Immunohistochemical examination of an orbital alveolar soft part sarcoma

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Abstract

Background: A 32-year-old male patient had a 6-week history of left-sided proptosis. Computer tomography revealed a 16×15×15 mm smooth and well-defined mass between the optic nerve and the medial and superior rectus muscles in the left orbit.

Methods: The tumour was excised via a cranio-medial orbitotomy approach.

Results: Histopathological examination, immunohistochemistry and electron microscopy findings were consistent with an alveolar soft part sarcoma. Immunohistochemical staining showed positive immunoreactivity for neuron-specific enolase, vimentin, p53 (30%), p21 (10%) and cyclin D1 (20%), and negative immunoreactivity for CD45, cytokeratins, S-100 protein, glial fibrillary acidic protein, synaptophysin, chromogranin, calcitonin, serotonin, thyreoglobulin, desmin, myosin, actin, HMB-45, pRB, p16 and BCL-2. The growth fraction of the tumour cells was 3%. At examination 4 years after surgical excision, there was no evidence of local recurrence or for metastases.

Conclusion: Alveolar soft part sarcoma of the orbit is a rare malignant tumour best controlled by surgery. The unpredictable behaviour of these neoplasms, however, indicates the need for long-term follow-up.

Introduction

Alveolar soft part sarcoma (ASPS) is an uncommon malignant soft tissue tumour, representing approximately 1% of all soft tissue tumours [22]. Although it has been reported in other locations, most cases of ASPS occur in the deep soft tissue of the extremities of young adults [39] and in the head and neck region in children [65]. When occurring in the latter location, the tongue and orbit are sites of predilection. To date, approximately 50 cases have been described in the orbit [1, 2, 11, 13, 26, 29, 30, 32, 34, 42, 53, 55, 65, 71], the largest series being reported by Font et al. [26] and, recently, by Simmons et al. [65]. Although electron-microscopic investigations have been performed on orbital ASPS, immunohistochemical studies are limited. We describe the results of an extensive immunohistochemical study of an additional case of an orbital ASPS.

Case history

A 32-year-old male patient presented with a 6-week history of progressive proptosis of the left eye. The patient was of good health and had no prior history of ocular or systemic disease.

The ophthalmological examination revealed a slight proptosis of 5 mm of the left eye (Fig. 1) without any manifestation of conjunctival injection or signs of congestion. An orbital mass or swelling could not be palpated. The intraocular pressure, visual acuity and visual fields in both eyes were normal and ocular motility was not restricted. The remaining examination of the anterior and posterior segments was unremarkable.
Computer tomography demonstrated a 16×15×15 mm smooth and well-defined mass between the optic nerve and the medial and the superior rectus muscles in the left orbit (Fig. 2). The mass demonstrated high signal intensity relative to muscle on T1-weighted MRI and flow voids on angiography. All other orbital structures were unremarkable. Abdominal, retroperitoneal and thoracic CT did not reveal any evidence of a possible primary extra-orbital tumour.

As the nature of the orbital mass could not be determined clinically, the tumour was removed via a cranio-medial orbitotomy approach. Intraoperatively, it was well defined, encapsulated and not adherent to adjacent orbital structures.

Methods

Following excision, the tumour was immediately placed in 4% buffered formalin and processed through paraffin for conventional histology. Stains included haematoxylin and eosin, periodic acid–Schiff (PAS) before and after diastase digestion, van Gieson, Prussian blue for iron and the modified Grimelius stain (2% silver nitrate) for cytoplasmic argyrophil granules.

Additional slides were stained for immunohistochemical studies using a panel monoclonal and polyclonal antibodies that are reactive in paraffin sections. An antigen retrieval method using a pressure cooker was performed before immunohistochemical staining [56]. The staining consisted of a first-stage incubation with the following primary monoclonal antibodies: CD45; a pan-cytokeratin marker (MNF-116); vimentin; S-100 protein; glial fibrillary acidic protein (clone 6F2; GFAP); neurone-specific enolase (NSE); synaptophysin; chromogranin; calcitonin; serotonin; thyreoglobulin; desmin; myosin; actin; HMB-45; cyclin D1 (clone P2D11F11); retinoblastoma protein (pRB; clone G3–245 which binds to an epitope between amino acids 300–380 of human Rb; p53 (clone DO7 which recognises both wild-type and mutant p53 proteins); p21 (clone DCS-60.2); P21 (clone DCS-50.1A7); BCL-2 (clone 124; Dako, Denmark); and MIB-1 (antigen Ki-67, which reacts with a DNA-associated antigen in the nuclei at all phases of the cell cycle except the resting phase [27]; the antibody was kindly provided by Dr J. Gerdes, Borstel, Germany. The antibodies were made visible with an indirect immunoperoxidase method for p53, whereas the alkaline phosphatase anti-alkaline phosphatase (APAAP) method was used to demonstrate the binding of the remaining antibodies [16]. The number of MIB-1- and p53-positive cells was determined by counting the number of cells with clear nuclear positivity for these markers per 5×100 tumour cells using the 40× objective (Olympus, BH2).

Electron-microscopic studies were performed on tissue retrieved from the paraffin block. Blocks were processed through Araldite and examined on a Jeol 1200 EXII transmission electron microscope at 80 kV.

Results

Macroscopically, the excised tumour measured 15 mm³, was soft in consistency and encapsulated by a thin pink-brown capsule. The cut surface was yellow-brown in colour and vessels were identified in the parenchyma. Histopathological examination disclosed a tumour with organoid pattern composed of nests of middle-sized round to polyhedral epithelioid cells with distinct cell boundaries. The cell nests were separated by thin fibrous septa containing delicate vascular channels. The reticulin fibres clearly outlined the cell nests, demonstrating the alveolar pattern of the tumour. The tumour cells had eccentrically placed nuclei often with a single prominent nucleolus and surrounded by abundant granular acidophilic and sometimes vacuolated cytoplasm (Fig. 3). Nuclear polymorphism was not present and only occasional mitotic figures were seen. Similarly, neither haemorrhage nor necrosis was a feature of this tumour. The modified Grimelius stain was negative for cytoplasmic argyrophil granules. PAS-positive diastase-resistant granules and crystals of varying size and shape were observed in the cytoplasm. On electron microscopy, classical ASPS crystals were not observed (where rhomboid, rod-shaped and oblong-shaped crystals with a regular lattice pattern), nor were there convincing electron-dense secretory granules. The tumour cell cytoplasm contained large numbers of mitochondria in an electron-dense granular matrix, and occasionally pseudocrystals with a finely filamentous content were identified (Fig. 4).

Immunohistochemical staining showed positive immunoreactivity for neurone-specific enolase, vimentin, p53 (30%) (Fig. 5), p21 (10%) and cyclin D1 (20%) (Fig. 6), and negative immunoreactivity for CD45, cytokeratins, S-100 protein, glial fibrillary acidic protein, synaptophysin, chromogranin, calcitonin, serotonin, thyreoglobulin, des...