Intracranial olfactory neuroblastoma: Evidence for olfactory epithelial origin

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Abstract

Aims: To determine the possible histogenesis of the intracranial variant of olfactory neuroblastoma.

Methods: Four specimens from three cases of intracranial olfactory neuroblastoma were studied by light microscopy and immuno-histochemistry, and electron microscopy in two cases.

Results: Light microscopical examination showed small cell tumour with additional features of epithelioid cells in one case and ganglion cells in another. Olfactory and Homer-Wright rosettes were present. All the specimens showed a uniform positive reaction to neurone specific enolase, S-100, and cytokeratin antibodies. Glial fibrillary acidic protein was absent. The salient electron microscopic features were the presence of cell junctions, cytoplasmic intermediate filaments, basal bodies and cytoplasmic processes. Dense cored vesicles were absent.

Conclusions: The results strongly support the view that intracranial olfactory neuroblastomas are of olfactory epithelial origin and differ from conventional neuroblastomas.

Methods

Four specimens from three Indian patients with olfactory neuroblastoma who presented to the neurosurgical unit of this hospital with an anterior cranial fossa mass were studied. Nasal or paranasal tumour was detected nine months after diagnosis along with a spinal metastasis in case 1 and at the time of presentation in case 2. The excised intracranial tumours from all the cases and the nasopharyngeal tumour from case 2 comprised the material of this study (table).

Light microscopic and immunohistochemical studies were performed on formalin fixed, paraffin wax embedded sections. Immunostaining was performed for the presence of neurone specific enolase (NSE), S-100, cytokeratin and glial fibrillary acidic protein (GFAP). The conventional indirect immunoperoxidase method was used with primary antibodies against polyclonal NSE (prediluted) (San Ramon, California, USA), polyclonal S-100 (prediluted) (San Ramon), monoclonal cytokeratin (prediluted anticytokeratin cocktail MAK-6, Triton Biosciences, Alameda, California, USA) and polyclonal GFAP (Dako, Santa Barbara, California, USA), diluted 1 in 1500. The MAK-6 cytokeratin cocktail used in this study provides qualitative demonstration of human cytokeratin numbers 8, 14, 15, 16, 18 and 19. Electron microscopy was performed on tissue removed from a paraffin block in case 1 and fixed in 3% glutaraldehyde in case 3. Ultrathin sections stained with lead citrate and uranyl acetate were examined using a Philips 201 electron microscope.

Results

Light microscopic morphology in the three cases was similar to some extent but each showed certain distinctive features. The common features were a lobulated appearance, small lymphocyte-like cells, and olfactory rosettes. The fibrous septae dividing the lobules were thin and contained capillaries. The small cells had scanty cytoplasm, round nuclei, well defined nuclear membrane, moderately dense chromatin and nucleoli (fig 1). These cells exhibited from two to five mitotic figures per high power field. A focally fibrillar background related to these cells was present in cases 2 and 3 but not in case 1 which consisted of a homogenous population of small cells only. Rosettes of the olfactory type with a well defined lumen were present in all the cases and case 1 also displayed several Homer-Wright

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Olfactory neuroblastoma is a relatively rare malignant tumour with a wide spectrum of morphological appearances and uncertain histogenesis. Difficulty in establishing a correct diagnosis may be experienced because of variable morphology, unusual location, or unfamiliarity. Recent immunohistochemical and ultrastructural studies have attempted to resolve the question of histogenesis and at the same time have provided useful diagnostic criteria.¹⁻⁹ The results of these studies have not been consistent, however, and further application of these techniques continues to add new information about the nature of olfactory neuroblastoma.

Case No	Age/sex	Primary site	Treatment	Follow up
1	28 M	Anterior cranial fossa	Surgery radiation	Spinal metastasis, ethmoidal tumour nine months after operation
2	22 M	Anterior cranial fossa Nasal cavity nasopharynx	Surgery	Died
3	26 M	Anterior cranial fossa	Surgery, radiation	Optic atrophy, no tumour 1 year postoperatively

Figure 1 Case 1: lobulated tumour consisting of more or less uniform cells and no fibrillar background. Note rosette with lumen (Haematoxylin and eosin).

pseudorosettes. Focal necrosis was present in all the cases. The intracranial portion of the tumour in case 2 contained small numbers of another cell type towards the periphery of some of the lobules. These were well defined larger discrete cells with abundant hyaline cytoplasm and relatively smaller eccentrically placed nuclei (fig 2). A nasopharyngeal specimen of the same tumour did not contain these cells. These are subsequently referred to as ganglion cells.

Cells of epithelial appearance in solid nests or mixed with small cells were observed only in case 3. These were larger with a moderate amount of cytoplasm, relatively larger nuclei with lighter chromatin, and attached to each other (fig 3). These are subsequently referred to as epithelioid cells. The nasopharyngeal tumour in case 2 showed a polypoidal mass

Figure 2 Case 2: intracranial tumour showing clusters of large cells (ganglion cells) between lobules of small cells (Haematoxylin and eosin).

covered with intact surface epithelium and olfactory rosettes bearing small cell tumour in the submucosa (fig 4).

IMMUNOHISTOCHEMICAL FINDINGS

Immunostaining was positive for NSE, S-100, and cytokeratin, and negative for GFAP in all the four specimens. NSE positivity was observed in scattered tumour cells throughout the lesions and did not follow any specific distribution pattern. Dense staining was observed in the positive cells while other small cells were totally negative. The fibrillar background, when present, also stained positively. The ganglion cells of case 2 and the epithelioid cells of case 3 were weakly positive. The S-100 positivity exhibited a consistent and characteristic distribution pattern. The positively stained cells were located almost exclusively at the periphery of the lobules. The cytoplasmic processes of these cells were also positive and merged with the connective tissue fibres of the septae but could be clearly distinguished from these under high magnification. Occasional positive cells found within the lobules could be traced to the periphery in adjacent sections. These cells could not be identified in the routinely stained sections. The ganglion cells and the epithelioid cells were S-100 negative.

Cytokeratin positive cells were also distributed throughout the tumours. In the small cell areas, apart from strongly positive scattered cells, positive staining was particularly prominent at the centre of the rosettes, both the olfactory and the Homer-Wright types. Because of this, much larger number of Homer-Wright rosettes were visible in cytokeratin preparations of case 1 than in haematoxylin and eosin stained sections. The ganglion cells and the fibrillar material were cytokeratin negative, but the epithelioid cells were strongly positive. The nasopharyngeal specimen stained strongly positive for cytokeratin in the surface epithelium and the tumour underneath (fig 4). A fragment of brain tissue outside the tumour capsule in case 3 stained positively for astrocytes to S-100 and GFAP. Apart from the epithelioid cells showing positive staining for both NSE and cytokeratin, there was no topographic overlap between cells reacting to different antibodies.

ELECTRON MICROSCOPIC FINDINGS

Preservation of structure was poor in case 1. The features confidently identified were the prominent cell junctions and centrioles or basal bodies in several cells. Several blocks examined in case 3 showed predominantly one cell type—oval or polygonal cells with moderate amounts of cytoplasmic organelles. These cells were closely packed with prominent cell junctions extending over a considerable area (fig 5). True desmosomes were not identified. Folded basal lamina-like material was observed at the periphery of groups of cells but not the plasma membrane of cells. Abundant polyribosomes, rough endoplasmic reticulum, and mitochondria were consistently present.

A number of cells contained centrioles or basal bodies and a few bundles of intermediate

Figure 3 Case 3: contrasting appearance of darkly stained small cells and larger paler epithelioid cells (Haematoxylin and eosin).

filaments (fig 5). The second cell type was represented by portions of cytoplasm or cytoplasmic processes clearly outlined by plasma membrane. Polyribosomes, rough endoplasmic reticulum, mitochondria and centrioles were present as in the other cells, but the distinguishing feature was the presence of a large number of tightly packed microtubules. These cells or processes were seen in round or longitudinal profiles in which nuclei were not identified. Dense cored granules and synaptic complexes were not seen in any cells of the two cases.

Figure 4 Case 2: nasopharyngeal tumour showing strong cyokeratin staining of surface epithelium and submucous tumour particularly outlining the lumina of olfactory rosettes (immunoperoxidase).

Discussion

Although these three cases were observed within a short period, no racial or geographic factors are implicated. The purpose of this report is mainly to support the view that olfactory neuroblastoma with primary intracranial presentation differ from the more common examples in the nasal cavity and related areas.

Most of the reported olfactory neuroblastoma have morphological, immunohistochemical, and electron microscopic features similar to those of childhood neuroblastomas of the adrenal glands and the sympathetic nervous system.^{1 7-11} Another group of olfactory neuroblastoma with epithelial-type cells have been described as neuroendocrine carcinomas.¹⁰ The morphological feature specific for olfactory neuroblastoma is the presence of olfactory rosettes.^{5 10 12 13} Despite variation in morphology, all the four specimens from our cases had this feature, conforming to the accepted diagnostic criteria of olfactory neuroblastoma. In case 1 there was no fibrillar background, and, but for the rosettes, the diagnosis of olfactory neuroblastoma would have been difficult. The findings in case 2 more closely resembled childhood neuroblastoma but the olfactory rosettes confirmed the diagnosis of olfactory neuroblastoma. The nature of the ganglion cells remains uncertain. Although true ganglion cells have been reported in olfactory neuroblastoma,^{10 11 14} and the cells in our cases were weakly NSE positive, their morphological resemblance to neurones was inappropriate. Negative reaction to S-100 and GFAP precluded a Schwann cell or glial cell nature. The epithelioid cells were similar to those reported in a small number of olfactory neuroblastoma.^{3 5 8 10 13} These cells were indeed epithelial because of their strong cyokeratin expression.

Despite the heterogenous light microscopic features all the four specimens had the same immunohistochemical findings. Choi and Anderson reported NSE and S-100 positivity in all the 10 cases they studied.¹ Similar results have subsequently been reported by other workers.²⁻⁶ Our results are similar and we also observed the characteristic distribution pattern of S-100 positive cells so far reported only by Choi and Anderson.^{1 2} These cells have generally been regarded as supporting cells like the Schwann cells,^{2 3} but their exact relevance is not clear. Normal olfactory epithelium is negative for S-100.^{2 15} NSE positivity, although sometimes present in non-neuronal cells and their tumours,¹⁶ supports the neuronal nature of some of the cells in olfactory neuroblastoma in the present context. Unfortunately, neurofilament protein was not sought in our material which could have further confirmed the neuronal nature of these cells.

Cyokeratin expression in olfactory neuroblastoma has previously been reported in only a small number of cases.³⁻⁵ Axe and Kuhajda,⁴ in an attempt to define the immunostaining pattern of various small cell nasal tumours including olfactory neuroblastoma, studied these tumours with a series of antisera

Figure 5 Case 3: electron micrograph illustrating tumour cells with cell junctions and cytoplasmic organelles including centrioles or basal bodies (uranyl acetate and lead citrate).

to intermediate filaments, neuroendocrine, and tissue specific antigens. Cytokeratin was negative in all and neurofilament protein was positive in only one out of eight cases of olfactory neuroblastoma. Taxy *et al* reported cytokeratin expression in a proportion of morphologically different groups of olfactory neuroblastoma and coexpression of neurofilament protein in two cases.³ In this respect our results are similar to the case reported by Takahashi *et al*⁵ which also presented primarily as an intracranial tumour. The common cytokeratins detectable by the antibodies used in these two studies are cytokeratins 8, 18, and 19. It is, however, impossible to be more specific as to the precise cytokeratin involved. In the present series cytokeratin expression was noted in all the cases irrespective of morphological variation, but differed in distribution pattern. A positive cytokeratin reaction in the centre of both Homer-Wright and olfactory rosettes was a consistent finding in our cases. This characteristic distribution pattern has not been emphasised previously, although cytokeratin positivity in the centre of olfactory rosettes and 150 kilodalton neurofilament protein in rosette-like areas was observed in the one case reported by Takahashi *et al*,⁵ but these authors failed to show coexpression of both the antigens in the same locations. Results of our, as well as previous, studies show a far greater degree of positive correlation between cytokeratin and NSE expression than cytokeratin and neurofilament protein.^{3,4,6} It is likely, therefore, that in keeping with the normal olfactory sensory epithelium, some tumour cells in olfactory neuroblastoma attain complete epi-

thelial differentiation while other cells retain a variable degree of neuronal characteristics. Our ultrastructural data are limited and mainly point towards the presence of ciliated epithelial cells and some possible neuritic elements. Although Schwann cells were not precisely identified, the finding of folded basal lamina-like material and uniform S-100 expression would be in keeping with the findings of previous studies that similar cells occur in olfactory neuroblastoma.¹⁻³

Our results strongly support the suggestions made by Hassoun *et al*⁸ and Takahashi *et al*⁵ that olfactory neuroblastoma are derived from the olfactory epithelium and not the neural crest. This is particularly relevant for a subgroup of olfactory neuroblastoma with an intracranial component.

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