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Pineal parenchymal tumors: cell differentiation and prognosis

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Abstract. Eleven pineal parenchymal tumors were studied using various antibodies specific to the central nervous system and cell-proliferation-related antigen MIB-1 in order to examine the divergent types of cell differentiation and also evaluate prognosis. Electron microscopy was also performed. All tumors were immunohistochemically positive to chromogranin A and αB crystallin, and were also highly positive to retinal S protein. Pineocytoma cells contained microtubules, intermediate filaments, glial bundles, clear-centered vesicles and synaptic apparati. Pineoblastoma cells also had microtubules and neurofilaments, but glial filaments and definite synapses were not identifiable. Pineal parenchymal tumors were considered to be of pinealocyte origin, and there was a continuous spectrum of divergent cell differentiation between pineocytoma and pineoblastoma cells. The MIB-1 labeling index correlated well with histological malignancy, neuronal differentiation evaluated immunohistochemically by both neurofilament protein and synaptophysin, and cases with seeding potentials. Although histopathological features of neuronal development were, until recently, seen as the hallmark of benign prognosis in pineal parenchymal tumors, they are now thought to be only one of the pieces of evidence that may be used for purposes of prognosis.

Key words: Pineal parenchymal tumors – Cell differentiation – MIB-1 – Prognosis

Introduction

Neoplasms that originate from the pineal parenchyma are extremely uncommon, and many clinical, biological and histological features of these tumors remain elusive because of the small number of reported cases and the high degree of pathological variability. In the past, histological features were the cornerstone of tumor diagnosis: neuronal differentiation in this tumor group has been reported to be benign (Rubinstein 1980) but, according to recent studies, this has not been universally accepted (Schild et al. 1993). As the enclosure of the skull provides a limited space for intracranial expansion, clinical manifestations of an intracranial malignancy depend largely on the rate of tumor growth. Thus, the prognosis should correlate with the size and proliferative potential of the tumor. Anti-Ki-67 clone MIB-1 has been studied on microwave-processed formalin-fixed paraffin sections, and its utility has been discussed (Shibuya et al. 1993). MIB-1 indices were used to evaluate prognosis.

We describe here a series of 11 pineal parenchymal tumors that were studied by immunohistochemistry and electron microscopy in order to examine the divergent types of cell differentiation and their relationship with prognosis. The possible tumor origins are also discussed.

Materials and methods

Preparation of specimens. Five histologically confirmed pineocytomas (PC), four pineoblastomas (PB) and two mixed PC/PB were studied by immunohistochemistry and electron microscopy. The series included 8 male and 3 female patients with an age range of 15–60 years (mean 41 years). Gross total excision was performed except in 1 case, which had shown multiple tumor lesions along the cerebral spinal fluid (CSF) passage prior to initial excision. Clinical follow-up evaluations were performed on 8 patients.

Histochemical analysis. Light-microscopic histopathological examinations were carried out on 10%-formalin-fixed, paraffin-embedded sections, which were stained with standard hematoxylin and esoin. Additional special stains included Bodian's silver impregnation, reticulin silver impregnation, phosphotungstic acid/hematoxylin and Fontana-Masson impregnation for melanin. Immunohistochemical studies were carried out with antibodies against neurofilament protein (NFP; 70 kDa, 160 kDa and 210 kDa), synaptophysin, chromogranin A, glial fibrillary acidic protein (GFAP) and myelin basic protein supplied by DAKO (Carpinteria, Calif., USA), and anti-Ki-67 clone MIB-1 (MIB-1; Immunotech, Marseille, France). Antibodies against αB crystallin and

Abbreviations: PC, pineocytoma; PB, pineoblastoma; NFP, neurofilament protein; GFAP, glial fibrillary acidic protein

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retinal S protein were also used, supplied through the courtesy of Dr. K. Kato, Institute for Developmental Research (Aichi, Japan), and Dr. A. Mizuno, Jikei University School of Medicine (Tokyo, Japan). Each antiserum was raised in rabbits against highly purified bovine lense αB antigen, and against bovine S antigen (Kato et al. 1991; Wacker et al. 1977). Sections were immunostained using the avidin-biotin complex method. Immunohistochemical methods for MIB-1 using microwave pretreatment were performed by the methods described by Shibuya et al. (1993). Conventional electron-microscopic examinations were performed on eight specimens fixed in 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide, dehydrated in graded solutions of ethanol and embedded in epoxy resin.

Evaluation of immunohistochemistry. The MIB-1 labeling index was calculated from each slide as the percentage of positive nuclei in tumor cells. Between 10 and 15 areas of each tumor were randomly selected for determination, corresponding to a total number of neoplastic cells ranging from 1000 to 1500. All vascular components and hematogenous cells were excluded. The mean labeling indices obtained were compared with the unpaired Student's *t*-test to determine statistical significance.

Results

Pathological findings

Light microscopy

The overall organization of PC was lobular, and delineated by delicate interstitial tissue which included thin-walled capillaries and reticulin fibrils (Fig. 1). Scattered calcified deposits were also frequently seen. These features closely re-

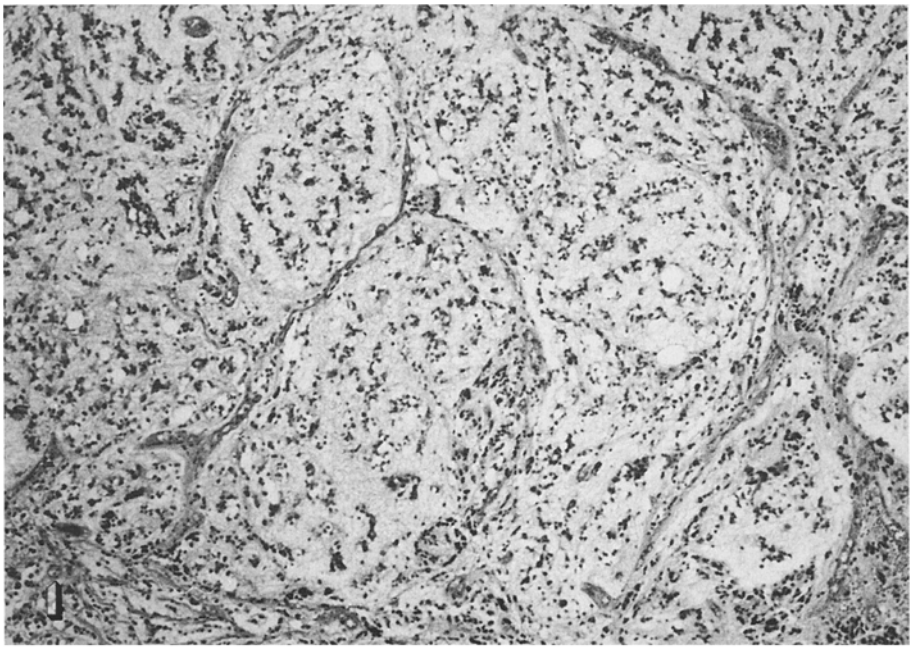

Fig. 1. Light-microscopic study of a pineocytoma. The tumor cells are divided into lobular compartments by delicate interstitial tissue containing blood capillaries (H & E stain, ×80)

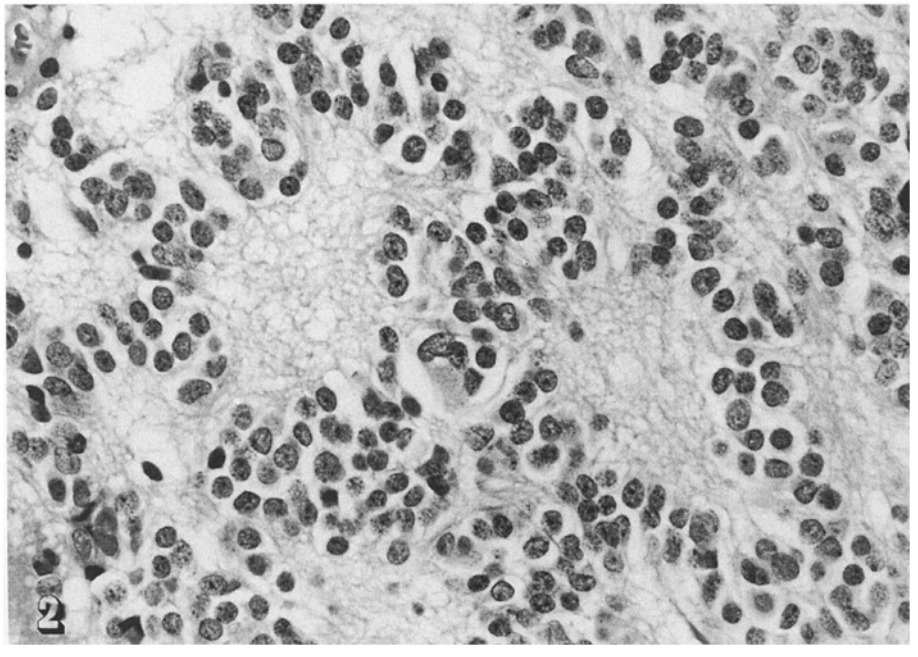

Fig. 2. Light-microscopic study of a pineocytoma. Tumor cells form large clear "pineocytomatous rosettes" (H & E stain, ×480)

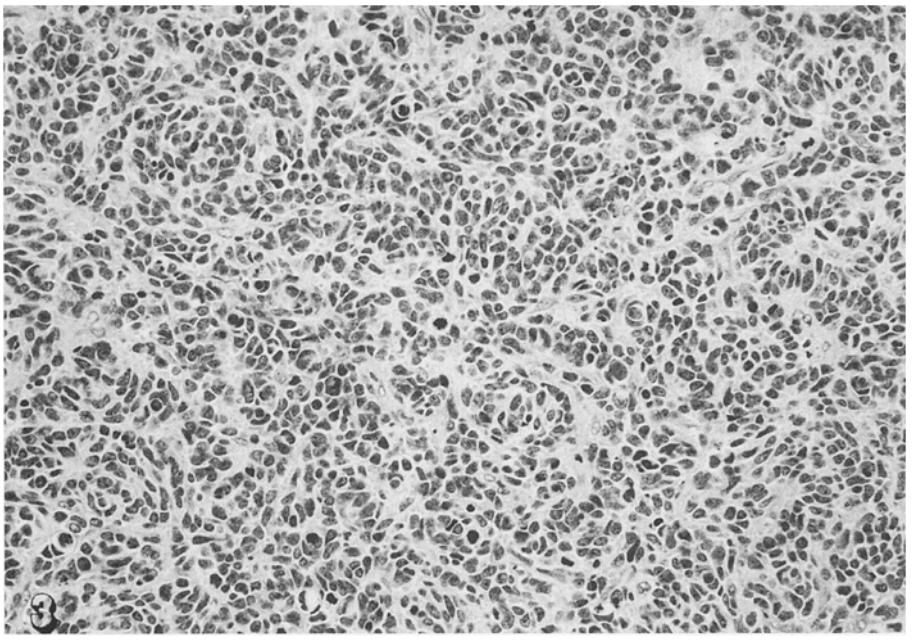

Fig. 3. Light-microscopic study of a pineoblastoma. Small and irregular palisading tumor cells form Homer-Wright rosettes. Mitotic figures are also evident (H & E stain, ×230)

sembled those of normal pineal glands. Several rosette formations were also noted, the most common being pineocytomatous rosettes comprised of tumor cells clustered around large acellular areas (Fig. 2). Perivascular rosettes known as fleurettes and microcyst-like tubular formations were also seen. PC was characterized by cytologically benign mature cells, with easily distinguishable cell margins and tumor cells of various sizes, often including mononucleated and multinucleated giant cells. There was no vascular endothelial cell proliferation and mitotic figures were rarely observed. The nuclei were slightly hyperchromatic, round or elongated, and contained coarse chromatin nodes. Nucleoli were not evident in smal tumor cells, but were more apparent in large tumor cells.

PB were highly cellular and richly vascularized in comparison to PC, and frequently displayed associated focal hemorrhage and necrosis. The tumor cells were distributed in patternless sheets (Fig. 3), although 1 PB had an incomplete lobular arrangement. Poorly developed Homer-Wright, perivascular, and Flexner-Wintersteiner rosettes were found in 2 tumors. Tumor cells were generally uniformly small in size with scanty and ill-defined cytoplasm. The nuclei were small and irregular, frequently convoluted and hyperchromatic, with a thickened nuclear membrane. In addition, a large number of mitotic figures were noted.

Significant histological variability was noted between tumors, such that transitional features of both tumor types were often seen. The two tumor types were therefore not always clearly distinguishable. Mixed PC/PB had histological features of both tumor types: the basic lobular pattern and pineocytomatous rosettes, with areas of high cellularity.

Special stains and immunohistochemistry

Fine argyrophilic fibers and club-shaped structures were revealed by Bodian staining, usually in pineocytomatous rosettes directed towards the center (Fig. 4A). NFP immunostaining also demonstrated fine neuronal fibrils with enlarged termini within the centers of these pineocytomatous rosettes (Fig. 4B). The tumor cell cytoplasm was also stained

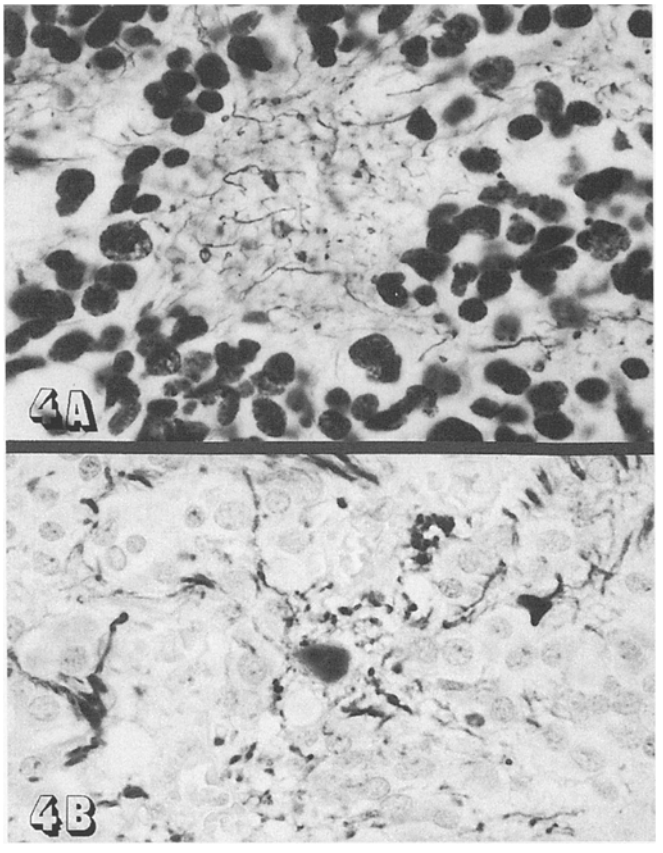

Fig. 4A, B. Light-microscopic and immunohistochemical study of a pineocytoma. **A** Club-like expansions can be seen directed towards the center of a pineocytomatous rosette (Bodian stain, ×640). **B** Neurofilament-protein (NFP)-positive fibrils and an enlarged ending in a pineocytomatous rosette (NFP immunostaining, ×450)

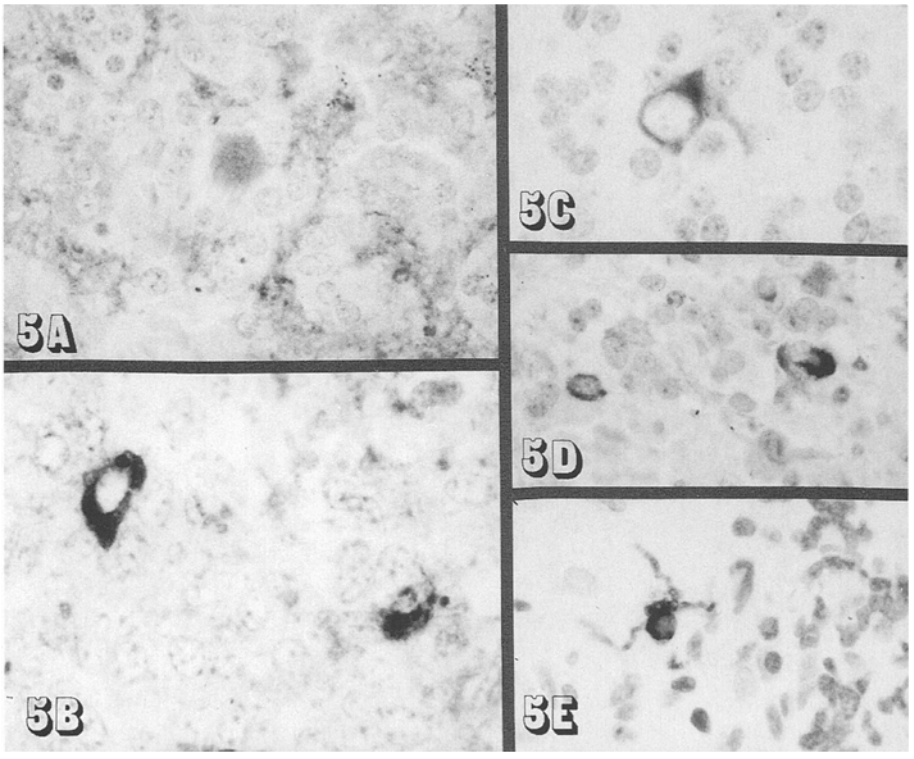

Fig. 5A–E. Immunohistochemical studies of pineocytomas. **A** Synaptophysin is stained in the tumor cell membrane and in the center of a pineocytomatous rosette (synaptophysin immunostaining, ×430). **B** Retinal-S-protein-positive cells (retinal S protein immunostaining, ×660). **C** An αB-crystallin-positive cell (αB-crystallin immunostaining, ×620). **D** Chromogranin-A-positive cells (chromogranin A immunostaining, ×460). **E** A glial-fibrillary-acidic-protein (GFAP) positive cell producing neuroglial fibrils (GFAP immunostaining, ×540)

in 1 PC, 2 PB, and 2 mixed PC/PB. Synaptophysin was demonstrated in 3 PC, 2 PB, and 2 mixed PC/PB, usually located on the cell membrane. Accumulation of synaptophysin inside the pineocytomatous rosettes was also found in 2 tumors (Fig. 5A). Retinal-S-protein-positive cells were located in all cases except for 1 PB (Fig. 5B). αB Crystallin and chromogranin A were positive in all pineal parenchymal tumors, αB crystallin being found particularly in large tumor cells with conspicuous nucleoli (Fig. 5C, D). Many pineal parenchymal tumors contained phosphotungstic-acid/hematoxylin-positive neuroglial fibrils, which also shared immunoreactivity with GFAP (Fig. 5E). Melanin granules were located in 2 PC, 1 PB, and 1 mixed PC/PB by Fontana-Masson staining, and these were confirmed by bleaching studies.

Immunohistochemical characteristics are summarized in Table 1, which also lists semiquantitative evaluations of immunoreactivity. Immunoreactivity for myelin basic protein was negative in all the tumors studied.

Electron microscopy

Rosette formations were demonstrated in all PC studied, in which cell processes were elongated and directed towards the center (Fig. 6A). Each tumor was characterized by various degrees of neuronal differentiation, along with variable cytoplasmic differentiation in each individual cell. The PC perikaryons contained numerous mitochondria and Golgi complexes, while the processes were filled with microtubules and intermediate filaments. Desmosomes and synaptic structures (Fig. 6B) as well as processes containing clear-centered vesicles and glial bundles (Fig. 6C) were evident, consistent with the immunohistochemical data.

PB tumor cells were smaller and more homogeneous, and had scanty cytoplasm compared with PC cells, although numerous cell processes were also observed. The rosettes in PB tended to be smaller than those in PC, where a small number of tumor cells were arranged radially around a central lumen

Table 1. Immunohistochemical staining of pineal parenchymal tumors

Diagnosis	Patient age (years)/sex	NFP	SY	RSP	αB-Crystallin	Chromo-A	GFAP	MBP
PC	32/M	+	+	+	+	+	+	–
PC	45/F	–	+	+	+	+	+	–
PC	60/M	–	–	+*	+*	+	–	–
PC	37/M	–	+*	+*	+	+	–	–
PC	53/M	–	–	+*	+	+	NS	–
PC/PB	40/M	+	+	+	+	+	+	–
PC/PB	51/M	+	+	+*	+	+	+	NS
PB	34/M	+*	–	+*	+	+*	+	–
PB	36/F	–	+	–	+	+*	–	–
PB	15/M	+*	+	+*	+	+	+	NS
PB	45/M	–	NS	NS	NS	NS	–	NS

PC, pineocytoma; PB, pineoblastoma; PC/PB, mixed pineocytoma/pineoblastoma; NFP, neurofilament protein; SY, synaptophysin; RSP, retinal S protein; Chromo-A, chromogranin A; GFAP, glial-fibrillary-acidic protein; MBP, myelin basic protein; NS, not studied; –, negative; +*, few positive; +, positive

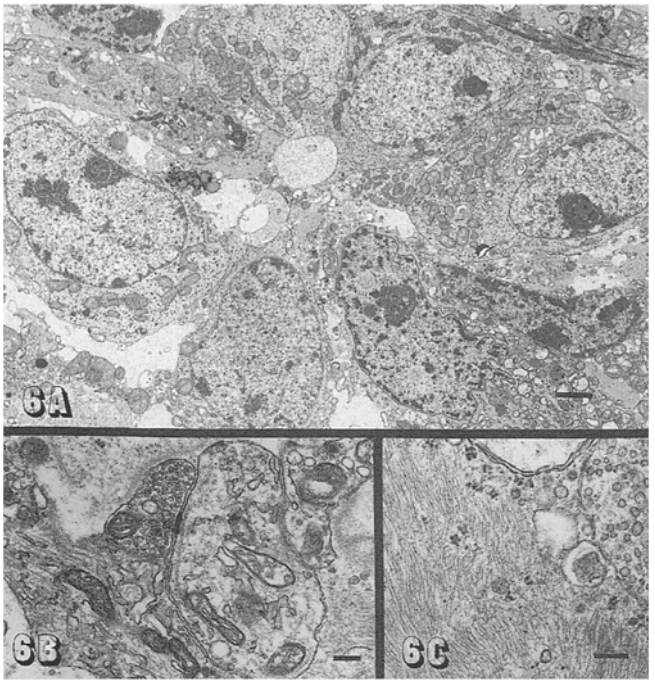

Fig. 6A–C. Electron-microscopic study of pineocytomas. **A** A small rosette found in a pineocytoma (*bar* = 1 μm). **B** Synaptic apparatus (*bar* = 500 nm). **C** Clear-centered vesicles and glial filament bundles (*bar* = 200 nm)

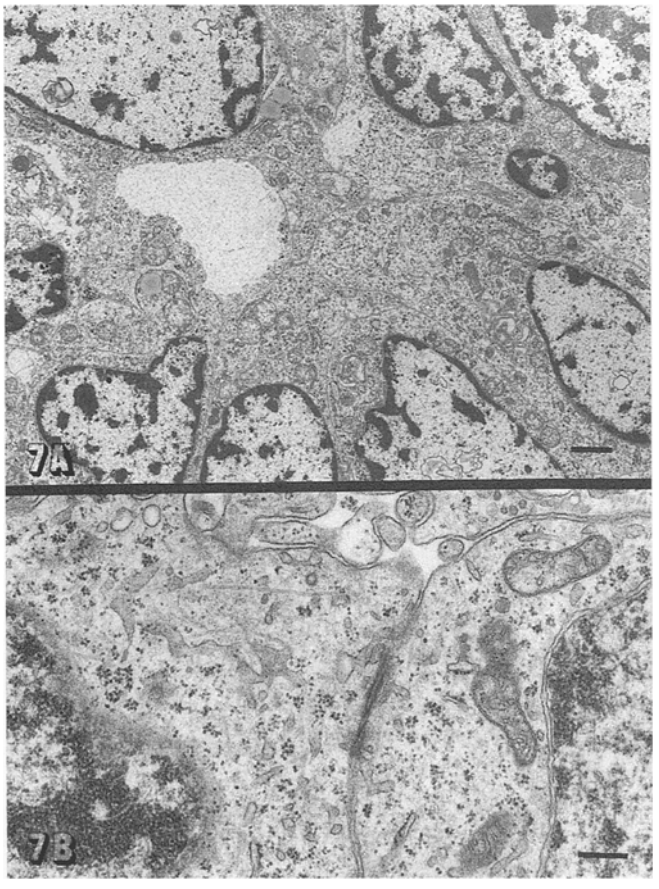

Fig. 7A, B. Electron-microscopic study of pineoblastomas. **A** Tumor cells are arranged around a central lumen, with the processes pointing towards the center (*bar* = 1 μm). **B** A desmosome is evident between two tumor cells (*bar* = 300 nm)

(Fig. 7A). The nuclei were elongated and polygonal, and chromatin was distributed in large irregular clusters and perinucleonemal structures. The nucleoli were generally conspicuous. Some cells contained microtubules and neurofilaments, but no glial filaments were found despite GFAP positivity in 2 PB. Unlike PC, no definite synapses were visible in PB, although desmosomes were present (Fig. 7B). Mitotic figures were also seen in these electron-microscopic studies.

Survival analysis

Eight of the cases were male (73%), in which a male dominance was recognized. The length of follow-up was calculated from the date of initial surgical treatment and ranged from 6 to 63 months (median 19 months). One patient, histologically diagnosed as having a mixed PC/PB, had died 6 months after primary excision of the tumor through local recurrence and leptomeningeal seeding. Another PB case, which had multiple tumor lesions along the CSF passage at the time of initial surgery, showed a decrease in size of the multiple tumor lesions after radiation. No other cases showed recurrence or leptomengineal seeding during the follow-up period.

Correlation with light-microscopic features and MIB-1 labeling indices is presented in Table 2. Comparison of the mean MIB-1 labeling indices showed a statistically significant difference between PC and PB ($P<0.05$), and between cases with leptomeningeal seeding and those without ($p<0.05$). The mean MIB-1 labeling index of cases with pineocytomatous rosettes was 1.33±1.61% compared with 5.87±4.26% in cases without rosettes, in which there was no statistical significance. The immunohistochemical results of neither NFP nor synaptophysin revealed any statistical significance in MIB-1 indices when compared independently. However, the mean labeling index of neuronal differentation evaluated by both NFP and synaptophysin showed a statisti-

Table 2. Light-microscopic features and MIB-1 labeling index

Histological features	MIB-1 labeling index (%)	*t*-test
Pineocytoma	0.27±0.08	$P<0.05$
Pineoblastoma	6.49±2.02	
Mixed pineocytoma/pineoblastoma	3.53±3.21	
Pineal parenchymal tumors		
With leptomeningeal seeding	8.67±1.95	$P<0.05$
Without leptomeningeal seeding	1.52±0.86	
Immunohistochemistry		
NFP^+	1.57±1.58	NC
NFP^-	4.17±4.02	
SYN^+	2.49±1.81	NC
SYN^-	5.08±4.26	
NFP^+ and SYN^+	0.62±0.58	$P<0.01$
NFP^- and SYN^-	4.49±1.82	

Mean MIB-1 labeling indices were compared with the unpaired Student's *t*-test
NFP, neurofilament protein; SYN, synaptophysin; NC, no correlation

cally significant difference ($P<0.01$). Other markers did not correlate with the MIB-1 labeling index.

Discussion

According to the Japanese Brain Tumor Registry, the incidence of PC is 0.1% of all primary intracranial tumors, and that of PB is 0.2% (Committee of the Brain Tumor Registry of Japan 1990). Each individual tumor demonstrated extensive histological variability, sometimes possessing features characteristic of both PC and PB. Many transitional cases have been reported (Numoto et al. 1992a), prompting the addition of the new category of mixed PC/PB to the revised WHO histological classification of primary brain tumors (Keihues et al. 1993). Pineal parenchymal tumors created a continuous histopathology spectrum; the more primitive PB tumors, the more differentiated PC tumors, and the intermediate mixed PC/PB tumors. Pineal parenchymal tumors had differentiating capabilities that were apparently determined by the pineal parenchyma from which they originate. Of the two main neuroepithelial cell types, the pinealocytes were considered to be responsible for neoplastic transformation in pineal parenchymal tumors. For example, pineal parenchymal tumors were characterized by compartmentalization into lobular structures by means of fibrovascular tissue, thus resembling normal pineal bodies. The presence of lobulated structures, pineocytomatous rosettes, and argyrophilic club-shaped expansions also suggested that these tumors arose from pineal glands (Herrick and Rubinstein 1979).

The histological structure of normal pineal bodies comprises two main neuroepithelial cell types; pinealocytes, and astrocytes. The mononucleated and multinucleated giant cells found in pineal parenchymal tumors were identical to those found in normal pineal glands. These are thought to occur only rarely in other neuroectodermal tumors, which are a reactive feature following radiation treatment. However, since none of the patients in the current series had undergone any other treatment prior to tumor resection, the appearance of these giant cells could not be ascribed to radiation therapy. These cells were thought to originate from pinealocytes, confirming probable pineal parenchymal tumor origin (Borit et al. 1980).

Large amounts of melanin are found in fetal epithelial pineal cells, which decrease during postnatal development except in the pineal parenchymal cells, which are known as pinealocytes in adults (Tapp and Huxley 1972). The function of pineal melanin and the reason for its transient appearance is unknown. Melanin pigment is found in pineal parenchymal tumors and a wide range of neuroepithelial tumors (Dooling et al. 1977). This phenomenon of melanin production is consistent with pinealocyte tumor origin and the capability for neuroectodermal differentiation. Further studies of these melanin-containing pineal parenchymal tumors may provide information regarding pineal transformation and function of the pineal body.

Herrick and Rubinstein (1979) have previously classified PC into those with neuronal differentiation, those with astrocytic differentiation, and those with potential for both neuronal and astrocytic differentiation. Okuda et al. (1988) have reported a PB with neuronal differentiation that was not previously classified. However, Herrick and Rubinstein originally defined neuronal differentation by silver impregnation, unlike the immunohistochemical methods used by Okuda and his colleagues. Schild et al. (1993) have reported that only electron microscopy has diagnostic value. In the present study, all pineal parenchymal tumors expressed divergent types of differentiation, which were confirmed by both immunohistochemical and ultrastructural studies. Immunohistochemically, NFP existed inside the pineocytomatous rosettes and also in the tumor cell cytoplasm. In addition, the presence of synaptophysin, a 38-kDa glycoprotein originally isolated from the presynaptic vesicles of bovine neurons, suggests that these tumors possess the potential for neuronal differentiation (Wiedenmann and Franke 1985). Chromogranin A is a glycoprotein stored in the secretory granules of neuroendocrine cells. Previous studies have revealed that expression of chromogranin A in the central nervous system of human embryos occurs earlier than the expression of neuron-specific enolase, synaptophysin or NFP (Kleinert 1991). Chromogranin A was present in all the pineal parenchymal specimens in this study, indicative of an earlier level of neuronal differentiation than signified by the presence of NFP or synaptophysin. αB-Crystallin is a small heat-shock protein found in the eye lens (Klementz et al. 1991), which was located in all specimens characteristically in the large neuron-like tumor cells. Retinal S protein, which is related to photoreceptors (Perentes et al. 1986), is also reported to be rarely present in medulloblastomas and retinoblastomas (Kramm et al. 1991). In the present study, retinal S protein was positive in cases positive for both chromogranin A and αB-Crystallin. The relationship between these antigens and their presence in neuroectodermal tumors remains to be determined, nevertheless neuronal differentiation with some progression towards neurosensory photoreceptors was surmised. Because of the high positivity of these markers, they were also thought to be helpful in the diagnosis of pineal parenchymal tumors.

The other neuroepithelial component, astrocytes are known to be GFAP-positive, fibrillary and reactive, to increase with age and to play a role in gliosis (Papasozomenos 1983). It is possible that these pineal astrocytes represent precursors of astrocytic tumors that can also arise in this region. Tumors with interstitial-tissue-positive cells were excluded, since these were considered to be reactive astrocytes rather than neoplastic. Despite significant differences in GFAP immunoreactivity, 6 tumors demonstrated neoplastic glial differentiation. One tumor contained clusters of GFAP-positive tumor cells, and the others contained positive cells scattered throughout the tumor. Glial differentiation was consistent with pinealocyte tumor origin, since tumor cells could be expected to possess the characteristics of both of the neuroepithelial cell types that construct the pineal gland.

Electron-microscopic studies demonstrated discrepancies in the content of intracellular organs between individual tumor cells. For example, some cells seemed more primitive while others appeared more differentiated, demonstrating various levels of intratumoral cell differentiation. The results correlated well with those obtained by immunohistochemistry, revealing neurofilaments, glial filament bundles, synapses, and clear-centered vesicles. Although neurosensory photoreceptor differentiation was suggested by retinal S pro-

tein immunoreactivity, microtubular tonofilaments with a 9+0 configuration, suggestive of photoreceptor differentiation (Kline et al. 1979), were not observed in our series.

In the past, the relationship between histological features and prognosis has been studied. Tumors with pineocytomatous rosettes or neuronal differentation have been considered as "benign hallmarks", and reported to be benign or to be associated with a long recurrence-free period (Borit et al. 1980; Rubinstein 1980; Schild et al, 1993). Therefore, immunohistochemical and electron-microscopic studies of cell differentiation in these tumors are considered important for the evaluation of future prognosis. However, pineal parenchymal tumors with these benign hallmarks sometimes have a fatal prognosis (McGrogan et al 1992; Numoto et al. 1992b), and some factor that could not be evaluated by standard histopathological techniques was thought to have affected prognosis.

The estimation of the proliferative potential of human brain tumors is very important when predicting the prognosis of patients, as the enclosure of the skull provides a limited space for intracranial expansion. In the present series, a noninvasive method using a monoclonal antibody applicable to paraffin-embedded specimens was considered. There are many reports on profilerating cell nuclear antigen (PCNA) studied in brain tumors, although recent reports are doubtful about its specifity, since PCNA is also known to react with G0 cells (Figge et al. 1992). Therefore, in this study we have used MIB-1, which is also applicable to formalin-fixed paraffin sections. It reacts with the Ki-67 nuclear antigen, which is associated with cell proliferation, increases during the S phase, reaches a maximum during the G2/M phases, and is absent in the resting G0 cells (Cattoretti et al. 1992; Landberg et al. 1990). The MIB-1 labeling index was significantly higher in PB compared with PC, and also in tumors with clinical manifestations of seeding potential compared with those that did not show seeding. MIB-1 was especially thought to be useful for pineal parenchymal tumors, which demonstrated a continuous histopathological spectrum between PC and PB. MIB-1 labeling indices were studied in cases with the benign hallmarks of pineocytomatous rosettes, and also neuronal differentiation. The extensive markers used in this study were also considered. The labeling indices tended to be lower in cases with pineocytomatous rosettes; however, this was not statistically significant. In the past, neuronal differentiation was defined by silver impregnation, or immunohistochemical demonstration of NFP or synaptophysin. However, there is no study of their relationship with prognosis. MIB-1 labeling indices of NFP- or synaptophysin-positive cases tended to be lower, but this was not statistically significant, whereas neuronal differentiation defined by both NFP and synaptophysin inversely correlated well with proliferation indices. Therefore, neuronal differentiation should be evaluated by both NFP and synaptophysin. MIB-1 labeling is easily applicable to archival histological material, useful in assessment of cell kinetics, and could be used as an additional prognostic factor.

PB and mixed PC/PB are known to easily disseminate through the CSF passage, which differs from the biological behavior of PC (Schild et al. 1993). Studies on the expression of adhesion molecules and their relationship with metastasis have been carried out on other neuroectodermal tumors (Figarella-Branger et al. 1990). Studies on pineal parenchymal tumors may be useful in the assessment of future dissemination and prognosis. Further investigations of the biological behavior of pineal parenchymal tumors are necessary to assess the utility of these prognostic parameters.

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References

Borit A, Blackwood W, Mair WGP (1980) The separation of pineocytoma from pineoblastoma. Cancer 45:1408–1418

Cattoretti G, Becker MHG, Key G, Duchrow M, Schluter C, Galle J, Gerdes J (1992) Monoclonal antibodies against recombinant parts of the Ki-67 antigen (MIB-1 and MIB-3) detect proliferating cells in microwave-processed formalin-fixed paraffin sections. J. Pathol 168: 357–363

Committee of the Brain Tumor Registry of Japan (1990) Vol 7. Brain Tumor Registry of Japan, Tokyo, pp 13–56

Dooling EC, Chi JG, Gilles FH (1977) Melanotic neuroectodermal tumor of infancy. Cancer 39:1535–1541

Figarella-Branger DF, Durbec PL, Rougon GN (1990) Differential spectrum of expression of neural cell adhesion molecule isoforms and L1 adhesion molecules on human neuroectodermal tumors. Cancer Res 50:6364–6370

Figge C, Reifenberger G, Vogeley KT, Messing M, Roosen N, Wechsler W (1992) Immunohistochemical demonstration of proliferating cell nuclear antigen in glioblastomas: pronounced heterogeneity and lack of prognostic significance. J Cancer Res Clin Oncol 18:289–295

Herrick MK, Rubinstein LJ (1979) The cytological differentiating potential of pineal parenchymal neoplasms (true pinealomas). A clinicopathological study of 28 tumours. Brain 102:289–320

Kato K, Shinohara H, Kurobe N, Inaguma Y, Shimizu K, Ohshima K (1991) Tissue distribution and developmental profiles of immunoreactive αB crystallin in the rat determined with a sensitive immunoassay system. Biochim Biophys Acta 1074:201–208

Keihues P, Burger PC, Scheithauer BW (1993) Neuroepithelial tumours. In Sobin LH (ed) Histological typing of tumours of the central nervous system, 2nd edn. Springer, Berlin Heidelberg New York, pp 11–30

Kleinert R (1991) Immunohistochemical characterization of primitive neuroectodermal tumors and their possible relationship to the stepwise ontogenetic development of the central nervous system. 1. Ontogenetic studies. Acta Neuropathol 82:502–507

Klementz R, Frohli E, Steiger RH, Schafer R, Aoyama A (1991) αB-crystallin is a small heat shock protein. Proc Natl Acad Sci USA 88: 3652–3656

Kline KT, Damjanov I, Katz SM, Schmidek H (1979) Pineoblastoma: an electron microscopic study. Cancer 44:1692–1699

Kramm CM, Korf HW, Czerwionka M, Schachenmayr W, Grip WJ (1991) Photoreceptor differentiation in cerebellar medulloblastoma: evidence for a functional photopigment and authentic S antigen (arrestin). Acta Neuropathol 81:296–302

Landberg G, Tan EM, Roos G (1990) Flow cytometric multiparameter analysis of proliferating cell nuclear antigen/cyclin and Ki-67 antigen: a new view of the cell cycle. Exp Cell Res 187:111–118

McGrogan G, Rivel J, Vital C, Guerin J (1992) A pineal tumour with features of "pineal anlage tumour". Acta Neurochir (Wien) 117: 73–77

Numoto RT, Tanaka J, Hasumura M, Fukuda T, Yasue M, Kamio M, Hashimoto T, Nakamura N (1992a) Immunocytochemical and elec-

tron microscopic studies of pineal parenchymal tumors. Brain Tumor Pathol 9:75–80

Numoto RT, Tanaka J, Hasumura M, Fukuda T, Miyazaki Y, Matsumoto M, Akachi K, Kanki T, Hashimoto T, Nakamura N (1992b) A case of a cerebellopontine angle pineoblastoma. Neurol Surg 20:899–903

Okuda Y, Taomoto K, Saya H, Ijichi A, Kokunai T, Tamaki N, Matsumoto S (1988) Pineoblastoma with neuronal differentiation: immunohistochemical and immunocytochemical studies. J Neurooncol 6: 193–198

Papasozomenos SC (1983) Glial fibrillary acidic (GFA) protein-containing cells in the human pineal gland. J Neuropathol Exp Neurol 42:391–408

Perentes E, Rubinstein LJ, Herman MM, Donoso LA (1986) S-antigen immunoreactivity in human pineal glands and pineal parenchymal tumors. A monoclonal antibody study. Acta Neuropathol 71:224–227

Rubinstein LJ (1980) Cytogenesis and differentation of pineal neoplasms. Hum Pathol 12:441–448

Schild SE, Scheithauer BW, Schomberg PJ, Hook CC, Kelly PJ, Frick L, Robinow JS, Buskirk SJ (1993) Pineal parenchymal tumors: clinical, pathologic, and therapeutic aspects. Cancer 72:870–880

Shibuya M, Utsunomiya Y, Osamura Y (1993) Immunohistochemical determination of the proliferating cells with monoclonal antibody MIB-1 on paraffin-embedded section using antigen retrieval method. Pathol Clin Med 11:373–377

Tapp E, Huxley M (1972) The histological appearance of the human pineal gland from puperty to old age. J Pathol 108:137–144

Wacker WB, Donoso LA, Kalsow CM, Yankeelov JA, Organisciak DT (1977) Experimental allergic uveitis. Isolation, characterization and localization of a soluble uveitopathogenic antigen from bovine retina. J Immunol 119:1949–1958

Wiedenmann B, Franke WW (1985) Identification and localization of synaptophysin, an integral membrane glycoprotein of Mw 38 000 characteristic of presynaptic vesicles. Cell 41:1017–1028