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Case report

Clear cell ependymoma

A histological variant with diagnostic implications

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Summary. Four cystic brain tumours, one from the frontal lobe, one in the third ventricle and two in the cerebellum, were studied histologically including immunostaining and electron microscopy. Anatomically, all the tumours were located within or adjacent to the ventricular system. By light microscopy, the tumours had a largely honeycomb pattern and were made up of clear cells. Some of the clear cells showed positive for GFAP. Leu 7, Factor VIII and NSE were negative. Electron microscopy of the areas with clear cells revealed densely packed polyhedral cells with clear cytoplasm. They had well developed intercellular junctions, microvilli and some cilia which confirmed their ependymal derivation. Perivascular pseudorosettes or papillary features were only seen in limited areas, where GFAP was strongly positive in the tumour cell processes. As these tumours mimic oligodendroglioma and cerebellar haemangioblastoma, they are called as "clear cell ependymoma" to obviate such errors in diagnosis.

Key words: Ependymoma – Clear cells – Oligodendroglioma – Haemangioblastoma – Pathology

Introduction

Brain tumours with a honeycomb pattern with clear cells having round nuclei and perinuclear halos could pose a problem in differential diagnoses. One such tumour was revealed to be an ependymoma by electron microscopy and was reported briefly by one of us (Kawano et al. 1983). Subsequently, we came across three more cases, which further confirmed the presence of such ependymoma and may indicate that this variant of ependymoma occurs frequently.

The diagnostic importance of this type of ependymoma encourages us to report its clinicopathological features.

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Materials and methods

The tumour tissues obtained by surgery and by autopsy were fixed in 10% formalin. Paraffin-embedded 5 micrometer-thick sections were stained with haematoxylin and eosin (H & E), phosphotungstic acid haematoxylin (PTAH), silver impregnation for reticulin, periodic acid Schiff (PAS), mucicarmine and fat stain with oil red-O.

Immunostaining for glial fibrillary acidic protein (GFAP), Leu 7, factor VIII and neuron specific enolase (NSE) were performed in all the cases with the PAP technique using antibodies produced by Dako.

For electron microscopy, the tissues were immediately fixed in 2% glutaraldehyde, post-fixed in osmic acid, dehydrated by serial graded alcohol and embedded in plastic.

Case reports

Case 1. A 22-year-old man was found to have a right frontal cystic tumour with computed tomography (CT). The tumour, which was vascular and well demarcated from the surrounding brain tissue, was removed and the patient was discharged from hospital following post-operative radiation and chemotherapy. The clinical course and the pathological findings at this stage have been reported previously (Kawano et al. 1983). Two years later, a mass was detected in the left cerebellopontine angle. This was surgically removed and was found to have the same histology as the initial surgical specimen. The patient died of a recurrence 52 months after his first operation. Autopsy revealed tumour in the left cerebellopontine angle and diffuse subarachnoid spread at the base of the brain.

Case 2. This 5-year-old girl showed progressive hemiparesis and vomiting prior to admission. A CT showed a large cystic tumour with calcification in the third ventricle. This highly vascular lesion was partially resected and she received 5000 rads of post-operative radiation therapy. Seven months later, the tumour recurred and 3000 rads of radiation was added. The patient died of the tumour, 20 months after the operation. Autopsy was not performed.

Case 3. A 26-year-old man had a one year history of progressive dizziness and headache. After admission, angiography revealed a highly vascular mass with early venous filling within the right cerebellar hemisphere. During operation, it was found to be cystic with a mural nodule which was dark red in color and was well demarcated from the brain tissue. The cyst contained xanthochromic fluid. The nodule, which was located in the cerebellar tonsil and extended to the ventricular roof was removed. Three years after the operation, the patient became paraplegic. Radiological study showed recurrence of the tumour in the cerebellum and a spinal tumour at Th-10 level. Both tumours were excised and they disclosed the same histology as the initial surgical specimen. On the basis of immunostaining and ultrastructural studies, the initial diagnosis of haemangioblastoma was changed to ependymoma. He received 5200 rads of radiation to the posterior cranial fossa and 2200 rads to the spinal cord. Shortly afterwards, two other metastatic tumours appeared in the temporal lobe and sacral spinal cord, which were treated with additional radiation therapy. The patient is now ambulatory at home, 9 years after his first operation.

Case 4. A 55-year-old woman with a short history of headache and disturbance of finger movement was revealed to have a cystic tumour with a mural nodule in the paramedian cerebellar hemisphere. The mural nodule was strongly enhanced by contrast medium in CT scan and was vascular in angiography. At operation, the cyst contained xanthochromic fluid. The mural nodule was brightly red in colour and well delineated from the surrounding brain tissue. There was no continuity with the ventricular wall. Post-operatively, the patient received 5600 rads of radiation therapy. She shows no neurological abnormalities four years after the operation.

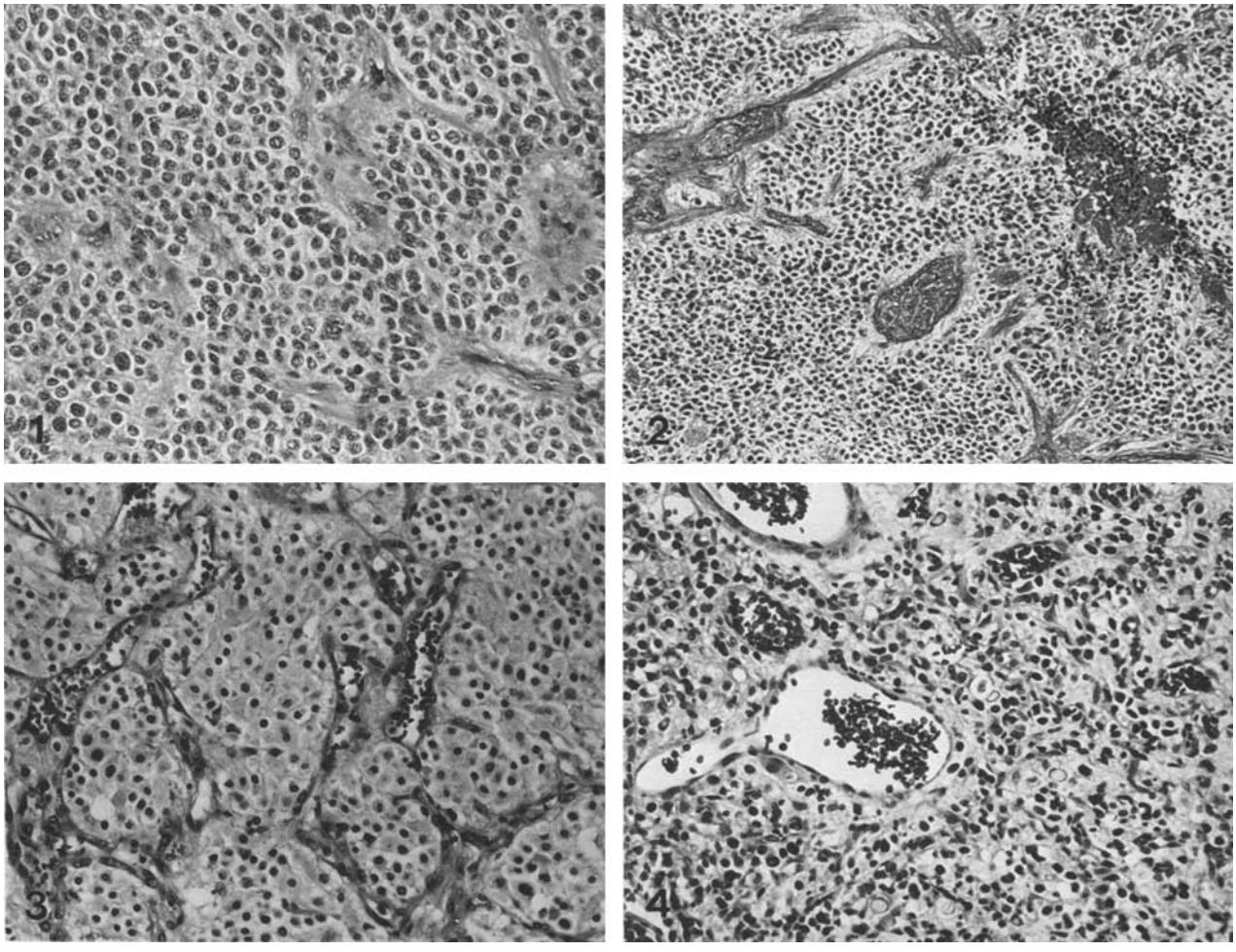


Fig. 1. *Case 1.* The representative histological features showing a honeycomb pattern with clear cells. Note a slight tendency of perivascular arrangement of the tumour cells. Vascular endothelial proliferation is present. H & E, × 500

Fig. 2. *Case 2.* The perivascular arrangement of the tumour cells is more easily identifiable than in case 1. A necrotic area and prominent vascular endothelial proliferation are shown. H & E, × 250

Fig. 3. *Case 3.* A highly vascular tumour with an interpolated clear cell cluster resembling a cellular type of haemangioblastoma. The clear cell cytoplasm is homogenous and faintly eosinophilic. Moderate endothelial proliferation is noted. H & E, × 400

Fig. 4. *Case 4.* The common finding mimicking the capillary type of haemangioblastoma is shown. Some of the tumour cell nuclei are ballooned. H & E, × 170

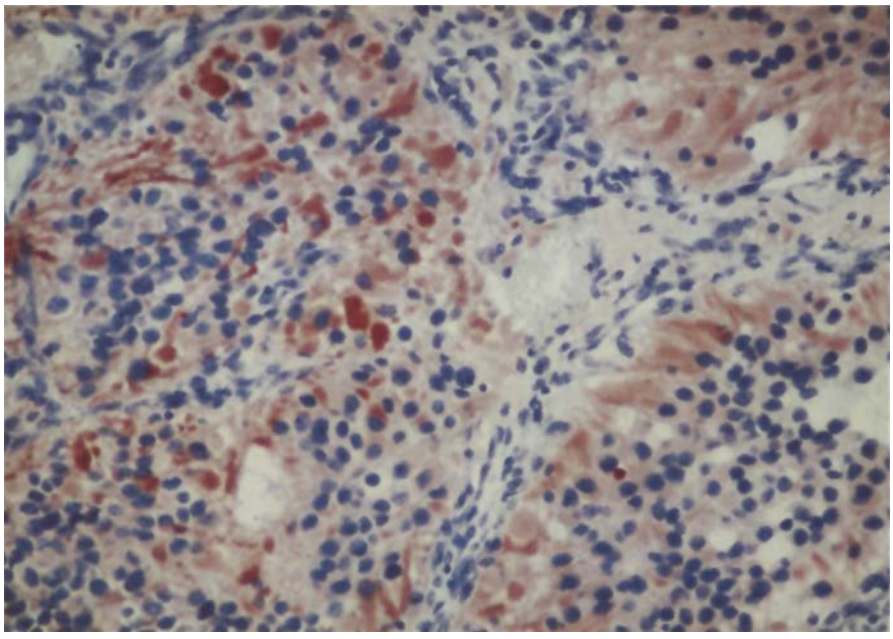


Fig. 5. Immunostaining for GFAP of case 3 showing many positive cells in both clear cells and perivascular tumour cells. Stained with 3-amino-9-ethyl-carbazole. Original magnification, ×45

Results

All the tumours were sharply demarcated from the surrounding brain tissue. Remarkable findings common to these cases was that most of the tumour tissue consisted of clear cells which had round cell bodies with central nuclei and clear cytoplasm (Figs. 1–4). The cells occurred in clusters intersected by capillaries showing a honeycomb pattern. The cytoplasm of many tumour cells was homogenous and faintly eosinophilic (Fig. 3).

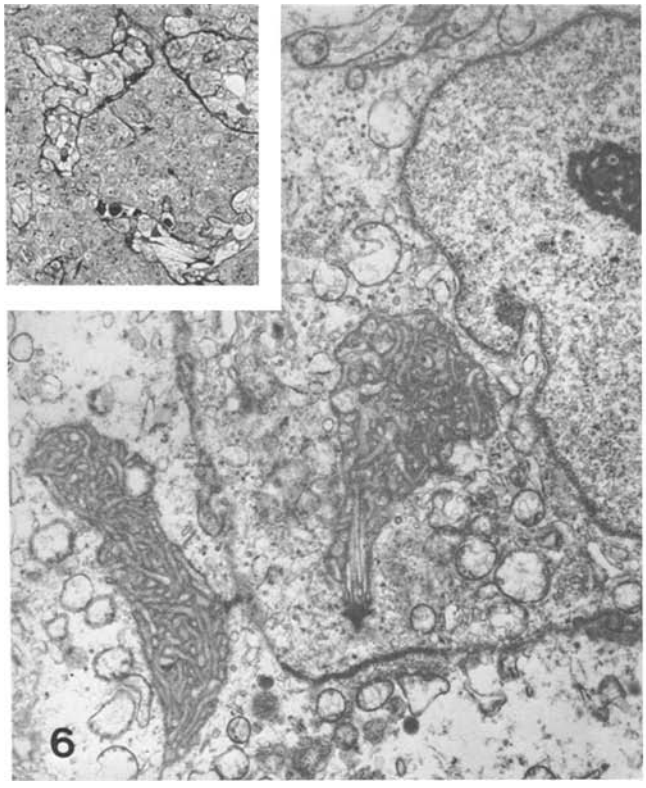
In limited areas, the tumour cells showed a perivascular arrangement. The cell processes which extended toward blood vessels were often short and difficult to identify (Figs. 1, 2). Unipolar tumour cells with long and slender cell processes around blood vessels were observed only in case 3 and 4 after extensive samplig of the tissues. No ependymal rosettes were seen in any of the four cases. Case 1 showed only one area where the tumour cells formed epithelial and papillary structures.

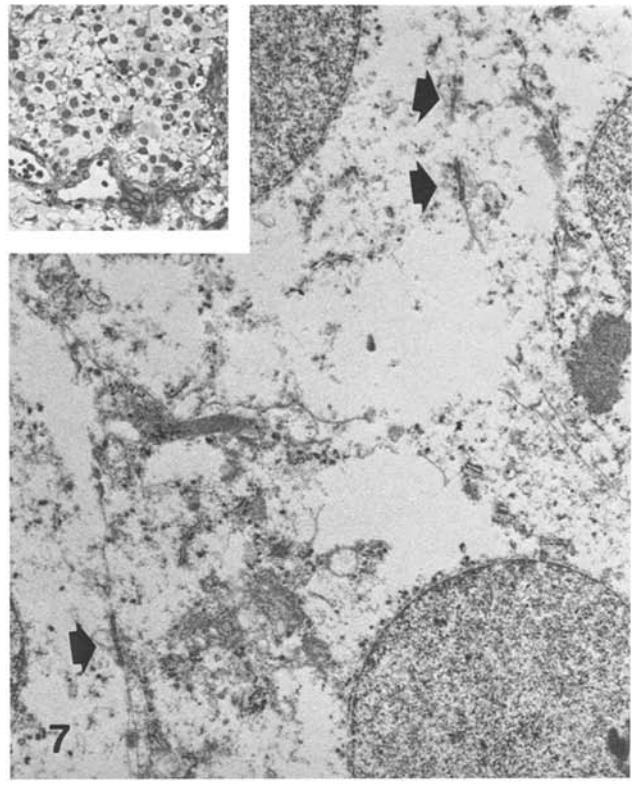
Nuclear pleomorphism was generally moderate. Tumour giant cells were rarely seen. Mitotic figures and necrotic foci were observed in cases 1 and 2 (Fig. 2). Microcystic degeneration was seen in cases 3 and 4. There was a slight tendency in the clear cells to have paler and irregular nuclei compared with those cells in perivascular arrangement. All the tumours were highly vascular and exhibited prominent endothelial proliferation (Figs. 1–3). Reticulin fibers were generally limited to blood vessel walls and in regions where prominent mesenchymal proliferation was present. PAS positive and diastase-reactive granules were seen in some tumour cells. Mucicarmine staining was negative. PTAH stained cell processes around blood vessels. Fat stain revealed only a few sparse positive cells.

Immunostaining for GFAP showed positive clear cells, which distributed diffusely or clustered within the tumour tissue (Fig. 5). In limited areas with perivascular pseudorosettes, the tumour cell processes were strongly positive for GFAP. Leu 7, factor VIII and NSE were not detected in all the cases.

Electron microscopy was most valuable in two cases, case 1 (Fig. 6) and 4 (Fig. 8) where the specimens were well preserved for the study. There were round or polyhedral tumour cells which were densely packed with diminutive extracellular spaces. Cytoplasmic processes were rarely seen. Junctional complexes such as desmosomes and gap junctions were well developed. Plasmalemma was loose and the organelles such as Golgi's apparatus, rough endoplasmic reticulum (RER), mitochondria and glycogen granules were present. There were many clusters of microvilli or cilia, and no intermediate filaments observed in case 1. In case 4, microvilli and intermediate filaments were noted occasionally.

Despite poor preservation of the tissue in case 2 and 3, definite and abundant desmosomal junctions were identified (Fig. 7). Occasionally, micro-





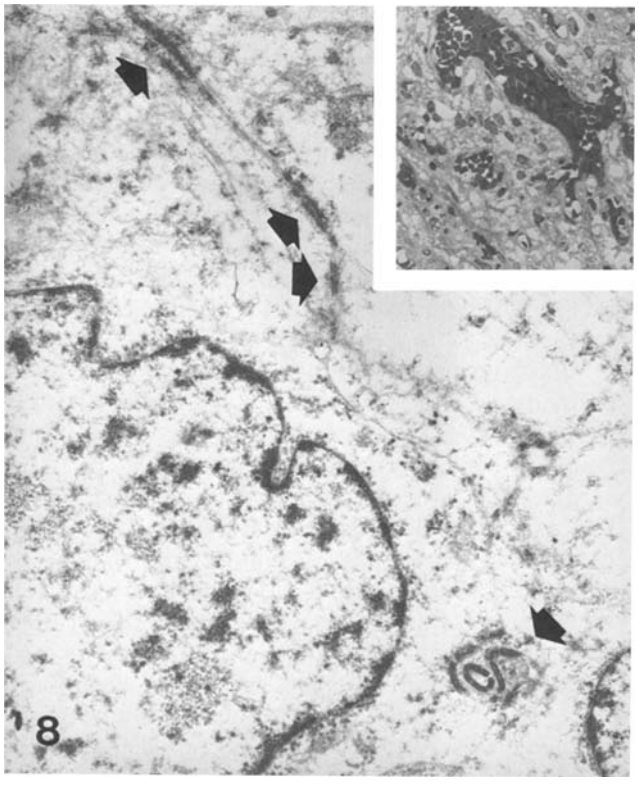


Fig. 6. *Case 1.* Electron micrograph of the clear cells showing well developed intercellular junctions, clusters of microvilli and a cilium. ×13000
Inset: 1 micrometer thick section indicating the area studied with electron microscopy. Toluidine blue stain, ×170

Fig. 7. *Case 3.* Low power view showing densely packed polyhedral tumour cells. Abutting plasma membranes develop many intercellular junctions (*arrows*). ×7000
Inset: The area selected for electron microscopy study. Toluidine blue stain, ×140

Fig. 8. *Case 4.* Tumour cell cytoplasms are loosened and contain some glycogen granules. Desmosomal junctions are well developed (*arrows*). ×14000
Inset: The area selected for electron microscopy study. Toluidine blue stain, ×150

Table 1. Clinical and histological findings of the cases

Case	1	2	3	4
Age	22	5	26	55
Sex	M	F	M	F
Location	frontal	IIIrd vent.	cerebellum	cerebellum
Cyst	+	+	+	+
Subarachnoid metastasis	+	−	−	−
Survival	4 y 4 mos	1 y 8 mos	9 y 6 mos, alive	4 y, alive
Histology				
Predominant tumour cell	clear cell	clear cell	clear cell	clear cell
Perivascular arrangement	rare	partly	rare	partly
Rosettes	−	−	−	−
GFAP	+	+	+	+
Leu 7	−	−	−	−
Factor VIII	−	−	−	−
NSE	−	−	−	−
Electron microscopy				
Intercellular junctions	++	++	++	++
Microvilli	++	+	−	+
Cilia	+	−	+	−
Intermediate filaments	−	+	+	+

++: abundant, +: positive, −: absent

villi, intermediate filaments or cilia were also noted. Organelles suggesting neuronal origin, such as abundant microtubules, clusters of RER, neurosecretory granules or synaptic complexes were not observed in any of the cases.

The results of pathological studies were consistent with those on ependymomas reported in the previous literature both immunohistochemically (Duffy et al. 1979; Marsden et al. 1983; Sonneland et al. 1985) and ultrastructurally (Goebel and Cravioto 1972; Hirano et al. 1975; Luse 1960; MeiLiu et al. 1977; Raimondi et al. 1962; Vraa-Jensen et al. 1976). Based on these, all four tumours were diagnosed as pure ependymomas. Clinical and histological features of the cases are summarized in Table 1.

Discussion

Clear cells, oligodendroglia-like cells, are known to occur frequently in ependymomas (Arendt 1975; Fokes and Earle 1969; Kepes 1971; Kernohan and Fletcher-Kernohan 1937; Kritcheff et al. 1964; MeiLiu et al. 1976; Russel and Rubinstein 1977; Shuangshoti and Panyathanya 1973; Svien et al. 1953; Zülch and Schmid 1955). Clear cells sometimes predominate in the tumour tissue, exhibiting a honeycomb pattern (Kałuża and Adamek 1984; Klein 1953; Zülch and Schmid 1955). The nature of these clear cells has been controversial. Some have speculated that they were oligodendroglial in origin (Hart et al. 1974; Kernohan and Fletcher-Kernohan 1937; Russel and Rubinstein 1977) and others have suggested that they are of ependymal derivation without showing satisfactory evidence (Kepes 1971; MeiLiu et al. 1976; Raimondi et al. 1962; Shuangshoti and Panyathanya 1973; Svien et al. 1953). In the present paper, using immunostaining and electron microscopy, the clear cells in question were clearly revealed to be ependymal in origin. As this type of ependymoma seems to occur frequently and has diagnostic significance, we currently use the term "clear cell ependymoma" for these tumours.

A unique histological feature of the present cases was that most of the tumour tissues consisted of clear cells with a honeycomb pattern. Tumours to be excepted from this type of ependymoma may be classified as oligodendroglioma. Occasional oligodendrogliomas have been reported (Earnest et al. 1950; Van Tassel et al. 1986) to contain areas of ependymoma and these tumours bear a close resemblance to our present cases.

Cerebellar haemangioblastomas also deserve special mention, because two of the tumours in this study were located in the cerebellum and resembled haemangioblastoma macroscopically as well as microscopically. Bonnin et al. (1983) reported four cases of mixed capillary haemangioblastoma and glioma, and explained that the haemangioblastic component may have originated from neoplastic transformation of the exuberant vascular stroma in the glial tumour. In our cases, the vascular proliferation which simulated a haemangioblastoma was considered to be within the range of reactive change. In that respect, we agree with Arendt (1975) and Klein (1953) that, microscopically, ependymoma can often mimic cerebellar haemangioblastoma. Accordingly, it is possible that haemangioblastomas which have been diagnosed only by light microscopy may have contained "clear cell ependymoma".

Other tumours to be considered are central neurocytoma (Hassoun et al. 1982) and metastatic renal cell carcinoma. These, however, can be easily differentiated from the present type of ependymoma with immunostaining and electron microscopy.

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