b** T2-weighted image. MR images demonstrated the tumor in the anterior cranial fossa to be mostly heterogeneous, and slightly hypodense on T1-weighted images and slightly hyperdense on T2-weighted images, and enhanced inhomogeneously by the intravenous infusion of gadolinium-DTPA

headache. After a computerized tomography (CT) scan revealed a large intracranial mass lesion, he was referred to our hospital. On admission, the patient was alert and had no neurological deficits. The mass appeared on the CT scans as a large well-circumscribed tumor that extended bilaterally from the midline with a broad base in the anterior cranial fossa. The tumor consisted mostly of an isodense mass, which was strongly enhanced after intravenous injection of contrast medium, with a cystic component. No destruction of the bone structures in the anterior cranial fossa was detected on plain craniograms or bone window CT scan. No tumor was demonstrated in the nasal and paranasal cavity. Magnetic resonance (MR) imaging demonstrated the tumor to be mostly heterogeneous and slightly hypodense on T1-weighted images, slightly hyperdense on T2-weighted images, and enhanced inhomogeneously by intravenous infusion of gadolinium-diethylenetriamine pentaacetic acid (DTPA) (Fig. 1). On a cerebral angiogram, the vascularity into the tumor was found to be fed through the anterior skull base from the external carotid artery; however the vascularity was not great.

Operation and postoperative course

The patient underwent gross total removal of the tumor by bifrontal craniotomy. The tumor was found to be located wholly within the intracranial cavity and outside the dura. There was no dural invasion or bone destruction in the anterior cranial base. The patient's postoperative course was uneventful. The patient underwent radiation therapy, with a total dose of 50 Gy. Two years after the surgery he had had no recurrence and was in excellent health.

Material and methods

Surgical specimens were fixed in 10% formalin, embedded in paraffin, and stained with hematoxylin-eosin (H&E). Immunohistochemical studies for the presence of cytokeratin, epithelial membrane antigen (EMA), synaptophysin, neuron-specific enolase (NSE), MIC 2, myoglobin, vimentin, p53 protein, vascular endothelial growth factor (VEGF), glial fibrillary acid protein (GFAP), and MIB-1 (Ki-67) to determine cell proliferation were carried out using the above paraffin-embedded sections. The avidin-biotin-peroxidase complex (ABC) method was used with deparaffinized sections for immunohistochemical detection.

Samples for electron microscopic examination were fixed in 2% glutaraldehyde with cacodylate buffer, postfixed in 2% osmium tetraoxide, dehydrated, and embedded in Epon. Ultrathin sections were cut with a diamond knife on a porter type I ultramicrotome, and stained with uranyl acetate and lead citrate prior to observation with a JEM 1200 EXII electron microscope (JEOL, Tokyo, Japan), using a 20- or 50-μm ϕ objective aperture at an accelerating voltage of 80 kV. Transmission electron micrographs were taken at original magnifications of 3000 to 20 000 times.

Results

Light microscopic findings

Histologically, the tumor mostly consisted of compactly arranged sheets and large nests of small, round-to-oval cells with scant cytoplasm surrounded by fibrovascular stroma. Tumor cells had relatively hyperchromatic nuclei and mitotic figures were frequently observed. Necrosis and hemorrhage were occasionally seen in small scattered foci of the tumor. Tumor cells were often found in a characteristic tubular arrangement (Flexner rosettes), which consisted of cylindrical cells radially oriented toward a central lumen, and Homer-Wright rosettes (Fig. 2a). Cilia were occasionally found on the luminal surfaces of the tumor

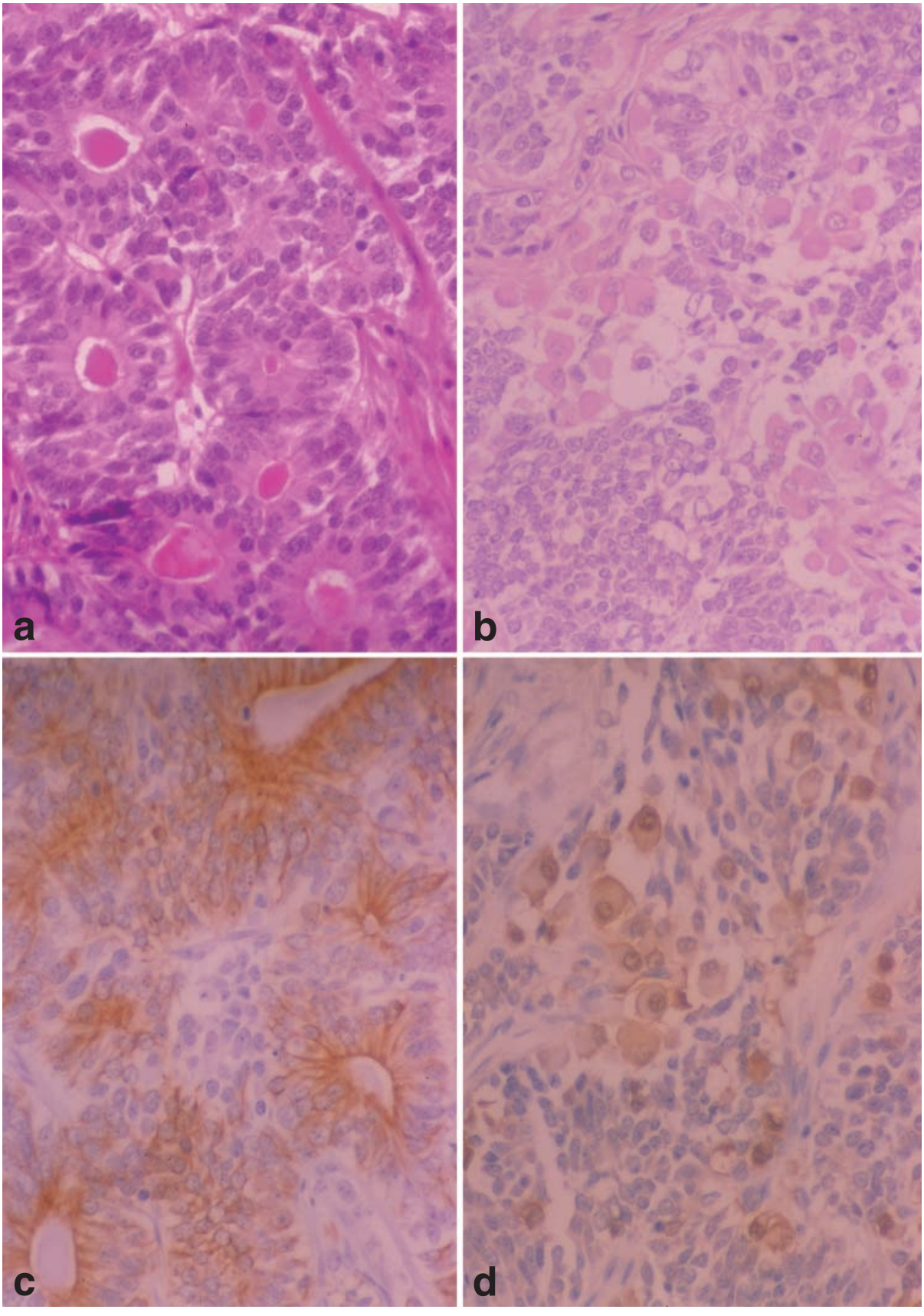

Fig. 2a–d. Micrographs. **a** Showing characteristic tubular arrangement (Flexner rosette), which consisted of cylindrical cells radially oriented toward a central lumen. **b** Oval cells were frequently observed to have abundant eosinophilic cytoplasm and a nucleus sometimes displaced toward the periphery of the cell body. **c** Micrograph reveals strongly positive immunoreactivity for cytokeratin in the cytoplasm of most of the tumor cells. **d** Micrograph shows positive reaction for myoglobin in the tumor cells, with a relatively large amount of cytoplasm, findings which were consistent with rhabdomyoblasts. **a** and **b** H&E, ×200; **c** cytokeratin ×200; **d** myoglobin ×200

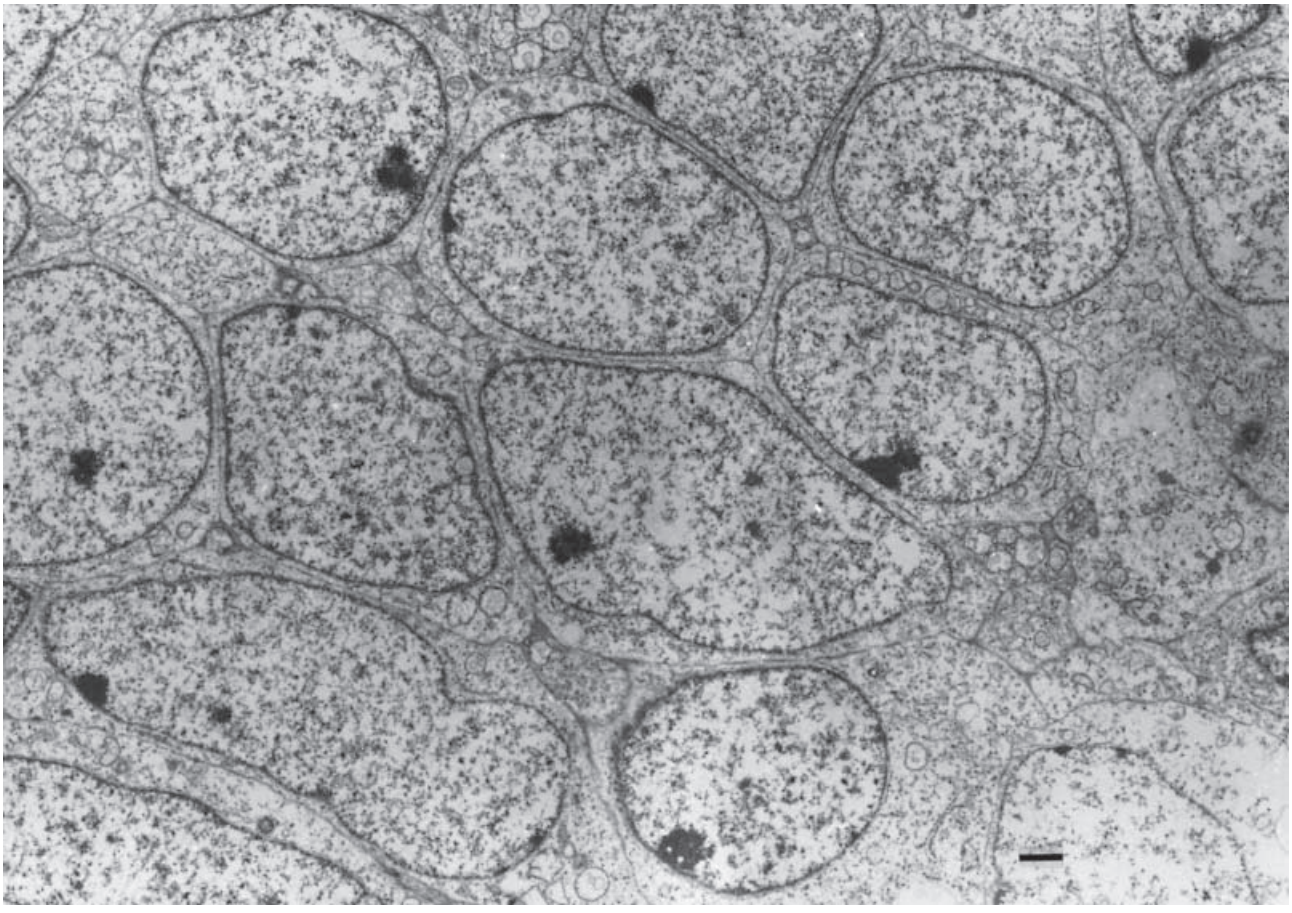

Fig. 3. The tumor was mainly composed of round-to-oval cells, with scant cytoplasm, with a high nuclear/cytoplasmic ratio. Tumor cell processes were often presented between the tumor cell bodies. *Bar* 1 μm

cells, forming a tubular arrangement. Most of the tubular rosettes had positive materials for periodic acid schiff (PAS) staining in the lumen. In some of the tumor areas, oval cells were frequently observed to have abundant eosinophilic cytoplasm and a nucleus sometimes displaced towords the periphery of the cell body (Fig. 2b). These cells were suspected to be rhabdomyoblasts.

Immunohistochemical findings

Immunohistochemical studies of this tumor have demonstrated positive reactions for both neurogenic and epithelial markers. Immunoreactivity for cytokeratin and EMA, as epithelial markers, was strongly positive in the cytoplasm of most of the tumor cells, and, in particular, cytokeratin immunoreactivity was detected extensively in the tumor cells forming tubular rosettes (Fig. 2c). A relatively large number of tumor cells were shown to be positive for NSE. However, most of the tumor cells forming tubular rosettes were not reactive for NSE. Synaptophysin, one of the neurogenic markers, was weakly positive in some of the tumor cells. S-100 protein-positive cells were found in some of the tumor cells without any particular arrangement, including some of the tumor cell cytoplasm of the fibrous component in the tumor tissues. Most of the tumor cells had positive immunoreactivity for vimentin. Neither MIC 2 nor glial fibrillary acidic protein (GFAP) were detected in the tumor cells.

Immunoreactivity for myoglobin was frequently found to have a positive reaction in the eosinophylic cytoplasm of relatively large tumor cells (Fig. 2d). The MIB-1 index, a marker for proliferative potential, showed high values, of 20%–40%, in small round cells with scanty cytoplasm. Ten percent to 20% of the small round cells had immunoreactivity for p53 protein. About 10% of the tumor cells showed immunoreactivity for VEGF in part of the tumor tissue.

Ultrastructural findings

The tumor was mainly composed of round-to-oval cells which had a high nuclear/cytoplasmic ratio, and the organelles in their scant cytoplasm were usually poorly developed (Fig. 3). Tumor cells frequently showed a rosette-like arrangement, and between the tumor cell bodies, tumor cell processes were often presented, particularly at the center of a rosette-like arrangement. Numerous junctional complexes, such as desmosome-like structures and tight junctions, were observed between the processes and the cell

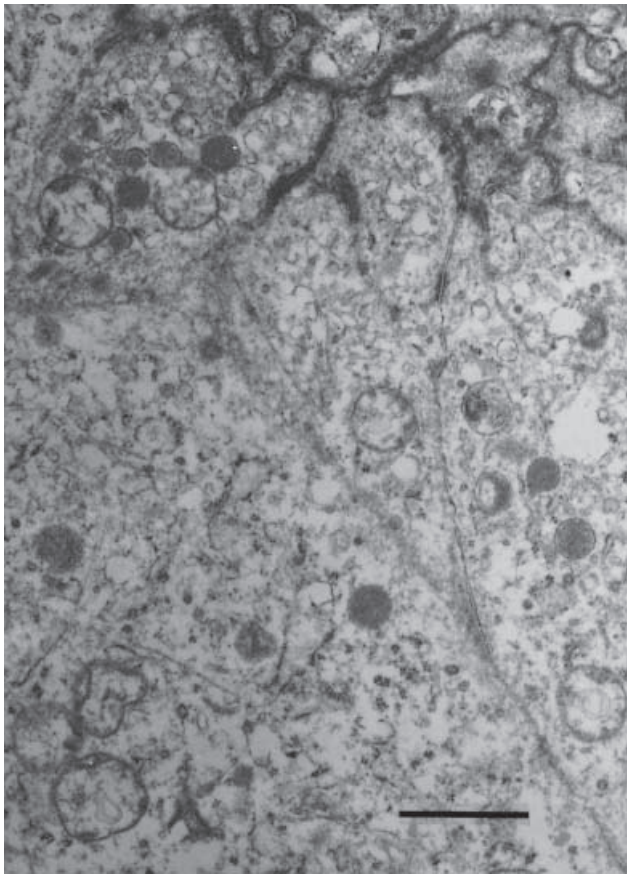

Fig. 4. Numerous junctional complexes were observed between processes and cell bodies, and interdigitation of plasma membrane was noted. Dense-cored vesicles appeared in some tumor cell processes. *Bar* 1 μm

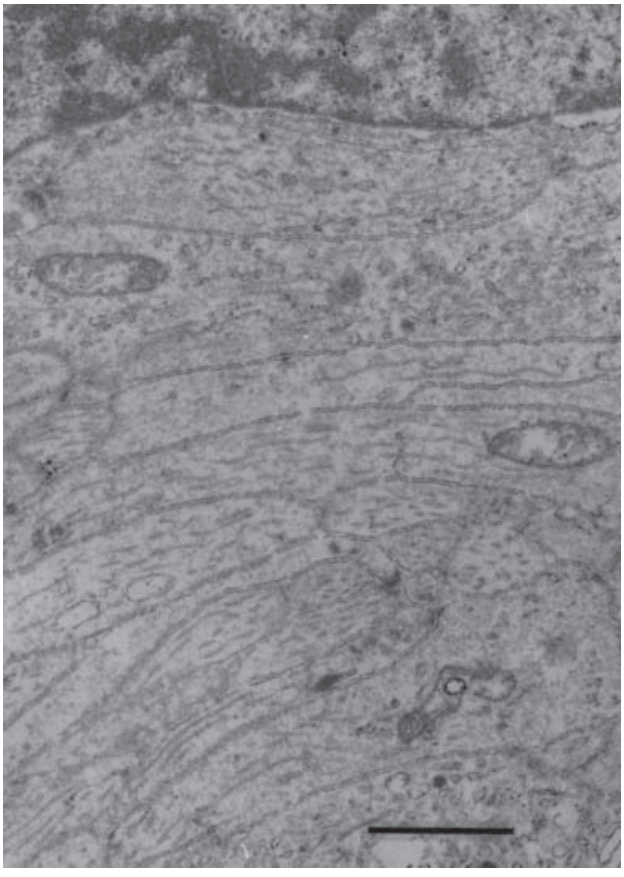

Fig. 5. The tumor cell processes frequently demonstrated numerous microtubules in the cytoplasm. *Bar* 1 μm

bodies, and interdigitation of plasma membrane was noted (Fig. 4). At the margin of the nests, the tumor cell bodies and processes were abutting on a basement membrane separating them from connective tissues. Tumor cell processes frequently demonstrated numerous microtubules, this being essentially confirmatory of the neuronal nature of this neoplasm (Fig. 5). The tumor cell processes included relatively well-developed cell organelles such as mitochondria, rough endoplasmic reticulum (ER), and lysosomes. However, dense-cored vesicles were scarcely seen in them.

Oval cells, which had eosinophilic cytoplasm and, sometimes, the nucleus displaced towards the periphery of the cell body on microscopic findings, were sometimes found, and they were consistent with rhabdomyoblasts in terms of immunohistochemical findings and ultrastructural features. These oval cells were observed to have basal lamina around them and, infrequently, intercellular junctions were seen between them. In the cytoplasm of these tumor cells, there were numerous filaments forming bundles, and the filament bundles occasionally included small aggregates of Z-band material (Fig. 6). These tumor cells also revealed findings of thick filament-ribosomal complexes, in which a group of thick filaments were arranged in parallel, and rows of ribosomes were situated between them (Fig. 7). These tumor cells were diagnosed as rhabdomyoblasts.

Discussion

There have been few studies, with electron microscopy and immunohistochemical methods, of olfactory neuroepithelioma.[3,6–12] Referring to its histogenesis, Hassoun et al. (1981)[3] and Takahashi et al. (1987)[9] reported that olfactory neuroepithelioma (esthesioneuroepithelioma) is derived from the bipotential, undifferentiated basal cells of the olfactory epithelium which develops from primordial placodes that are cytoarchitecturally similar to brain vesicles or neural tubes. On the other hand, olfactory neuroblastomas are considered to be derived from the APUD cell nests of the ethmoidal and maxillary sinuses.[13–15] But there has still been persistent controversy in terms of conclusions on their histogenesis. The present case was localized as an extradural mass without bone destruction in the anterior cranial fossa, and no tumor infiltration was seen in the paranasal sinus and nasal cavity. The origin of the present case could actually be the olfactory epithelium and, more precisely, the bipotential basal cells of this epithelium.

It has been characterized that olfactory neuroepithelioma reveals the features of bipotential differentiation into neurons and epithelial cells, and has numerous true rosettes (Flexner) of various sizes, which were different from olfac-

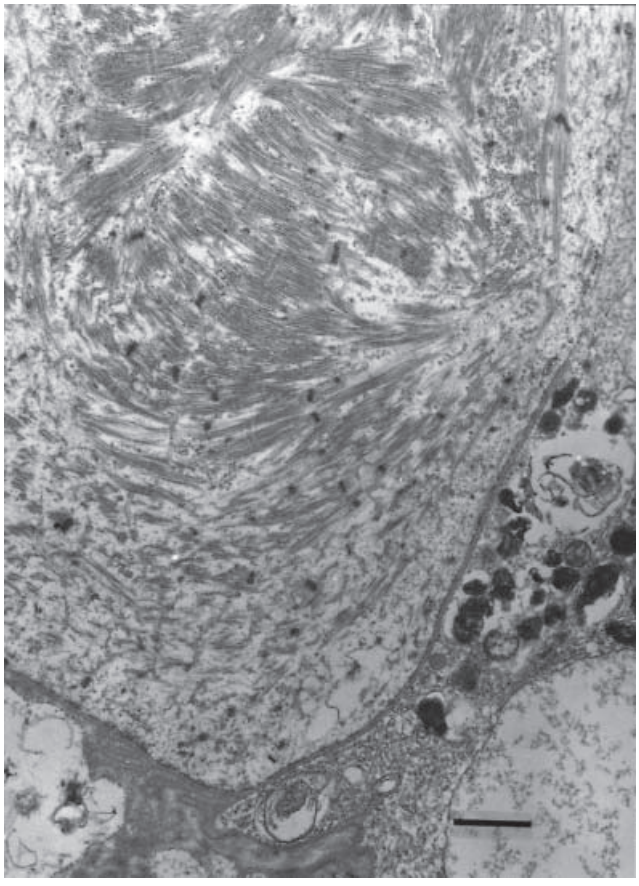

Fig. 6. Numerous filaments, forming bundles, were found in the cytoplasm of the tumor cells, and the filament bundles occasionally contained small aggregates of Z-band material, which was characteristic of rhabdomyoblasts. *Bar* 1 μm

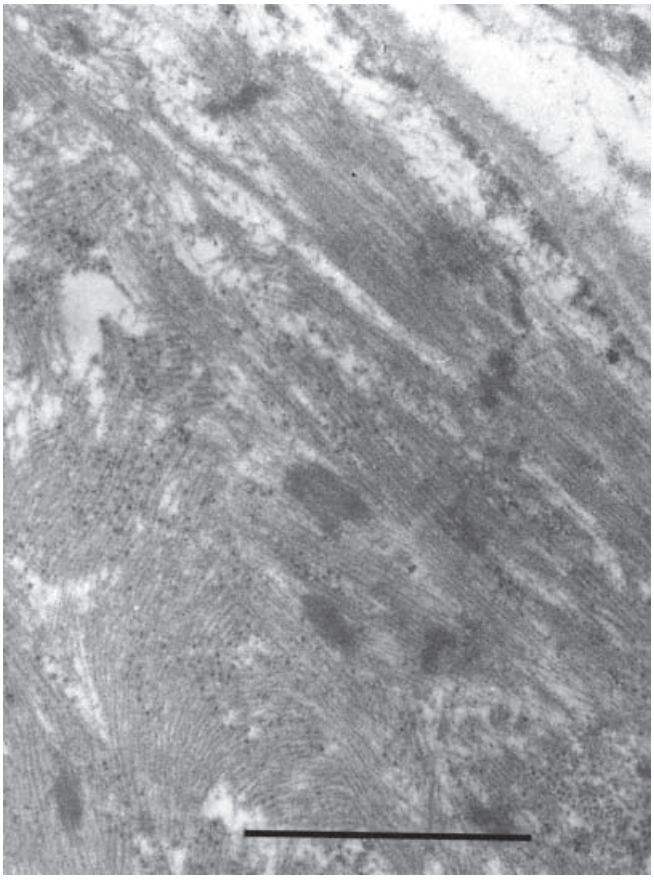

Fig. 7. These tumor cells also revealed filament bundles with Z-band materials, and thick filament-ribosomal complexes, in which a group of thick filaments were arranged in parallel, and rows of ribosomes were situated between them. *Bar* 1 μm

tory neuroblastoma on light microscopic findings. Kudo et al.,[6] Hassoun et al.,[3] and Takahashi et al.[9] reported that two cell types clearly demonstrated characteristic ultrastructural features. The first cell type showed cilia and basal bodies with well-developed cytoplasmic organelles. They also bore processes resembling neurites. The second cell type was found in tubular arrangements with numerous microvilli and apical junctional complexes. However, they emphasized the absence of dense-cored secretory granules in these cells. The present case also showed the characteristics of epithelial cells, with numerous junctional complexes between the tumor cells and rosette-like arrangement of the tumor cells, and the cell processes between tumor cells frequently included microtubules, suggesting neuronal differentiation.

Immunohistochemical studies of olfactory neuroepithelioma were reported in only four cases in the previous literature.[9–12] All of the tumors revealed positive immunoreactivity for keratin, EMA, NSE, S-100, and vimentin. Immunoreactivity for synaptophysin and neurofilament was indicated to be different depending on the case. The present tumor demonstrated positive immunoreactivity for keratin, EMA, NSE, S-100, and vimentin, and synaptophysin in small numbers of tumor cells. MIB-1 indices were 43.8% (case 25 in Hirose et al.'s report[11]) and 3.5% (Utsuki et al.[12]) in two cases in the previous reports, and 20%–40% in the present case. Hirose et al.[11] noted that longer survival rates were significantly related to: (1) a low (<10%) Ki-67 (MIB-1) index and (2) a higher incidence of S-100 protein-positive cells, in their 26 olfactory neuroblastomas. The expression of p53 protein was detected immunohistochemically in 62% of the 26 cases of olfactory neuroblastoma, which included 1 case with biphasic (neuronal and epithelial) differentiation and 1 case with predominantly epithelial features, in the study of Hirose et al.[11] Our case also showed p53 protein expression, in about 10% of all tumor cells, by an immunohistochemical method.

It has been characterized that rhabdomyoblasts are relatively large cells with abundant eosinophilic cytoplasm, and hyperchromatic nuclei and mitotic figures tend to be abundant.[16] Immunohistochemical staining shows positivity for myoglobin and desmin within rhabdomyoblasts.[16] The present tumor was a rare case of olfactory neuroepithelioma combined with rhabdomyoblasts with ample eosinophilic cytoplasm and showing positivity for myoglobin, one of the myogenic markers. It was confirmed to have the

characteristics of filament bundles with Z-band material and thick filament-ribosomal complexes in the cytoplasm in regard to the ultrastructural features.[17,18]

It is well known that several tumors besides rhabdomyosarcomas contain neoplastic rhabdomyoblasts. Myogenic differentiation has been reported in intracranial neuroectodermal tumors such as medulloblastoma and medulloepithelioma.[19,20] This divergent differentiation was explained by the capacity of the neural crest to differentiate into various mesenchymal cell types, including skeletal muscle cells.[21] Slootweg and Lubsen[5] first reported the presence of rhabdomyoblasts in a case of typical olfactory neuroblastoma, and these cells exhibited positivity for vimentin, actin, and myosin in ample cytoplasm, resembling the findings in our case.

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