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Neuron-associated class III β-tubulin isotype, retinal S-antigen, synaptophysin, and glial fibrillary acidic protein in human medulloblastomas: a clinicopathological analysis of 36 cases*

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Summary. Surgical specimens from 36 medulloblastomas (25 classic and 11 desmoplastic) were studied by peroxidase-antiperoxidase (PAP) immunohistochemistry with antibodies against the class III β-tubulin isotype (β-tubulin), synaptophysin, retinal S-antigen (S-Ag), and glial fibrillary acidic protein (GFAP). We found that neoplastic cells expressed β-tubulin in 91% of the tumors (23 classic and 10 desmoplastic), synaptophysin in 75% (19 classic and 8 desmoplastic), S-Ag in 44% (11 classic and 5 desmoplastic), and GFAP in 11% of medulloblastomas (2 classic and 2 desmoplastic). Synaptophysin and β-tubulin positivities were observed in undifferentiated neoplastic cells, in cells forming neuroplastic rosettes, and in pale islands, while S-Ag immunopositivity was noted in undifferentiated cells, occasionally in β-tubulin-negative neuroblastic rosettes, and exceptionally in pale islands. Large pale islands, in two desmoplastic medulloblastomas, exhibited distinct patterns of immunoreactivity to the above markers, suggesting neuronal and glial differentiation in the central area, and intense neuritic development in the peripheral zone. Our findings confirm the predominant capacity of medulloblastoma cells to differentiate along neuronal cell lines and indicate that large pale islands, in desmoplastic medulloblastomas, represent well-organized areas for neuronal and, to a lesser degree, astroglial differentiation. Furthermore, it appears, in our cases, that immunohistochemical features do not represent clear-cut prognostic indicators in patients with medulloblastomas.

Key words: Medulloblastoma – Primitive neuroectodermal tumor – Cell differentiation – Immunohistochemistry – Prognosis

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A large number of morphological, immunohistochemical, ultrastructural and in vitro studies have provided evidence that medulloblastoma cells can undergo neuronal and, to a lesser degree, glial differentiation [2, 5–7, 17–21, 24, 25, 35]. Furthermore, it has been suggested that the expression of photoreceptor-specific proteins by neoplastic cells in a number of medulloblastomas [1, 23] could be regarded as an indication of their differentiation potential along the photoreceptor cell lineage, and a special subtype of medulloblastoma displaying photoreceptor-specific characteristics could, thus, be defined [8, 29].

The possible correlation between histological and/or immunohistochemical features in medulloblastomas and the prognosis of the disease is controversial, and various studies have shown diametrically opposed results [4, 5, 8, 13, 22, 26, 27].

In this report, we present and discuss the results obtained in 36 cases of medulloblastoma using a panel of immunohistochemical markers with well-established specificities. In addition, an attempt has been made to correlate the immunoreactive patterns of medulloblastomas with the clinical course and survival of patients.

Material and methods

Surgical specimens from 36 medulloblastomas (25 classic and 11 desmoplastic) were studied. All specimens were biopsies from the files of the following institutions: Department of Neurosurgery of the Kyoto Prefectural University of Medicine, Japan (12 cases); Institute of Pathology of the Cantonal Hospital Aarau, Switzerland (11 cases); Institute of Pathology of the Basle University School of Medicine (9 cases); and Department of Pathology of Patras School of Medicine, Greece (4 cases). Tissues had been fixed in 10% formalin and embedded in paraffin, and 5-μm-thick sections were stained routinely with hematoxylin and eosin, and reticulin, or were used for the immunohistochemical reactions. The peroxidase-antiperoxidase (PAP) method of Sternberger and associates [37] was applied with the following rabbit polyclonal (pAb) and mouse monoclonal (mAb) antibodies: (1) anti-glial fibrillary acidic protein (GFAP) pAb (Dako Co., Santa Barbara,

Calif., USA; lot 015, diluted 1:800); (2) TUJ1 mAb [11], which recognizes an epitope on the class III β-tubulin isotype, prepared by one of us (AF) (diluted 1:800); (3) MAbA9-C6 mAb (MAbA9-C6 [9]), which defines an epitope of the retinal S-antigen (S-Ag) (courtesy of Dr. L. A. Donoso, Wills Eye Hospital, Philadelphia, Pa., USA, diluted 1:2000); and (4) SY-38 mAb, an anti-synaptophysin mAb (Boehringer Mannheim Biochemica, Mannheim, FRG; lot 10644536-01, diluted 1:100). The three-step (for pAb) and four-step (for mAb) PAP methods used have been described previously [30, 32]. As positive controls, surgical specimens from human glioblastomas (for GFAP), necropsy specimens from human retina (for TUJ1 mAb and MAbA9-C6), and necropsy specimens from rat adrenals (for SY-38 mAb) were immunostained in parallel. Negative controls were obtained either by omitting the primary antibodies or by applying rabbit serum instead of pAb and mouse serum instead of mAb.

Sections from three medulloblastomas were immunostained according to a previously described double-labeling protocol [31] for β-tubulin and S-Ag.

Results

Clinical data and immunohistological findings are summarized in Table 1. Of the tumors, 25 (69%) were classified as classic medulloblastomas. Their histological picture was essentially that of a homogeneous highly cellular tumor with abundant mitoses and with relatively little vascular stroma. The cells were closely packed, usually with scanty, poorly defined cytoplasm and round, oval or carrot-shaped hyperchromatic nuclei. Rosettes of the Homer Wright type (neuroblastic rosettes) were observed in 8 of the 25 classic medulloblastomas and, in 1 example, ganglionic differentiation was present. The appearance and distribution of these ganglion cells precluded their being interpreted as non-tumor cells of the adjacent cerebellar parenchyma.

Table 1. Main clinical, histopathological and immunohistochemical features

Patient			Cerebellar location	Histological variant	Immunoreactivity[b]				Outcome[c]	Survival[c]
No.	Sex	Age[a]			GFAP	TUJ1	SY-38	S-Ag		
1	M	3	V	C	R	+	−	−	?	?
2	F	11	V	C	R	+++	−	−	?	?
3	F	10	V	C	R	++	++	++	A	1y
4	M	10	V	C	R	++	++	+++	?	?
5	M	4	V	C	R	+++	++	++	A	2y
6	M	5	V	C	R	++	++	−	D	7mo
7	M	8	V	C	R/N	+++	+	++	?	?
8	M	3	V	C	R	++	++	+	D	5y3mo
9	M	6	V	C	R	+++	+++	−	D	3y3mo
10	M	14	V	C	R	++	++	−	A	16y3mo
11	M	5	V	C	R	+++	+++	+	D	3y3mo
12	F	9	V	C	R	++	++	++	D	1y
13	M	10	V	C	R	+++	+	−	D	2y
14	M	40	V	C	R	+++	+++	−	A	3y6mo
15	M	32	H	C	R	+++	+	−	D	2y
16	M	2	V	C	R	+++	+++	+++	?	?
17	F	14	V	C	R	+++	−	−	A	6y8mo
18	F	19	V	C	R	+++	+++	++	?	?
19	M	14	V	C	R	++	+++	++	D	4y
20	M	4	V	C	R	+++	++	++	?	?
21	M	33	?	C	R	−	−	−	?	?
22	M	3	H	C	R	+++	−	−	A	7y7mo
23	F	9	V	C	R/N	+	+	−	A	3y6mo
24	F	11	V	C	R	+	+++	−	A	3y5mo
25	M	15	V	C	R	−	−	−	A	2y7mo
26	F	12	H	D	R	+++	−	−	?	?
27	M	1	V	D	R	++	++	−	A	18y8mo
28	M	3	V	D	R	++	++	++	D	3days
29	F	6	V	D	R	++	++	+	D	4y9mo
30	M	3	V	D	R	+++	+++	−	D	10mo
31	M	10	V	D	R	++	++	+	D	5y4mo
32	F	15	H	D	R/N	+++	+++	−	D	7y8mo
33	M	11	V	D	R/N	++	+++	+++	D	9mo
34	M	27	H	D	R	+++	−	−	A	6y10mo
35	M	21	?	D	−	++	++	+	?	?
36	F	40	V	D	−	−	−	−	D	5y

M, Male; F, female; V, vermis; H, hemisphere; C, classic; D, desmoplastic; R, positivity in reactive astrocytes; N, positivity in neoplastic cells; ?, patient lost to post-operative follow-up; A, alive; D, dead; y, year(s); mo, months

[a] In years

[b] Number of immunoreactive cells with antibodies to glial fibrillary acidic protein (GFAP), to β-tubulin (TUJ1), to synaptophysin (SY-38), and to retinal S-antigen (S-Ag): −, no reactivity; +, a few immunopositive cells; ++, moderate number of immunopositive cells; +++, large number of immunopositive cells

[c] Outcome/survival at the time of review

Focal and, occasionally, abundant desmoplasia, due to seeding in the adjacent leptomeninges, was noted in 7 tumors. Eleven (30.5%) tumors were classified as desmoplastic medulloblastomas: all of them exhibited the characteristic lobular pattern of reticulin-free island with reduced cell density, surrounded by reticulin-rich desmoplastic areas of dense cellularity. Regarding the incidence of the classic and desmoplastic variants of medulloblastoma, there were no significant differences related to the median age and sex of the patients (classic variant: 18 males and 7 females, mean age 10.6 years; desmoplastic variant: 7 males and 4 females; mean age 13.5 years).

On control slides of adult human retina, TUJ1 mAb was found to stain weakly all layers of neuroretina and most intensely the layer of the optic nerve fibers (Fig. 1A), while only the entire photoreceptor cell layer (external nuclear and rod and cone layers) displayed strong S-Ag immunopositivity (Fig. 2B).

Of the 25 classic medulloblastomas, 23 expressed β-tubulin positivity. The number of immunoreactive cells and the intensity of the reaction varied considerably from one case to the other. Large areas of undifferentiated cells (Fig. 1C, F), neuroblastic rosettes (Fig. 1D), and neoplastic ganglion cells (Fig. 1E) stained with TUJ1 mAb. Both the perinuclear cytoplasm and the cytoplasmic processes exhibited thin diffuse and/or fibrillary reactivity (Fig. 1C, E, F) and, more rarely, a heavily fibrillary pattern was noted in neuroblastic rosettes (Fig. 1D). However, in 2 classic medulloblastomas, a small number of small neuroblastic rosettes remained consistently negative, contrasting with the surrounding β-tubulin-positive neoplastic cells (Fig. 1F). In 3 other cases, only a few scattered cells and/or small clusters of cells were found to be β-tubulin positive.

Of the 25 classic medulloblastomas, 11 (44%) contained S-Ag-positive cells. In 2 neoplasms, only a few isolated immunopositive cells were noted, while in others, large areas of undifferentiated neoplastic cells expressed strong S-Ag positivity (Fig. 1G). Immunostaining was present in the cell cytoplasm and membrane, and in the nucleus. Large Homer Wright rosettes did not display S-Ag positivity but, in 2 examples, small neuroblastic rosettes stained intensely for S-Ag (Fig. 1H). In successive sections, the S-Ag-positive neuroblastic rosettes were found to be β-tubulin negative (Fig. 1F), but they stained weakly for synaptophysin (Fig. 1J).

Synaptophysin positivity was observed in 19 of the 25 classic medulloblastomas (76%). The immunostaining was mainly cytoplasmic, and neuroblastic rosettes displayed thin fibrillary immunopositivity (Fig. 1I, J). Undifferentiated cells occasionally showed focal perinuclear positivity.

Immunoreactivity for GFAP was observed in all classic medulloblastomas. GFAP-positive cells were found at the periphery of the tumor, around vessels, and scattered in the neoplasm (Fig. 1K). On the basis of their cytological appearance they were interpreted as being reactive and/or entrapped astrocytes. However, in two cases, GFAP cytoplasmic positivity was found in isolated (Fig. 1L) or groups (Fig. 1M) of cells which appeared otherwise identical to the adjacent non-immunoreactive neoplastic cells.

In two classic medulloblastomas, large reticulin-free areas of dense cellularity were observed adjacent to and/or surrounding other less compact reticulin-free neoplastic areas (Figs. 1N–P; 2A–C). In both cases, the immunohistochemical investigation revealed that, while β-tubulin positivity was present only in the less compact tumor cells (Figs. 1N, 2A) and S-Ag positivity only in the areas of dense cellularity (Fig. 1O, 2B), synaptophysin immunoreactivity was expressed by neoplastic cells in both areas (Figs. 1P, 2C).

Of the 11 desmoplastic medulloblastomas, 10 displayed β-tubulin reactivity, similar to that observed in the classic variant. Moreover, consistent β-tubulin positivity was observed in the reticulin-free areas (pale islands). In small pale islands, a thin and delicate β-tubulin-positive network was present, while in the large reticulin-free areas, strong fibrillary immunopositivity was, in addition, noted at the periphery, adjacent to the surrounding reticulin-rich desmoplastic zone (Fig. 2E, G).

Synaptophysin was expressed in eight desmoplastic medulloblastomas, predominantly in neuroblastic rosettes and in pale islands. Strong diffuse cytoplasmic positivity was found in the latter, the immunostaining becoming weaker at the periphery of the large pale islands (Fig. 2F, H). Successive sections, immunostained with TUJ1 mAb and SY-38 mAb, respectively,

Fig. 1. Human retina (**A, B**) and classic medulloblastomas (**C–P**) immunostained for class III β-tubulin isotype (β-tubulin; **A, C–F, N**), retinal S-antigen (S-Ag; **B, G, H, O**), synaptophysin (**I, J, P**) and glial fibrillary acidic protein (GFAP; **K–M**). All sections were counterstained with hematoxylin. **A** Strong β-tubulin positivity in the layer of the optic nerve fibers (*bottom*) and weak immunostaining in all the other layers of neuroretina. **B** Retinal S-Ag positivity restricted to the entire photoreceptor cell layer (*top*). **C** Undifferentiated medulloblastoma cells expressing intense β-tubulin positivity. **D** Homer Wright rosettes displaying strong fibrillary β-tubulin positivity. **E** Ganglion cell with cytoplasmic β-tubulin immunoreactivity, surrounded by undifferentiated cells. **F** Two small β-tubulin-negative neuroblastic rosettes (*upper right* and *lower left*) surrounded by β-tubulin-positive undifferentiated cells (section from the same area as in **H** and **J**). **G** Large group of undifferentiated medulloblastoma cells expressing S-Ag positivity. **H** Two small S-Ag-positive neuroblastic rosettes (section from the same area as in **F** and **J**). **I** Large Homer Wright rosettes displaying thin synaptophysin positivity. **J** Two small neuroblastic rosettes displaying weak synaptophysin positivity (section from the same area as in **F** and **H**). **K** GFAP-positive reactive astrocyte with numerous elongated cell processes. **L** GFAP-positive neoplastic cell with a long cell process (*upper right*). The nucleus of the immunoreactive cell appears identical to the nuclei of the adjacent non-immunoreactive cells. **M** Group of GFAP-positive neoplastic cells exhibiting strong immunostaining in their cytoplasm and cytoplasmic processes. **N–P** Successive sections from the same area of a classic medulloblastoma. Areas of low cellularity expressing β-tubulin (**N**), while S-Ag positivity is present in areas of dense cellularity only (**O**). Both areas, of low and high cellularity, display synaptophysin immunopositivity (**P**). **A, B, G** ×250, **C–F, H–M** ×620, **N–P** ×100

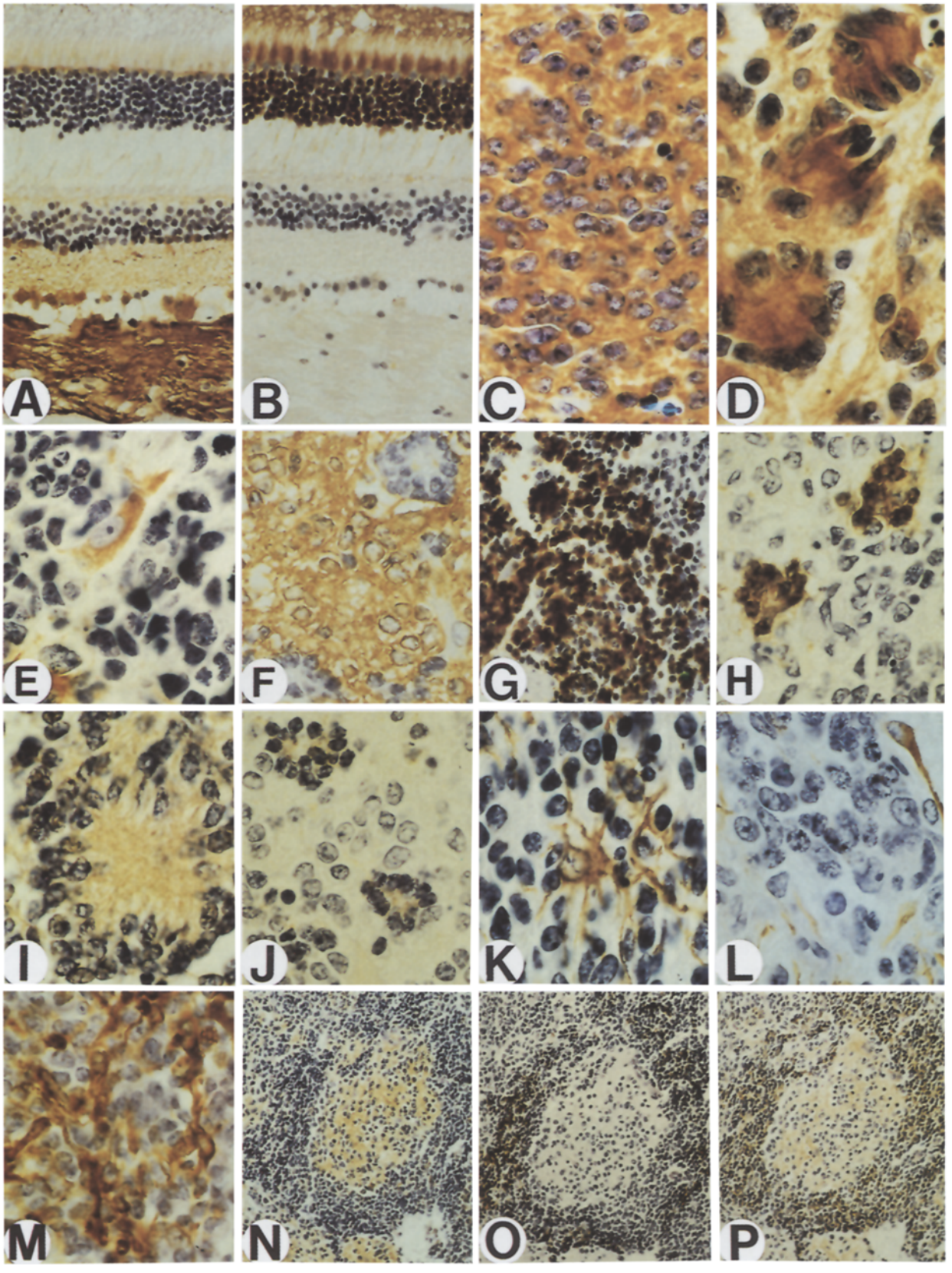

Fig. 1

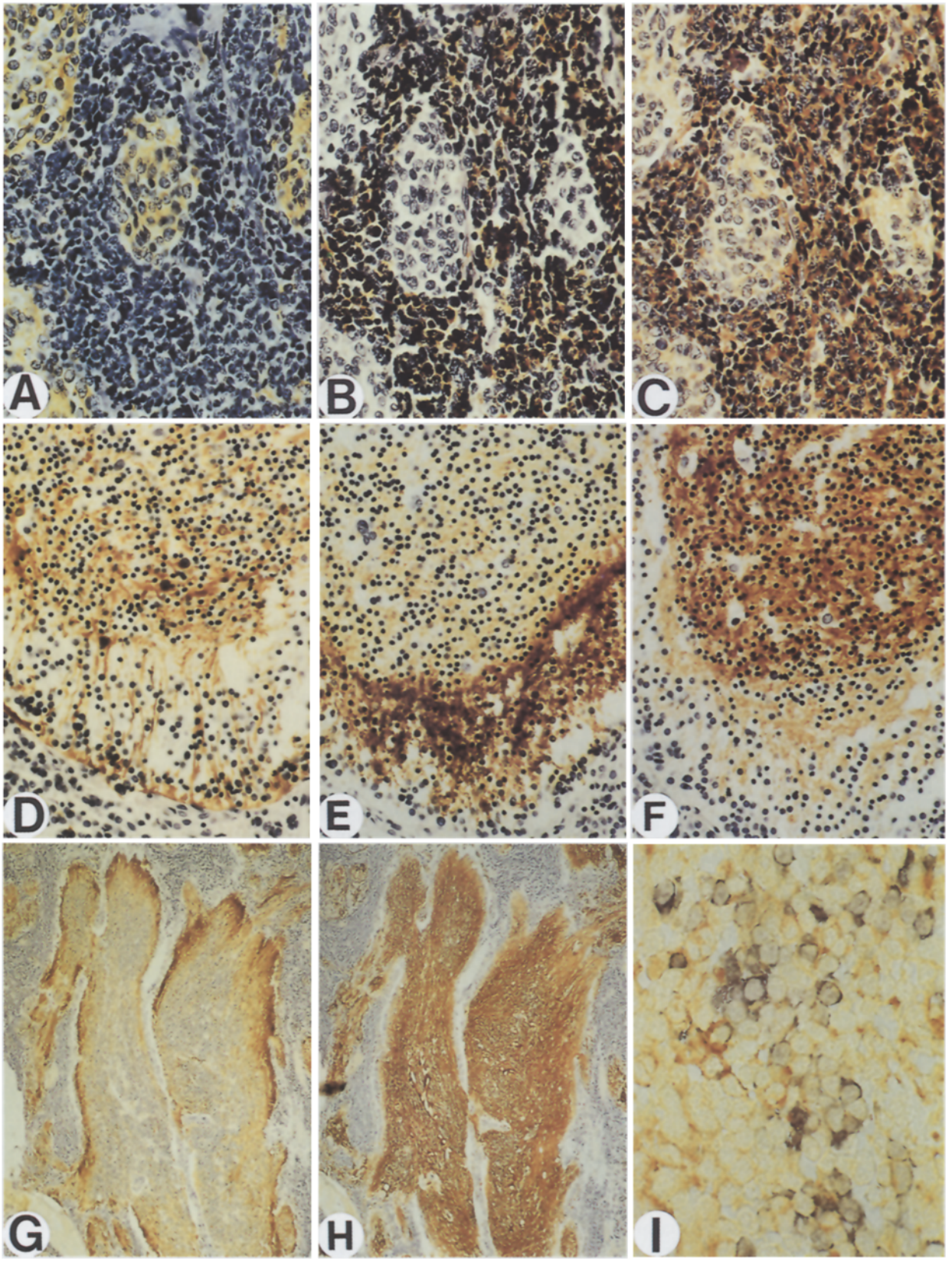

Fig.2 (for legend see next page)

revealed that in the large pale islands the center displayed a delicate β-tubulin-positive network with strong synaptophysin cytoplasmic positivity, while the periphery expressed intense fibrillary β-tubulin positivity and weak diffuse synaptophysin positivity (Fig. 2E–H).

GFAP-positive foci of reactive gliosis and scattered reactive astrocytes were noted in nine desmoplastic medulloblastomas. Moreover, in two cases, consistent GFAP positivity was observed in some of the large pale islands (Fig. 2D). The thin astroglial cytoplasm and the intermingled stellated cell processes formed a delicate GFAP-positive network in the center of the large islands, while radially oriented gliofibrillary cell processes were clearly observed at the periphery (Fig. 2D). In these same areas, at the periphery of the large pale islands, the cell density was lower than that of the more central areas (Fig. 2D), and strong β-tubulin (Fig. 2E, G) and weak synaptophysin (Fig. 2F, H) positivities had previously been noted. In these examples, there was a sharp immunohistochemical delineation between the large pale islands and the adjacent zones of desmoplasia (Fig. 2D–H), and a thin gliofibrillary limiting membrane appeared to be the thinner outer limit of the pale islands (Fig. 2D). In the reticulin-free areas, the nuclei of the GFAP-positive cells were indistinguishable from those of the GFAP-negative neoplastic cells.

Retinal S-Ag-positive neoplastic cells were present in five desmoplastic medulloblastomas. In four of these neoplasms, a few scattered cells (in three cases) and small groups of cells (in one case) were found to be S-Ag positive. In these four medulloblastomas, the immunoreactive cells were randomly distributed, located in undifferentiated neoplastic areas. In the fifth example, cells displaying strong cytoplasmic S-Ag positivity were found in reticulin-free areas. On double-labeled sections, most of the S-Ag-positive cells inside the pale islands were found to be β-tubulin negative, and vice-versa (Fig. 2I).

Table 2. Main immunohistochemical features in 26 medulloblastomas in regard to 5-year survival

Immunopositivity[a] in neoplastic cells	>5-year survival[b] (total: 9 patients)		<5-year survival[b] (total: 17 patients)	
	D (4 patients)	A (5 patients)	D (11 patients)	A (6 patients)
GFAP	1		1	1
TUJ1	3	5	11	5
SY-38	3	2	11	5
S-Ag	2		6	2

[a] Medulloblastomas with neoplastic cell displaying immunopositivity for glial fibrillary acidic protein (GFAP), class III β-tubulin isotype (TUJ1), synaptophysin (SY-38), and retinal S-antigen (S-Ag)

[b] Patients dead (D) or alive (A) at the time of review

The 36 medulloblastomas occurred in 25 male and 11 female patients. Their ages ranged from 1 to 40 years, with a mean of 12 years. Of these patients, 27 (75%) were less than 15 years old at diagnosis, and 9 (25%) were older. Of the tumors, 29 (80.5%) were situated in the vermis, and 5 (14%) in the cerebellar hemispheres. The location was not designated for 2 tumors (5.5%). All patients had initially been treated by surgery (total or subtotal resection). Postoperative irradiation and/or chemotherapy was administered to 25 patients. Ten individuals (28%) were lost to postoperative follow-up. Of the other 26 patients (72%), 1 died in the immediate postoperative period, and 14 others (54%) died of recurrent or metastatic disease, 7 months to 7 years and 8 months after surgery. Eleven patients were known to be alive and free of recurrence at the time of review, for periods ranging from 1 to 18 years and 8 months after operation. The main immunohistochemical features of the above 26 medulloblastomas with respect to a 5-year survival period are summarized in Table 2.

Of the nine patients who survived for more than 5 years, only two suffered from S-Ag-positive medulloblastomas, and one patient suffered from a GFAP-positive medulloblastoma. On the other hand, five of the nine patients were known to be alive at the time of review, for periods ranging from 6 years and 8 months to 18 years and 8 months, and suffered from medulloblastomas which were both GFAP- and S-Ag-negative. Of these five cases, three were also negative for synaptophysin.

Of the 17 patients of the second group (i.e. with less than 5-year survival), 6 were alive at the time of review, for periods ranging from 1 year to 3 years and 6 months. Of these 17 cases of medulloblastoma, 16 were positive for both β-tubulin and synaptophysin (11 dead and 5 alive), 2 were found to express GFAP in neoplastic cells (1 dead and 1 alive), and 8 cases displayed S-Ag positivity (6 dead and 2 alive).

Fig. 2. Classic (**A–C**) and desmoplastic (**D–I**) medulloblastomas immunostained for class III β-tubulin (**A, E, G**), retinal S-Ag (**B**), synaptophysin (**C, F, H**) and (**D**). Double-labeling immunoperoxidase method for β-tubulin and S-Ag (**I**). All sections were counterstained with hematoxylin. **A–C** Successive sections from the same area of a classic medulloblastoma. Areas of low cellularity expressing β-tubulin (**A**), while S-Ag positivity is present in areas of dense cellularity (**B**). Both areas, of low and high cellularity, exhibit synaptophysin immunoreactivity (**C**). **D–F** Successive sections through a large pale island in a desmoplastic medulloblastoma. The peripheral zone (*lower part*) appears less cellular than the central area (*upper part*). **D** GFAP-positive network in the central area of the pale island and radially oriented gliofibrillary cell processes at the periphery. Presence of a thin gliofibrillary membrane delimiting the pale island from the adjacent zone of desmoplasia (*bottom*). **E** Thin delicate β-tubulin positivity in the central area, and strong fibrillary immunopositivity in the peripheral zone. **F** Intense immunostaining for synaptophysin in the central area, and weak immunoreactivity in the peripheral zone. **G, H** Successive sections through the large reticulin-free areas in a desmoplastic medulloblastoma. Presence of strong β-tubulin positivity in the peripheral zone (**G**) and intense synaptophysin immunostaining in the central area of the large islands (**H**). **I** β-tubulin-positive neoplastic cells (*brown*) intermixed with S-Ag-positive tumor cells (*gray*) in a reticulin-free area in a desmoplastic medulloblastoma. **A–F** ×250, **G, H** ×37, **I** ×620

Discussion

Tubulin, the major constituent of microtubules, is a dimeric phosphoprotein exhibiting a high degree of evolutionary conservation [15]. Besides its cytoskeletal role [3], it also contributes to the formation of synaptosomal membranes [16]. In the present study, β-tubulin positivity was observed in 23 of the 25 classic medulloblastomas, in Homer Wright rosettes and in large areas of undifferentiated cells. Of the 11 desmoplastic medulloblastomas, 10 displayed β-tubulin immunoreactivity, especially in pale islands. Our findings are in agreement with those of Katsetos and co-workers [20], who first reported an invariable pattern of immunoreactivity for β-tubulin in cells of the reticulin-free areas, and interpreted this finding as suggestive of early neuronal commitment in medulloblastoma. Moreover, in our cases, at the periphery of the large pale islands, where the neoplastic cell density was also lower, we noted strong fibrillar β-tubulin positivity and a sharp demarcation with the surrounding β-tubulin-negative desmoplastic zone.

In electron microscopy, the presence of parallel arrays of microtubules in the cytoplasmic processes of tumor cells in medulloblastomas, resembling those of growth cones [38] and of developing embryonic neurons [28], has been regarded as evidence of neuronal differentiation [10, 17]. This feature has also been observed in cells forming the pale islands, in desmoplastic medulloblastomas, by Katsetos and co-workers [19], who stressed the fact that this finding was highly characteristic of embryonic neurons.

Synaptophysin has been identified as an integral membrane glycoprotein of presynaptic vesicles in neurons and neuroendocrine cells [14]. In the neoplastic state, synaptophysin immunopositivity has been reported in neuroendocrine and other neuronal tumors [36, 40]. In medulloblastomas, synaptophysin expression has been reported in 94 % to 100 % of cases [5, 36], and has been considered as evidence of neuronal differentiation in these neoplasms. Synaptophysin positivity was observed in 19 of the 25 classic and in 8 of the 11 desmoplastic medulloblastomas in the present series. As previously reported by others [5], the immunostaining was mainly cytoplasmic and more prominent in neuroblastic rosettes and in pale islands. Furthermore, in the large pale islands of the desmoplastic variant of medulloblastoma, successive sections immunostained for β-tubulin and synaptophysin revealed different patterns of immunoreactivity: while the central area of the island displayed a delicate β-tubulin positive network and strong diffuse synaptophysin positivity, the peripheral zone expressed intense β-tubulin and weak synaptophysin positivity.

Retinal S-Ag is a highly uveitopathogenic 50-kDa soluble protein [39] that has been exclusively identified in the photoreceptor cells of the retina and in some pinealocytes of the pineal gland of various vertebrate species including man. In neoplastic conditions, S-Ag immunoreactivity has been demonstrated in human retinoblastomas in situ and in vitro and in pineal parenchymal tumors (see for review [29]). In medulloblastomas, S-Ag immunoreactivity has been reported in approximately 30 %–50 % of the classical variant [1, 8, 23, 29] and it was considered as an indication of a differentiation potential of the medulloblastoma cells along the photoreceptor cell lineage. In our study, immunopositivity for retinal S-Ag was observed either in isolated, randomly distributed, cells or in groups of undifferentiated cells in 11 classic and in 5 desmoplastic medulloblastomas. In 2 classic medulloblastomas, small neuroblastic rosettes stained intensely for S-Ag, and in 1 desmoplastic medulloblastoma, cells displaying strong S-Ag positivity were found in the reticulin-free areas. To our knowledge, this is the first time that retinal S-Ag immunoreactivity has been reported in desmoplastic medulloblastomas. In the above 3 cases, as well as in 2 other classic medulloblastomas where S-Ag-positive areas of dense cellularity were observed adjacent to b-tubulin-positive less-compact areas, S-Ag-positive neoplastic cells were found to be β-tubulin negative, while they stained weakly for synaptophysin. This pattern of immunoreactivity could be partly compared with that of the photoreceptor cells of the adult human retina: photoreceptor cells have been found to express S-Ag and synaptophysin (our data, [21]). Moreover, weak β-tubulin positivity has been detected in photoreceptor cells (our data, [21]) while we found that S-Ag-positive cells in medulloblastoma remained β-tubulin negative. It, thus, appears that in medulloblastomas, neoplastic cells differentiating along the photoreceptor cell lineage do not display all of the cytochemical characteristics of normal photoreceptor cells.

GFAP-positive reactive and/or entrapped astrocytes were observed in all classic and in nine desmoplastic medulloblastomas. Moreover, in two classic medulloblastomas, GFAP positivity was noted in isolated and/or groups of neoplastic cells. In two desmoplastic medulloblastomas, GFAP positivity was observed in some of the large pale islands, where a delicate gliofibrillar meshwork was noted in the center of the island, while radially oriented GFAP-positive cell processes occupied the peripheral zone. Large pale islands appeared to be well delineated from the adjacent desmoplastic tissue by a gliofibrillary limiting membrane. As previously discussed, strong β-tubulin and weak synaptophysin positivities had consistently been noted at the periphery of these reticulin-free areas.

Collectively, our morphological and immunohistochemical findings indicate that pale islands, in desmoplastic medulloblastomas, represent sites of predominant differentiation toward neuronal cell lines. Moreover, it is obvious that in the large islands, while the central area appears to be a focus of neuronal and glial development, the peripheral zone seems to be involved in an intense neuritic development.

It had previously been suggested by others [20], that astroglial cell proliferation and differentiation in the pale islands could be related to neuron-glial interactions. Our findings support the view that neurogenesis and neuritogenesis in the large pale island may be influenced and/or guided by glio-neuronal interactions.

We think that cell proliferation and differentiation in the large pale islands could be compared with the early stages of development of the telencephalon in primates, where outward migrating neuroblasts follow the elongated processes of radial neuroglia [34]. Similarly, in the developing cerebellar cortex, inward migrating granule cells were found to be directly apposed to vertically oriented Bergmann glial fibers [33]. In addition, decrease in the packing density of nerve cells is considered as a feature of maturation of cortical layering, and of gray matter in general [12]. It, thus, seems likely that the less cellular peripheral zone of the large pale islands represent areas of advanced neuronal maturation, where neuritogenesis and, eventually, dendritic growth take place.

The use of histological and immunohistochemical features of cell differentiation as prognostic indicators in patients with medulloblastoma is controversial and various studies have shown diametrically opposed results [4, 5, 13 26, 27]. In an extensive study of 330 medulloblastomas in children, Kleihues and co-workers [22] reported that there appeared to be no clear-cut evidence for improved survival in patients with tumors displaying morphological and immunohistochemical features of advanced neuronal differentiation. However, Czerwionka and co-workers [8] reported a trend toward better prognosis for patients suffering from medulloblastoma with photoreceptor-specific features.

Our investigation of 36 cases of medulloblastoma does not justify a conclusion that the immunohistochemical features of neoplastic cells represent clear-cut prognostic indicators in patients with medulloblastomas. It may be possible that our study could have been hampered by its retrospective nature and the inclusion of patients from several centers, with presumably different therapeutic approaches. However, in contrast to previously reported observations [8, 13], it appears obvious in our study, that neither the GFAP positivity in neoplastic cells, nor the presence of S-Ag seemed to be associated with a better prognosis of the disease. In addition, our findings indicate that β-tubulin could not be used as a prognostic indicator in medulloblastomas, since more than 91% of the tumors in our study were found to be immunopositive. Finally, despite the fact that the incidence of synaptophysin and S-Ag positivity appeared to be higher in the group of patients with a survival of less than 5 years, the small number of cases does not allow speculation on the prognostic value of the above markers.

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