ULTRASTRUCTURE OF A “PINEOCYTOMA”

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ABSTRACT

The commonest pineal gland tumor, the germinoma, is more apparently related to germ cells than to pineal cells. Although ultrastructural studies of this tumor have confirmed this similarity, comparable characterization of a pineocytoma has not been reported, due to its rarity. Here we define the electron microscopic features of a rapidly growing human pineocytoma and compare the tumor cells in the present study with normal mammalian pinealocytes, as well as with cells described in the literature of pineal body pathology. Two cell types populate the tumor: one resembles the neuron, while the second, less frequently encountered, is often indistinguishable from the fibrous astrocyte. Synaptic complexes and dense-cored vesicles were not conspicuous fine-structural elements. Whereas emphatic morphological differences exist between the germinoma and pineocytoma, the similarities in ultrastructure between the pineocytoma cells and adult mammalian pinealocytes are even more striking.

INTRODUCTION

The term pinealoma is used most often in reference to the commonest tumor arising in the region of the pineal body and apparently more akin to the germ-cell tumors of the gonads (germinomas or atypical teratomas) than to pineal parenchymal tumors. An uncommon neoplasm, closely resembling the adult pineal gland and believed to originate from pinealocytes, is the pineocytoma (13, 14). This interesting matter of origin and semantics has engendered considerable discussion regarding the source of germ-cell tumors (3, 6). Comparison of the structures of the germ-cell tumor, the normal mammalian pineal gland, and a pineocytoma (the “true pinealoma”) might have bearing on this issue. Fine-structural studies indicate that the germinoma is ultrastructurally identical to its gonadal counterpart (3, 8, 10, 11, 15). The electron microscopic features of mammalian pineal bodies have been well described (1, 2, 7, 16, 17), but until now (to our knowledge), there have been no published reports on the ultrastructure of a pineocytoma.

Recently, a patient with a pineocytoma afforded us the opportunity of examining surgically obtained tissue with the electron microscope. This tumor differs...
significantly from the germinoma and has many mammalian pinealocyte characteristics, as observed by other investigators and confirmed by us.

CASE REPORT

A 32-year-old woman had complained of frequent, severe headaches for "some time", and, 4 months before hospital admission, suffered the onset of diplopia. Medical evaluation at that time, including lumbar puncture, skull films, and a brain scan, resulted in a tentative diagnosis of multiple sclerosis, and she was treated with steroids with no noticeable improvement. She was then seen here at the University of California Medical Center (San Francisco) by one of us (C. B. W.). The sole neurological abnormality was loss of convergent gaze, with diplopia in all directions except upward and to the right. The patient's visual fields were intact, and there was no other neurological deficit. Radiological studies (angiography and pneumoencephalography) revealed a mass in the region of the pineal body. Through an infratentorial, supracerebellar approach, a golf-ball-sized, light grey, soft tumor mass was disclosed, and approximately 90% of this circumscribed but non-encapsulated tumor was removed.

The patient's postoperative recovery was satisfactory, and within the next 2 weeks, a 5-week course of radiation therapy (6,000 rads via bilateral opposed fields) was initiated. After completion of these treatments, she felt well for about a month.

Repeated angiograms yielded evidence of a 6-cm, moderately vascular pineal tumor, as well as moderate dilatation of the lateral ventricles. Therefore, 3 months after the original surgery, she underwent a second operation, through a transtentorial approach. The tumor was soft and the inferior border appeared to extend into the midbrain. After an estimated 50% removal, a ventriculoperitoneal shunt was performed. Unfortunately, the patient experienced a difficult postoperative period, remained severely obtunded, and required extended chronic nursing care until her death 1 year after the onset of her initial symptoms.

Methods

Tissue obtained at surgery for examination by light microscopy was fixed in 10% buffered formalin and embedded in paraffin. Sections were then stained with hematoxylin and eosin, phosphotungstic acid and hematoxylin (PTAH), hematoxylin-Van Gieson stain, Gomori's reticulin stain, the Bodian silver protargol method for axis cylinders, cresyl violet, and the Hortega silver carbonate method for the pineal gland as modified for paraffin-embedded material by DeGirolami (5).

Specimens for electron microscopy were fixed in 2% glutaraldehyde buffered to pH 7.4 with sodium cacodylate, embedded in Araldite, and sectioned on a Porter-Blum microtome. Ultrathin sections, stained with uranyl acetate and lead citrate, were examined and photographed in a Siemens 1A electron microscope.

RESULTS

Light Microscopy

The tissue from both surgical procedures was identical in appearance and thus will be described together. Multiple fragments were available, and although the cellularity varied from field to field, a distinctive arrangement of cells was evident. Spherical, pale, vesicular nuclei formed a circular pattern around an eosinophilic network, usually containing no PTAH-positive fibers. These rosettes rich in Hortega-silver-carbonate-positive fibers created the rather distinctive pattern typical of most areas of the biopsy specimens (Fig. 1). Numerous thin-walled vessels were visible, generally near the edges of rosettes. Discrete
cell borders were inconspicuous in the majority of the tumor cells, but distinct borders were evident in the infrequent larger cells, which contained nuclei with more prominent nucleoli and a granular basophilic material in the cytoplasm. This material stained faintly with the cresyl violet method for Nissl’s substance, and occasional processes apparently emanating from these latter cells were reactive to the Bodian silver stain.

Several areas of the tumor lacked the orderly arrangement of cells as described above—they were both more cellular and more variegated in nuclear configuration. In addition to nuclei identical to those in the aforementioned regions, smaller, hyperchromatic, fusiform nuclei were observed. Infrequent astrogial fibers, staining blue with PTAH, coursed through these areas. No blepharoplasts were identified. Nor was any significant amount of collagen detectable, and reticulin was confined to vessel walls.

In all areas of the tissue, nuclear pleomorphism was minimal and mitotic figures were not conspicuous. No significant differences were noted in the biopsy
specimen from the second surgery—specifically, no changes possibly attributable to the irradiation.

**Ultrastructure of the Tumor**

The closely packed cells of the tumor revealed the same relationship to vessels as that seen by light microscopy. The nuclei were separated from vessel walls by numerous cell processes, which expanded when they neared the basement membranes of capillaries (Fig. 2). Immediately adjacent to the basement membrane, other distinct cell processes contained bundles of densely packed, 6-9-nm fibrils, typical of astrocytes (Fig. 3). Characteristic astrocytic processes were also found coursing between the other cells and away from vessels as well. The processes of the other, predominant cell type were in close contact with one another, but rarely were junctional densities (without associated vesicles) discerned, and complete desmosomes were absent.

Individual tumor cells exhibited some degree of nuclear uniformity. Round-to-oval nuclear contour was often altered by deep invaginations of cytoplasm. The double-layered nuclear envelope enclosed a rather diffusely granular karyo-

![Image](http://jnen.oxfordjournals.org/)  

**Fig. 2.** Nuclei of four tumor cells around a capillary. A complex network of expanded cell processes surrounds the basement membrane of the vessel. × 4,500.
plasm, and nucleoli were not prominent (Fig. 4). As in light microscopic examination, mitoses were not observed in the ultrathin sections.

The perikaryon, though small in volume, was rich in organelles: Mitochondria tended to be large, with quite pale matrices. Cristae were in no way remarkable in appearance. In the Golgi complex, stacked lamellar cisternae were frequently associated with empty, fuzzy-coated or alveolate vesicles, ranging from 40 to 60 nm in diameter and clustered near the nuclei.Aggregates of ribosomes, irregularly directed microtubules and filaments, smooth and rough endoplasmic reticulum (ER), centrioles, occasionally cilia, and sparse particles of glycogen occupied the remainder of the perikaryon (Figs. 5 and 6).

Both surrounding the cell body and enclosing the cellular contents as they tapered to form processes, the cell membrane disclosed frequent invaginating vesicular structures. The surfaces of these caveolae were invariably coated. Farther from the perikaryon, cellular organelle contents were much less complex: Parallel, longitudinally-oriented, 20–25-nm microtubules in association with variable numbers of filaments were the conspicuous features; whereas large mitochondria and empty alveolate vesicles (80–100 nm in diameter) were the most common other organelles (Fig. 6). In sharp contrast to this sort of process, occasional less dense processes contained abundant filaments (8–10 nm in diameter), which were usually loose in arrangement, but sometimes appeared in densely packed bundles. Both types of process demonstrated recurrent caveolae
Fig. 4. Deeply infolded nucleus and scant perikaryon, characteristic of the tumor cells. The bulk of the tumor consists of closely approximated cell processes. × 12,400.

Fig. 5. The perikaryon of one tumor cell with a prominent cilium is adjacent to a more distal process of another cell (vertically-oriented in the center of the micrograph), containing abundant loosely-arranged filaments. × 22,400.
along the plasma membrane and alveolate vesicles scattered among the microtubules and filaments. Synaptic complexes were not seen.

Dense-cored vesicles, 250–300 nm in diameter, were extremely rare, and when detected, were only present in cell processes near a capillary basement membrane.

The capillaries—specifically, endothelial cells and basement membranes—were unremarkable. Tight junctions were not observed between adjacent endothelial cells; however, since tracer studies were not performed, comment on the presence or absence of such structures could not be conclusive.

Comparative Ultrastructure of a Baboon Pineal Gland

A recent opportunity to study the pineal body freshly obtained from a baboon enabled us to substantiate the findings of other investigators in the normal mam-
malian gland. In this animal, individual cells had scant-to-moderate amounts of perikaryon, the bulk of the tissue consisting of a splendid array of closely juxtaposed cell processes (Fig. 7). Golgi complex, smooth and rough ER, clusters of ribosomes, multiple mitochondria, and randomly-oriented microtubules were present in the perinuclear cytoplasm. In an occasional cell, cilia too originated here.

The most often-occurring cell process contained longitudinally-oriented microtubules 22–25 nm in diameter, with interposed sprinklings of 9-nm filaments and clear, coated vesicles. Loose collections of microfilaments characterized other processes, and occasional processes indistinguishable from fibrous astrocytes were observed (Fig. 8). Near blood vessels, they expanded, and some contained numerous coated (but empty) vesicles, which sometimes originated from infoldings of the plasma membrane.

**DISCUSSION**

This tumor, the pineocytoma, is sufficiently rare that no one clinician or investigator has had adequate experience to reach any firm conclusions about its biological behavior (4, 13, 14). Some of the tumors behave like low-grade gliomas, while others pursue a more aggressive course. In the case of our patient,
the tumor grew in accelerated fashion, with a rapid increase in size and evidence of ultimate brainstem involvement. The rather innocuous histological features of this tumor belie the clinical course it eventually took. The grouping of tumor cells into rosettes with complex fibrillar centers, plus the orientation about blood vessels and their rare, club-shaped, silver-positive processes, are the features which identify the nature of the tumor. Although pinealoma might be the ideal term for such a tumor, unnecessary confusion would probably result in regard to the classification of pineal tumors, since that designation has commonly been associated with the germinoma.

In the light of our present observations, further morphological distinctions can be made between the two neoplasms, the pineocytoma and the germinoma. The light microscopic differences are well known (13, 14), and several recent electron microscopic descriptions of the pineal germinoma have indicated the remarkable resemblance of pineal germinoma cells to those of similar gonadal tumors (3, 8, 10, 11, 15). Misugi et al. (ref. 8) termed their tumor an "ectopic pinealoma", but their published description seems to be that of a germinoma. The rather pale cytoplasm containing scattered mitochondria, few profiles of rough ER, rare microtubules but abundant glycogen and large (1,000-nm) membrane-bound vacuoles (either empty or containing osmiophilia-poor material)
are consistent features of both pineal and gonadal germinomas. Similarly pale nuclei with prominent nucleoli serve to link the morphology of these tumors even more closely. The small cells in both have characteristics which identify them as lymphocytes.

In contrast, the type, arrangement, and appearance of cellular organelles of the pineocytoma at the ultrastructural level very closely resemble those of cells in a variety of normal adult mammalian pineal glands. The number and orientation of microtubules in pinealocyte processes are strongly reminiscent of dendrites (9), suggesting kinship to neurons. In the baboon pineal body, we found coated vesicles, cilia, clusters of ribosomes, and prominent Golgi complexes, all consistent features of the pineal parenchymal cell. These were also conspicuous intracellular structures in our patient's tumor. The intracytoplasmic dense lamellae and associated small vesicles with the dimensions of synaptic vesicles ("synaptic ribbons") observed in some pinealocytes (7) were not seen in tumor cells in our material.

The lack of secretory vesicles or frequent dense-cored vesicles in pineal parenchymal cells was reflected in the pinealocytes of our tumor. This has been a somewhat frustrating (and negative) finding, in view of the convincing chemically supported evidence of a neuroendocrine function of the pineal gland. Melatonin and the enzymes associated with its derivation from 5-hydroxytryptophan have been demonstrable, but the structural correlate of this presumptive secretory activity is lacking. Although the presence of indoleamines has been demonstrated in the vesicles of nerve endings by electron microscopic and histochemical studies, this has not been accomplished in pinealocytes.

The ultrastructural similarity of our pineocytoma cells to neurons, as well as the presence of cell processes virtually indistinguishable from those of fibrous astrocytes, tempt us to adopt these two criteria as an implication of differentiation along these two cell lines. Some support for such a concept exists in the recent report of a ganglioglioma originating in a pineocytoma (12). Indeed, even a number of the light microscopic features of our tumor suggest such a differentiation. But it would be presumptuous to assert this claim on the basis of the structural evidence provided by microscopy alone in view of our sparse experience with the pineocytoma.

The relationship of germinoma cells and pineal parenchymal cells also remains to be determined. However, the evidence presented here establishes a solid link between pinealocytes and the cells of our tumor, justifying the term pineocytoma.

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REFERENCES