Electron microscopic and immunohistochemical findings in a case of olfactory neuroblastoma

Z M Du, Y S Li, B F Wang

Abstract
A case of olfactory neuroblastoma is reported. Light microscopic examination showed various arrangements of poorly differentiated tumour cells forming either uniform sheets or convoluted cords of multiple cell layers orientated toward a richly vascular stroma. Electron microscopic examination showed the presence of abundant cytoplasmic filaments and processes, and dense core endocrine vesicles ranging from 100–160 nm in diameter in both the perinuclear region and tumour cell processes. Immunohistochemical staining was positive in most of the tumour cells for neuron specific enolase, and in a few cells for S-100 protein, vimentin, and serotonin, but staining for desmin and keratin produced no reaction.

Results

Microscopic appearances
In haematoxylin and eosin stained sections, the tumour tissue showed poorly differentiated neuroblasts forming either uniform sheets against a delicately fibrillated background or convoluted cords of multiple cell layers orientated towards a richly vascular stroma (fig 1), with a suggestion of whorls or a rosettelike structure. The tumour cells were about 8–12 μm in diameter with round, oval, or kidney-shaped nuclei and eosinophilic cytoplasm. Argyrophilic staining showed abundant neuro fibrils and dendritic processes originating from the tumour cells. Argyrophilic granulates were also present in some cells. Tumour cell nests had infiltrated into the surrounding normal tissue. There was prominent necrosis of some tumour tissue which seemed related to poor blood supply.

Ultrastructural findings
The tumour cells contained convoluted nuclei with additional invaginations thus forming multiple nuclear pockets. The cytoplasm was poorly differentiated, except for abundant free ribosomes, with scant organelles (fig 2), although the mitochondria, endoplasmic reticulum, glycogen, lysosomes and autophagosomes were present to some degree. A prominent feature was the presence of dense core vesicles ranging from 100 to 160 nm in diameter and scattered mainly in the cytoplasmic processes (fig 3), though their number...
was small. Another characteristic was the presence of abundant cytoplasmic processes, axon-like vesicles, and compact bundles of cytoplasmic filaments, presumably neurofilaments. Ciliated structures, or basal bodies, were also detected in a few tumour cells (fig 4), indicating some differentiation towards mucosal epithelial cells.

IMMUNOHISTOCHEMICAL STAINING
Most of the tumour cells had a moderately positive reaction to the antibody against neuron-specific enolase. Positivity to S-100 protein was found mostly in the peripheral dendritic cells and their cytoplasmic processes extending into the intercellular spaces among other tumour cells (fig 5). Occasional positivity for vimentin and serotonin was expressed by only a few tumour cells but the fibrinoblasts and vascular endothelial cells were strongly positive for vimentin. Desmin and keratin were not expressed at all in tumour cells.

Discussion
The structure features of olfactory neuroblastoma have been detailed in many reports. The presence of abundant axons, of dense core vesicles of various sizes, numerous cytoplasmic processes, microtubules and synaptic complexes, all offer evidence of the neuronal nature of this neoplasm. The described number and size of the dense core vesicles has varied greatly in previous reports, possibly related to the variable endocrine activity of different tumours.

A separate variant, regarded as an example of true aesthesioneuroepithelioma originating from sensory neurons and sustentacular cells of the olfactory epithelium, and devoid of dense core granules, has also been described. The dense core granules of tumour cells in our case were comparatively rare, but could be
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clearly demonstrated by electron microscopy, suggesting that the tumour was poorly differentiated. The presence of abundant cytoplasmic processes, clear centred vesicles, and compact bundles of cytoplasmic filaments, presumably neurofilaments, were typical of the described morphological features of olfactory neuroblastoma.

Cilia-like structures and basal bodies have been observed in some tumour cells, suggesting partial differentiation towards olfactory epithelium. Silva et al. reported that olfactory epithelium was observed in three of nine cases of olfactory neuroblastoma. The occasional association of olfactory neuroblastoma with adenocarcinoma and even squamous carcinoma has also been described. These exceptional mixed cell cases are regarded as instances of divergent neoplastic differentiation, presumably originating from undifferentiated basal cells. Olfactory epithelium consists of three cell types, basal cells, supporting cells, and sensory neurons, so it is logical that differentiation of epithelial cells arises in this tumour.

The most consistently positive immunohistochemical reaction is that for neuron-specific enolase. S-100 protein positive cells are found mostly in peripheral Schwann cells. Axe et al. reported that of eight cases of olfactory neuroblastoma, six were positive for NSE, five for SD-100 protein, and one for neuron filament protein. When keratin is expressed, it may be associated with frank squamous differentiation. We observed that most tumour cells were positive for NSE, and some dendritic cells were positive for S-100 protein, which confirmed the diagnosis of olfactory neuroblastoma.

Vimentin positivity is usually regarded as characteristic of sarcomas, but a positive reaction was found in a few tumour cells in this case. It was demonstrated by Schwob et al. that vimentin is the only intermediate protein present in most neurons in the olfactory epithelium, a feature which they share with the early, more undifferentiated stage of normal neuronal development. Vimentin positivity of tumour cells may be the expression of an embryonal antigen.

Silva et al. subdivided the tumour into neuroblastoma and neuroendocrine carcinoma, both groups showing different age incidences, recurrence rates, and metastatic potential. The main features of our case are more in accordance with neuroblatoma than neuroendocrine carcinoma, and the observed negative reaction to keratin and desmin suggested no component of adenocarcinoma or squamous carcinoma to be present.

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