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"Desmoplastic" versus "classic" medulloblastoma: comparison of DNA content, histopathology and differentiation

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Summary. A microfluorometric analysis was performed to analyse the DNA content of 42 medulloblastomas (MBs) and to seek correlations, if any existed, between the DNA distribution and ploidy values, neoplasm types (i.e. classic vs desmoplastic), histological features of aggressiveness, and immunocytochemical features indicating glial and/or neuronal differentiation. Thirty-one cases were classified as classic and 11 cases as desmoplastic MBs. Ten of 11 desmoplastic MBs had a near-diploid main mode and the remaining 1 case had a near-tetraploid main mode. Moreover, 10 of 11 (90%) cases showed a "monomodal" DNA distribution diagram. All these cases showed a uniform histology. In contrast, classic MBs represented a heterogeneous group of neoplasms. Twenty-two cases were near-diploid, 5 cases were near-tetraploid and 3 cases were near-triploid. The histogram type distribution showed a similar heterogeneity. Twelve of 31 (39%) cases had a monomodal histogram, 12 (39%) cases had a bimodal diagram and 7 (22%) cases a complex DNA distribution. There was a statistically significant difference ($P<0.001$) in terms of prevalence of DNA monomodal histograms between classic and desmoplastic MBs. Significant correlations were not observed among classic MBs between histological features of aggresiveness, type and degree of differentiation and DNA distribution. The present study indicates that desmoplastic MBs represent a homogeneous group of neoplasms in terms of histology and DNA distribution. In contrast, classic MBs are lesions with different degrees of histologically apparent aggressiveness and a complex DNA distribution.

Key words: Medulloblastoma – DNA – Microfluorometry

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Introduction

The term "medulloblastoma" (MB) characterizes a small cell neoplasm of the cerebellum which has often been subdivided into two chief variants, "classic" and "desmoplastic" (Rubinstein and Northfield 1964; Rubinstein 1974; Herpers and Budka 1985; Burger et al. 1987).

The term "desmoplastic MB" refers to a neoplasm showing areas with abundant reticulin intermingled with reticulin-free "pale islands" or "follicular structures" (Rubinstein and Northfield 1964; Herpers and Budka 1985; Burger et al. 1987; Katsetos et al. 1989). Both variants of MBs have been extensively studied with regard to histological composition and differentiation by means of electron microscopy and immunocytochemistry (Herpers and Budka 1985; Velasco et al. 1985; Burger et al. 1987; Katsetos et al 1988, 1989). These studies have indicated that desmoplastic MBs show neuronal or neuroblastic features, although a concurrent astroglial differentiation has been suggested by some observers (Herpers and Budka 1985; Katsetos et al. 1989). MBs are a highly malignant neoplasms, but a few studies suggest that histological variables suggestive of aggressiveness, such as presence of necrosis (Caputy et al. 1987; Kopelson et al. 1983) and a high number of mitoses (Kopelson et al. 1983), may affect the prognosis negatively. MBs have also been studied with regard to their DNA content by flow cytometry on fresh (Frederiksen et al. 1978; Hoshino et al. 1978; Lehmann and Krug 1980; Mörk and Laerum 1980) and paraffin-embedded tissues (Tomita et al. 1988; Yasue et al. 1989). However, these studies have not attempted to correlate the DNA distribution with histological variants, such as classic versus desmoplastic MB, and features of aggressiveness and differentiation. One of the reasons for this is the fact that flow cytofluorometry samples a large number of neoplastic cells. These same cells cannot be studied histologically and it is therefore difficult to correlate the flow

cytometric findings with the histological features of the neoplasm. To overcome these technical difficulties, in the present study we used a method by which the nuclei of cells identified in histological sections can be studied by microfluorometric analysis. This technique was applied to analyse the DNA content in 42 MBs to seek the correlation, if any existed, between the ploidy values, type of histogram and type of neoplasm (classic vs desmoplastic), histological features of aggressiveness and immunocytochemical findings indicating glial and/or neuronal differentiation.

Materials and methods

Forty-two MBs were obtained from the files of the Institutes of Anatomical Pathology of the University of Bologna, Ospedale Maggiore of Novara, Ospedale Maggiore Cà Granda of Milan and Ospedale Civile of Lecco (Table 1).

Formalin-fixed and paraffin-embedded tissues were obtained and stained routinely for haematoxylin and eosin (H & E) and reticulin. The histological evaluation of each case included the distinction between classic MB and the desmoplastic variant. Only cases with abundant proliferation of reticulin fibres surrounding reticulin-free structure were considered to be the desmoplastic vari-

Table 1. Clinicopathological, immunohistochemical and microfluorometric data

Case	Age (years)	Sex	Necrosis mitosis[a] vascular proliferation			Nuclear pleomorphism	Rosettes	Neurons	GFAP	NSE	Main ploidy	Histogram type
Classic medulloblastoma												
1	4	F	Neg	2	Neg	+	Neg	Neg	Neg	Neg	−4c	I
2	9	M	Pos[b]	6	Pos	++	Neg	Neg	Neg	Neg	−2c	I
3	29	M	Neg	6	Neg	++	Neg	Neg	Neg	Neg	−2c	I
4	6	F	Neg	2	Neg	+	Neg	Pos	Neg	Pos	−4c	I
5	6	M	Neg	12	Pos	++	Pos	Neg	Neg	Neg	−4c	I
6	11	M	Pos	23	Neg	+++	Neg	Neg	Neg	Pos	−4c	I
7	3	M	Pos	8	Neg	++	Neg	Neg	Neg	Neg	−2c	I
8	13	F	Pos	0	Neg	+	Neg	Neg	Neg	Neg	−2c	I
9	9	M	Pos	15	Pos	++	Pos	Pos	Neg	Pos	−3c	I
10	10	M	Neg	4	Neg	++	Neg	Pos	Neg	Pos	−3c	I
11	21	M	Neg	4	Pos	++	Neg	Neg	Pos	Neg	−2c	I
12	36	F	Neg	1	Neg	++	Neg	Neg	Neg	Neg	−2c	I
13	7	F	Neg	0	Neg	++	Neg	Neg	Neg	Neg	−4c	II
14	5	F	Neg	21	Neg	++	Neg	Neg	Neg	Neg	−2c	II
15	15	M	Neg	9	Neg	+	Neg	Neg	Neg	Pos	−2c	II
16	17	F	Neg	3	Neg	+	Pos	Neg	Neg	Neg	−2c	II
17	13	F	Pos	2	Pos	+++	Neg	Neg	Neg	Pos	−3c	II
18	18	F	Pos[b]	13	Neg	+++	Neg	Neg	Neg	Pos	−2c	II
19	5	F	Neg	5	Neg	++	Neg	Neg	Neg	Neg	−2c	II
20	3	M	Neg	1	Pos	++	Neg	Neg	Neg	Neg	−2c	II
21	8	M	Pos	3	Neg	+	Neg	Neg	Neg	Pos	−2c	II
22	9	M	Pos	2	Neg	++	Pos	Neg	Neg	Pos	−2c	II
23	40	F	Neg	1	Neg	+	Pos	Neg	Neg	Pos	−2c	II
24	26	F	Pos	5	Neg	+	Neg	Neg	Neg	Neg	−2c	II
25	8	F	Neg	3	Neg	++	Neg	Neg	Neg	Neg	−2c	III
26	9	M	Pos	7	Pos	++	Pos	Neg	Neg	Pos	−2c	III
27	9	M	Pos	7	Pos	++	Pos	Neg	Neg	Pos	−2c	III
28	33	M	Neg	6	Neg	++	Neg	Neg	Neg	Neg	−2c	III
29	13	F	Pos	9	Neg	++	Neg	Pos	Pos	Neg	−2c	III
30	20	F	Pos	1	Neg	++	Neg	Neg	Pos	Neg	−4c	III
31	14	M	Neg	11	Neg	++	Neg	Neg	Pos	Neg	−2c	III
Desmoplastic medulloblastoma												
32	20	M	Neg	4	Neg	++	Neg	Neg	Pos	Pos	−4c	I
33	32	M	Neg	8	Neg	+	Neg	Neg	Pos	Neg	−2c	I
34	37	F	Neg	1	Neg	++	Neg	Neg	Pos	Pos	−2c	I
35	14	F	Neg	0	Neg	+	Neg	Neg	Pos	Pos	−2c	I
36	18	F	Neg	2	Neg	+	Neg	Neg	Pos	Pos	−2c	I
37	18	F	Neg	5	Neg	++	Neg	Neg	Pos	Neg	−2c	I
38	26	F	Neg	5	Neg	++	Neg	Neg	Pos	Neg	−2c	I
39	21	M	Neg	4	Neg	++	Neg	Neg	Neg	Pos	−2c	I
40	24	M	Neg	1	Neg	+	Neg	Neg	Neg	Neg	−2c	I
41	15	F	Neg	2	Neg	+	Neg	Pos	Neg	Pos	−2c	I
42	14	M	Neg	8	Neg	+	Neg	Neg	Pos	Pos	−2c	II

GFAP, Glial fibrillary acidic protein; NSE, neuron-specific enolase

[a] Maximum total in 10 high power fields

[b] Necrosis with pseudopalisading

ant. For all 42 neoplasms the presence of necrosis (defined as multicellular areas of coagulative necrosis), number of mitoses per 10 high power fields (HPF), vascular proliferation (defined as glomeruloid endothelial proliferation), evidence of neuroblastic/neuronal differentiation as Homer-Wright rosettes, ganglion cells and fibrillary background was evaluated in H & E sections.

For immunohistochemistry, sections were deparaffinized and rehydrated through graded alcohols and water. The hydrogen peroxide-periodic acid-sodium borohydride sequence was performed to abolish endogenous peroxidases. The avidin-biotin-complex (ABC) method (Hsu et al. 1981) was used with overnight incubation at room temperature for primary antisera, 3,3-Diaminobenzidine was used as chromogen. Subsequently, sections were counterstained with Harris' haematoxylin, dehydrated and mounted with DPX (BDH, London, UK). The following primary polyclonal and monoclonal antibodies were used: rabbit anti-N neuron-specific enolase (NSE) (PAP kit; Dako, Santa Barbara, Ca, USA) diluted 1:12; mouse anti-neurofilament (triplet proteins) (Ortho Diagnostic Systems Milan, Italy) diluted 1:500; rabbit anti-glial fibrillary acidic protein (GFAP) (Dakopatts, a/b) diluted 1:400.

The method used for DNA analysis has been described in detail in a previous study (Giangaspero et al. 1987). From the H & E and immunostained sections of each case, areas were selected that were free of necrosis and contained, when present, specific structures such as Homer-Wright rosettes, ganglionic differentiation, reticulin-free pale islands, and GFAP- and NSE-positive cells. Sections from each case were then matched with the paraffin blocks and the selected region was removed with a scalpel blade, deparaffinized in xylene and rehydrated. Single cell suspensions were obtained after pepsin digestion (Hedley et al. 1983) by mincing and repeated syringing. Cells were mounted on glass slides, treated with 5N HCl for 1 h at 20° C, stained with 0.1% Schiff's reagent at pH 1.7 at 20° C for 20 min and mounted in DPX. This staining schedule for the Feulgen reaction met the requirements for microfluorometric DNA measurements at 450–490 nm excitation wavelength and 680 nm emission wavelengths (Prenna et al. 1974). Single-cell DNA measurements were performed on a Zeiss PMT microphotometer connected to a 48K computer (Apple Europlus). The microscope was equipped for fluorescence excitation with incident light from a stabilized HBO 50 W source. One hundred nuclei were selected randomly and measured in each slide. Lymphocytes obtained from lymph nodes processed in the same manner as the tumour tissue were used as a measure of the peri-diploid DNA content of nuclei. Appropriate adjustments were made to correct for differences in emission due to chromatin condensation (Duijndam and Van Dujin 1975) and cytoplasmic fluorescence (Benson et al. 1979), by comparing emission values of lymphocytes with cytological specimens containing non-neoplastic granules and Purkinje cells obtained from histologically normal cerebellar cortex present in the same paraffin block of a classic MB (case 30). Emission values of tumour cells were then transformed in "c" ploidy units by comparison with the mean value of control cells. One c-unit represented the DNA content of an aploid set of chromosomes; therefore a diploid value was denoted "2c".

A ploidy-frequency histogram of each tumour was obtained after identification of the main stem-line. With reference to the conventional phases of the cell cycle (Baserga 1981), five cell compartments could be identified in the histogram: the G1 peak; a compartment to the left of G1 [LG1], including cells with a DNA-ploidy lower than the main stem-line; compartment [G2], including cells with a DNA-ploidy twice that of the main stem-line; a compartment [S] between G1 and G2, including cells with an intermediate (S phase of cell cycle) DNA content; and a compartment [AG2] including cells with a DNA content higher than G2. In the histograms, the bars corresponding to G1 and G2 were made black. The histogram profiles of tumours were classified as: type I, monomodal (most of the cells in G1); type II, bimodal symmetrical (a consistent clustering of cells in G2); type III: complex (a high prevalence of values outside G1 and G2). Results were evaluated statistically using the chi-square method.

Results

The clinicopathological data are summarized in Table 1. Thirty-one cases were classified as classic MB. Of these, 16 were male and 15 female; their ages ranged from 3 to 40 years (mean 14.16). Eleven cases were classified as desmoplastic MB (5 males, 6 females; age range 14–37 years, mean 21.73).

Eleven cases were defined as desmoplastic MB on the basis of a distinct nodular pattern with islands of reduced density surrounded by more compact reticulin-rich zones (Fig. 1). Case 41 had larger lobulated reticulin-free areas separated by reticulin-positive septa in which the cells were arranged in aggregates or multiple parallel rows within an eosinophilic fibrillary matrix, in addition to nodular reticulin-free pale islands typical of a desmoplastic variant. Some cells had vesicular nuclei and prominent nuclei. This case had histological similarities with the cases reported as "cerebellar neuroblasto-

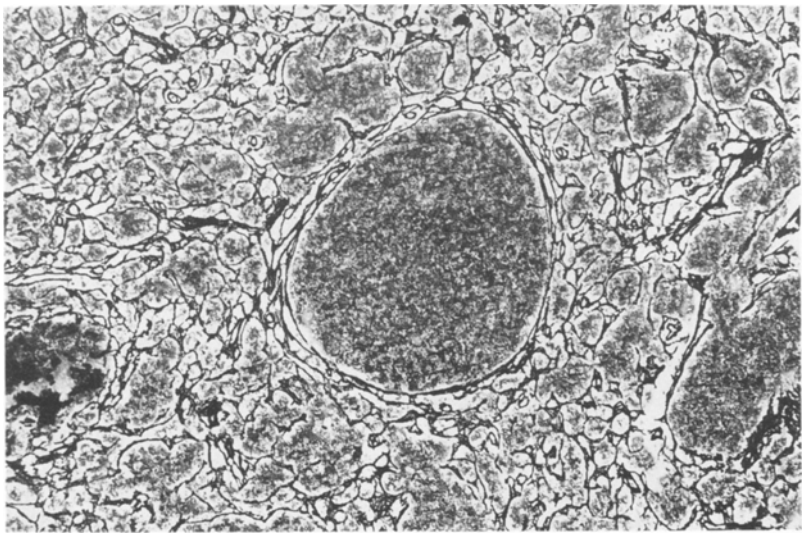

Fig. 1. Desmoplastic medulloblastomas (MBs) were identified on the basis of a distinct nodular pattern with islands of reduced density surrounded by compact reticulin-rich zones. Gordon-Sweet stain for reticulin, ×200

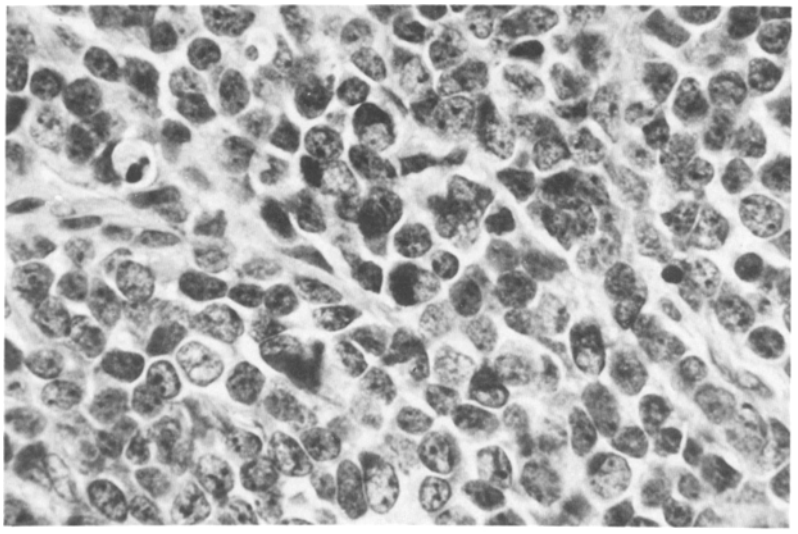

Fig. 2. Glial fibrillary acidic protein immunoreactivity of neoplastic cells in a classic MB (case 23). Streptavidin biotinylated complex, ×400

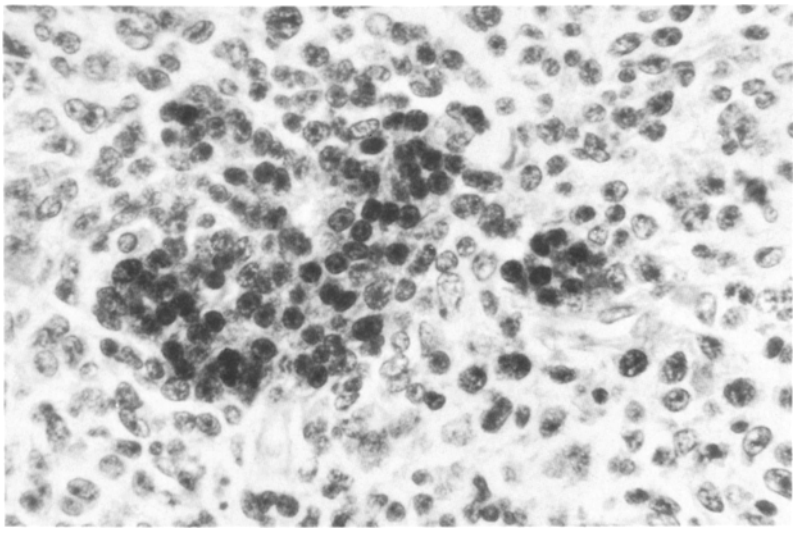

Fig. 3. Islands of neuron-specific enolase positive cells in a classic MB (case 22). This pattern of immunoreactivity was observed in 11 out of 32 cases of classic MB. Streptavidin biotinylated complex, ×400

ma" by Pearl and Takei (1981) and Yagishita et al. (1982).

No frank multicellular areas of necrosis vascular proliferation were observed in any of the cases. The number of mitoses ranged from 0 to 8 per 10 HPF. Cellular pleomorphism was mild or moderate and no case exhibited marked nuclear pleomorphism. Homer-Wright rosettes and mature ganglion cells were not observed in desmoplastic lesions. Immunocytochemically, 8 cases showed focal areas of GFAP-positive cells with the morphology of neoplastic cells. These cells were present in both internodular areas and reticulin-free nodules. Seven out of 11 cases showed NSE immunoreactivity, which was concentrated in the pale islands. Case 41, with neuroblastoma-like features, showed intense immunoreactivity in the fibrillar matrix and in tumour cells with larger and more vesicular nuclei.

No cases showed neurofilament protein (NFP)-positive cell, except for pre-existing axons and Purkinje cells.

Thirty-one cases were classified as classic MB. Leptomeningeal invasion with desmoplastic reaction but without reticulin-free pale islands was observed in 12 cases. Multicellular areas of necrosis were observed in 12 cases, and in 2 of them (cases 2 and 18) necrotic areas were surrounded by pseudopalisading. The number of mitoses per 10 HPF ranged from 0 to 23 and vascular proliferation was observed in 7 cases. Marked nuclear pleomorphism was present in 3 cases, moderate in 20 and mild in 10 cases. Neuroblastic rosettes were observed in 6 cases and neoplastic mature ganglion cells in 3 cases. GFAP-positive cells with the morphology of reactive astrocytes were present in 27 out of 33 cases.

In 4 cases, in addition to reactive astrocytes there were neoplastic cells with intense perinuclear immunoreactivity for GFAP (Fig. 2).

NSE-positive cells were seen in 11 out of 31 cases. NSE-positive cells were aggregated in clusters (Fig. 3). These cells showed larger, more clear nuclei compared with those of adjacent negative cells. NSE was detected in the fibrillar core of Homer-Wright rosettes and in rare scattered cells similar to adjacent neoplastic cells. As in desmoplastic neoplasms, no cases showed NFP-

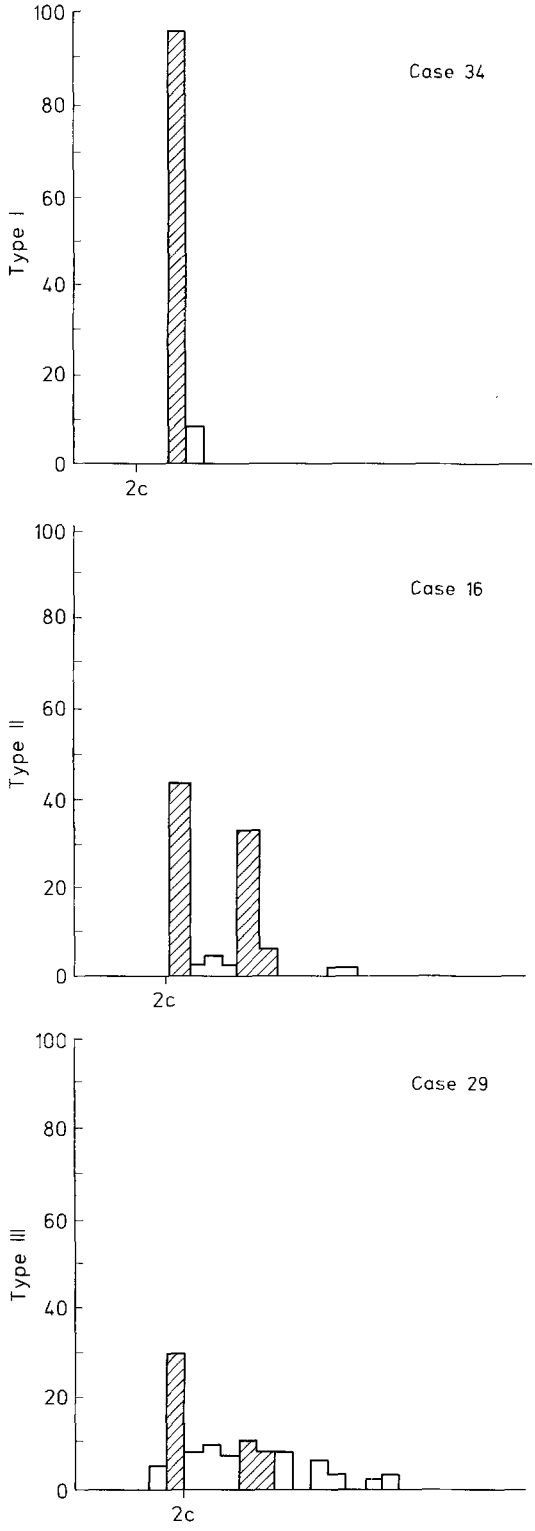

Fig. 4. The DNA distribution histograms were classified in three types on the basis of their complexity. The type I "monomodal" histogram was observed in 10 out of 11 desmoplastic MBs. In contrast, all the three types of histograms were present in the "classic" group of tumours

positive cells, except for pre-existing axons and Purkinje cells.

A clear and distinct mode was identified in all tumours and control specimens (G1). The internal granules and Purkinje cells used as a control preparation were

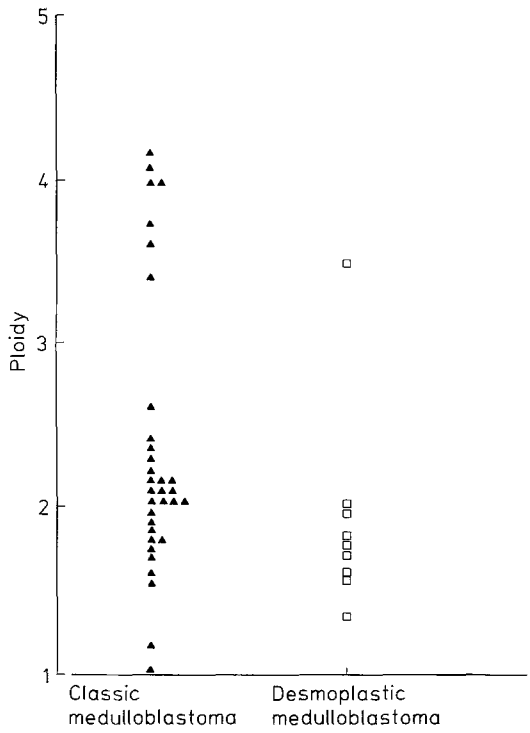

Fig. 5. Desmoplastic MBs were more homogeneous in terms of ploidy of the main population in contrast to the more widespread distribution of ploidy of the classic neoplasms

peri-diploid and peri-tetraploid respectively. The G1, which includes the basic stem cell population of the tumour (Atkin 1987), served as the least variable denominator to classify the histograms on the basis of increasing complexity. Three different histogram profiles were recognized (Fig. 4):

1. Type I "monomodal" histogram in which more than 70% of cells were concentrated in G1, indicating the presence in the neoplasm of a single pool of stem cells.
2. Type II "bimodal" histogram in which numerous cells clustered and peaked in the G2 area. This pattern of DNA distribution was consistent with the presence of second cell population characterized by a DNA content twice of G1.
3. Type III "complex" histograms in which there were numerous cells in the S area and numerous cells had values lower than G1 and higher than G2. This pattern indicated the presence of additional clones of proliferating cells.

Most of the classic MBs had the G1 cells grouped either nearby 2c or 4c values (Fig. 5). Twenty-two neoplasms had a near-diploid main mode with values ranging from 1.6 to 2.5; 6 tumours had a near-tetraploid G1 with values ranging from 3.6 to 4.1; and 3 neoplasms had an aneuploid (peri-triploid) G1 with values ranging from 2.6 to 3.1. In terms of DNA distribution histograms, 12 (39%) cases has type I "monomodal" histograms, 12 (39%) cases had the type II bimodal histogram and 7 (22%) cases the complex type III histogram (Table 1).

The desmoplastic MBs were predominantly near-diploid (10) with only 1 tetraploid. Moreover, 10 cases had

type I monomodal histograms and 1 case a type II bimodal histogram (Table 1).

The difference in the frequency of monomodal histograms in 10 desmoplastic (90%) and in 12 classic (39%) MBs was very highly significant ($P<0.001$).

Among the classic MBs no significant correlations were observed between DNA distribution and variables such as necrosis, number of mitoses, vascular proliferation, neuronal and/or glial differentiation.

Discussion

Although previous studies have focused on DNA content in MBs (Frederiksen et al. 1978; Hoshino et al. 1978; Lehmann and Krug 1980; Mörk and Laerum 1980; Tomita et al. 1988; Yasue et al. 1989), none have sought correlations between DNA distribution and morphological variables. Moreover, all these studies have been performed by flow cytometry on isolated cells. In the current study, particular attention was paid to the methodological approach in terms of selection of well-defined areas and adoption of a precise quantitative analysis that allowed optimal cell recognition. Analyses were done on cells from well-defined tumour areas not showing necrosis, vascular proliferation, or haemorrhage, all of which are factors capable of interfering with the DNA analysis (Giangaspero et al. 1987). Selected areas included reticulin-free nodules in desmoplastic variant, areas with neuroblastic rosettes and areas containing NSE- and GFAP-positive neoplastic cells. The cells were homogeneously dispersed and consequently the 100 cells selected for DNA analysis were representative of the cell population of the chosen tumour areas. The results obtained with this microfluorimetric method are comparable with those obtained with flow cytometry (Fallenins et al. 1987; Uyterlinde et al. 1989).

The DNA histograms were based on the prominent cell population of each tumour. This assured a fairly uniform class-interval variance among different tumours and shaped the graphs similarly to those obtained by flow-cytometric analysis.

The results of this study underscore the fact that most of MBs are predominantly near-diploid. In 31 out of 42 cases the main cell population has a DNA content that is near-diploid, indicating that the predominant cell components of these neoplasms have near-normal DNA content. These findings are in accord with previous data obtained with flow cytometry (Hoshino et al. 1978; Mörk and Laerum 1980; Lehmann and Krug 1980; Tomita et al. 1988; Yasue et al. 1989). In addition, cytogenetic studies performed in a small number of MBs have confirmed the prevalence of near-diploid neoplasms (Latimer et al. 1987; Bigner et al. 1988).

The present study indicates that when strict histological criteria are used to define the desmoplastic variant, this represents a homogeneous group of neoplasms in terms of morphology, immunocytochemical findings and DNA distribution. In addition to the nodular pattern all 11 cases of desmoplastic MB showed mild or moderate nuclear pleomorphism, low mitotic ratio, absence of multicellular areas of necrosis and no vascular proliferation.

The histologically "indolent" appearance shown by the present tumours has been well documented (Burger et al. 1987; Katsetos et al. 1989). GFAP-positive neoplastic cells were present both in the pale areas and in the internodular areas of desmoplastic MBs. Similar findings have been previously found by Burger et al. (1987) and Katsetos et al. (1988, 1989). In terms of DNA analysis 10 out of 11 cases of desmoplastic lesions had a near-diploid main population and the remaining 1 had a near-tetraploid stem-line. Moreover, all cases showed a monomodal DNA distribution diagram. This type of DNA histogram indicates the presence in the neoplasm of a single pool of stem cells without actively cycling heteroclones, suggesting a quite stable DNA content without gross genetic modifications.

In contrast to the desmoplastic variant, classic MBs represent a more heterogeneous group of neoplasms. They show different histological "grade" of malignancy and different type and degree of differentiation. Glial differentiation showed by GFAP immunoreactivity was observed in 4 out of 31 cases, whereas immunoreactivity for NSE suggesting neuronal differentiation was observed in 11 out of 31 cases. These results are similar to those previously reported on formalin-fixed paraffin-embedded material (Velasco et al. 1985; Burger et al. 1987). However a recent study by Gould et al. (1990) using fresh frozen material demonstrated that all MBs express neuronal markers such as synapthophysin.

Microfluorimetric analysis of DNA content in this group confirmed the heterogeneity of these neoplasms. Twenty-two cases were near-diploid, 5 cases were near-tetraploid and 3 cases had an aneuploid main mode in the near-triploid values. The histogram type distribution showed that 12 of 31 cases had a monomodal histogram similar to those observed in the desmoplastic cases, 12 cases had a bimodal diagram and 7 cases a complex DNA distribution diagram with accumulation of cells in the S areas, suggesting the presence of additional clones of cycling cells with DNA content intermediate between G1 and G2.

Chromosomal analyses in MBs (Latimer et al. 1987; Bigner et al. 1988) and in one established human MB cell line (Jacobsen et al. 1985) have showed the prevalence of tetraploid or near-tetraploid cell populations. Similarly Lehmann and Krug (1980) found that 3 of 5 MBs studied by flow cytometry with a diploid main population showed an additional fraction in the tetraploid compartment. These observations are consistent with our findings of the presence of clear evidence of cell accumulation in 4c (G2) in 6 classic MBs and 1 desmoplastic MB with a near-tetraploid main population, and in 12 out of 42 near-diploid classic MBs. The accumulation of these near-tetraploid cells may reflect a peculiar growth pattern of some MBs, which accounts for the different biological behaviour and response to therapy observed in these neoplasms (Jereb et al. 1982; Kopelson et al. 1983).

Our results did not show significant correlations between histological features of aggressiveness and DNA distribution. Similarly no correlation was observed regarding the type and degree of differentiation. Probably this lack of correlation between these morphological parameters and DNA content is related to relative low sensitivity of the method in detecting these differences, especially in a neoplasm which does not show the wide cytological variations observed in non-small-cell-malignant neoplasms.

Two recent studies of flow cytometry on paraffin-embedded material have indicated the prognostic importance of DNA ploidy in MBs (Tomita et al. 1988; Yasue et al. 1989). These authors have found that diploid MBs have a greater tendency to metastasize than aneuploid tumours. However, in both these studies no distinction is made between a classic and a desmoplastic variant. Whether these two variants have a different biological behaviour is an issue still open to discussion. Desmoplastic MB was initially thought to carry a more favourable prognosis than the classic variant (Rubinstein and Northfield 1964; Chatty and Earle 1971) but this belief has subsequently been refuted (Choux et al. 1983; Hubbard et al. 1989). The present study demonstrates that desmoplastic MBs share with most classic MBs a near-diploid main cell population; however, the two groups show a marked difference in terms of DNA distribution histograms. Ten of 11 (90%) desmoplastic tumours had a type I monomodal histogram with a single pool of them cells; in contrast, this simple DNA histogram was observed in only 12 of 31 (38%) ($P < 0.001$). The biological significance of these different DNA distributions in classic and desmoplastic MBs remains to be investigated.

The present study emphasizes that the desmoplastic and the classic MBs represent two distinct groups of neoplasms. The classic MBs are heterogeneous tumours with a different degree of histological parameters of aggressiveness and varying complexity of DNA distribution. In contrast, the desmoplastic MBs appear to be homogeneous in terms of morphology and DNA profile.

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