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Influence of preoperative dexamethasone therapy on proliferating cell nuclear antigen (PCNA) expression in comparison to other parameters in meningiomas

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Summary. We conducted a trial in 42 benign and malignant meningiomas to assess a possible influence of preoperative dexamethasone therapy on mitotic index, labelling indices of proliferating cell nuclear antigen (PCNA), progesterone receptor, epidermal growth factor receptor (EGF-R), c-erbB-2 oncoprotein, cathepsin D, gamma-gamma enolase as well as the mean number of silver-stained nucleolar organizer region-associated proteins (AgNORs). Tumors with preceding dexamethasone therapy for more than 1 day display significantly less immunohistochemical staining for PCNA. A correlation between the labelling index of PCNA and the degree of malignancy could not be identified. There was no significant effect of preoperative dexamethasone therapy on the other parameters. Our data suggest that dexamethasone may selectively inhibit the expression of PCNA in the G1/S-phase of the cell cycle. Thus, we emphasize the necessity to heed factors, e.g. dexamethasone, which may affect the expression of proliferating markers.

Key words: Proliferating cell nuclear antigen, Meningioma, Steroid hormone, Epidermal growth factor receptor, Cell proliferation

Introduction

Proliferating cell nuclear antigen (PCNA), an auxilliary protein of DNA polymerase-delta (Bravo et al., 1987), is located in the nuclear compartment of both normal and transformed proliferating cells (Takasaki et al., 1981). The monoclonal antibody PC-10 binds to a PCNA-epitope in formalin-fixed and paraffin-embedded sections. In contrast to other proliferation markers, e.g. Ki 67, this antibody greatly facilitates retrospective studies of the proliferative potential of tumors. Normal adult central and peripheral nervous system cells do not express PCNA (Hall et al., 1990). The exact function of this protein has not yet been clarified, but recent communication indicates a positive correlation between the labelling index of PCNA and the degree of malignancy in human tumors (Hall et al., 1990; Yu et al., 1990; Kamel et al., 1991). Different tumors may show heterogeneity in the distribution of immunostained cells, e.g. Glioblastoma (Allegranza et al., 1991). In glioblastomas, PCNA-expression is not correlated to patient survival (Frigge et al., 1991). Furthermore labelling indices may be altered by fixation artifacts, aberrant expression of PCNA (Benjamin and Grown, 1991) and anti-proliferative therapy (van Dierendonck et al., 1991).

The majority of patients with brain tumors receive a varying amount of glucocorticoids preoperatively to reduce the accompanying edema. In order to substantiate a possible relationship between preoperative glucocorticoid treatment and PCNA-expression in meningiomas, the clinical charts were reviewed and the findings correlated. The labelling indices of PCNA and immunohistochemical results obtained with human epidermal growth factor receptor (EGF-R), progesterone receptor (PgR), c-erbB-2 oncoprotein (c-erbB-2), human cathepsin D (CD) and human gamma-gamma enolase (gg-E) were compared. Furthermore silver-stained nucleolar organizer region-associated protein (AgNOR) was applied to the same tumors. C-erbB-2 oncoprotein and acidic lysosomal protease cathepsin D (an estrogen-regulated protein) are said to be of prognostic relevance in breast carcinoma, though overall agreement on this fact has not been achieved yet (Berger et al., 1988; Thorpe et al., 1989; Wright et al., 1989; Brouillet et al., 1990; Henry et al., 1990). Several authors found a correlation between the number of AgNOR and the grade of malignancy in meningiomas (Maier et al., 1990; Orita et al., 1990; Plate et al., 1990). Preoperative dexamethasone therapy might also influence the labelling indices of PgR, EGF-R, c-erB-2, cathepsin D

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and the AgNOR counts, though we do not know about any study on meningioma dealing with this problem. Furthermore, gg-EE was evaluated, a protein most meningiomas contain (Hitchcock and Morris, 1987) and which is not known to have any impact on the grade of malignancy or dexamethasone therapy.

Materials and methods

Tissue samples of the 42 tumors investigated, were retrieved from files at our institution. Clinical data relevant for this study are listed in table 1. Tumors were classified according to the criteria of Scheithauer (1990). «Ordinary meningiomas» without signs of malignancy were classified as grade I tumors, «atypical meningiomas» as grade II, and those with positive signs of malignancy as grade III. Malignant meningiomas with focal sarcomatous changes were classified as grade IV. Four of these five cases showed focal fibrosarcomatous differentiation, the growth pattern of the fifth case corresponded to a haemangiopericytoma with high mitotic activity. All grade IV tumors showed predominantly an immunohistochemical pattern typical of meningioma (combination of epithelial membrane antigen and vimentin positivity). All specimens were fixed in 10% phosphate-buffered formalin and embedded in paraffin wax. Sections were cut at 5 µm and stained with haematoxylin & eosin, Nissl, Masson's trichrome and periodic acid Schiff.

Preoperative dexamethasone therapy

The data on preoperative glucocorticoids were retrieved from the clinical charts (see table 1). The majority of patients received 4 mg dexamethasone three or four times a day. Accounting for a halflife of PCNA of approximately 20 h (Bravo and MacDonald-Bravo, 1987), a duration of preoperative therapy of less than 2 days was regarded as irrelevant for the PCNA index. Therefore, the treatment schemes were divided into two groups (Group I: 0-1 days, Group II: 2-7d). All patients with a duration of steroid application longer than 7d were classified as 7d. No patient had been subject to radiation, embolisation or hormonal therapy preoperatively.

Immunohistochemical studies

Prior to immunohistochemical studies all cases were reexamined and most representative sections were selected. To provide additional information, immunohistochemical investigations were carried out using the following monoclonal antibodies (mAB) and polyclonal antisera (pAS): mAB V9 (vimentin, 1:40); D-33 (desmin, 1:100); factor VIII-related antigen (1:100); EMA (epithelial membrane antigen, 1:10); Kp1 (CD 68, 1:100) and pAS S 100 protein (1:5000); and GFAP (1:5000)-Dakopatts, Hamburg, FRG. We additionally used mAB KL1 (cytokeratin 1:500, Dianova, Hamburg, FRG), mAB HMB-45 (1:10000, Enzo, New York, USA) and pAS type IV collagen (1:500, medac, Hamburg, FRG). Immunostaining was achieved using the alkaline phosphatase anti-alkaline phosphatase (APAAP) technique (Cordell et al., 1984) and the avidin-biotin

Table 1. Clinical data, grading and location of tumors and days of preoperative dexamethasone therapy in 42 meningiomas.

Case no.	Age(yrs)/Sex	tumor location	Subtype*	Preoperative dexamethasone therapy (days)
Grade I				
1	68/F	olfact/L	trans	7
2	72/F	sphenoid/L	menin	4
3	51/F	suprasellar	menin	7
4	72/F	spinal	psamm	0
5	52/F	sphenoid	angio	2
6	54/F	sphenoid/L	menin	7
7	35/F	frontal/L	menin	7
8	82/F	occip/R	fibro	5
9	77/F	sphenoid/L	menin	7
10	68/M	sphenoid/L	angio	7
11	77/F	frontal/R	menin	7
12	59/M	frontal/R	menin	6
13	43/F	tuber. sellae	angio	2
14	59/F	suprasellar	menin	5
15	55/M	frontotemp/R	menin	7
16	50/F	spinal	menin	3
17	46/F	sphenoid/R	menin	1
18	46/F	sphenoid/R	menin	1
Grade II				
19	41/M	parietal/L	atyp	0
20	69/F	par-occ/L	atyp	1
21	42/M	parietal/L	atyp	1
22	70/F	par-occ/L	atyp	1
23	72/F	frontopar/R	atyp	1
24	60/F	sphenoid/L	atyp	1
25	9/M	parietal/L	atyp	1
Grade III				
26	68/F	occip/L	menin	5
27	77/F	sphenoid/L	menin	1
28	74/M	occip/R	menin-haem	0
29	70/F	frontal	menin	3
30	74/F	tentorium	menin	2
31	48/M	temp-par/R	menin	7
32	60/M	falx	pap	2
33	29/M	par-occ/R	menin-haem	7
34	61/M	parasagit	pap	4
35	65/M	frontobas/L	menin	7
36	63/F	sphenoid/L	menin	7
37	50/F	parietal/L	menin	0
Grade IV				
38	70/F	frontotemp/L	menin-fibrosac	7
39	73/F	falx	menin-fibrosac	7
40	81/F	falx	menin-fibrosac	2
41	61/M	parasagit	menin-haem	7
42	71/M	bifrontal	menin-fibrosarc	7

*subtypes of meningiomas: trans= transitional; menin= meningothelial; psamm= psammomatous; angio= angiomatous; fibro= fibromatous; atyp= atypical; pap= papillary; menin-haem= mixed meningothelial-haemangiopericyic; menin-fibro= mixed meningothelial with fibrosarcoma-like structures. L= left, R= right.

complex (ABC) method (Hsu et al., 1981). Secondary antibodies and the APAAP-complex were obtained from Dakopatts. The biotinylated secondary antibody and the avidin biotinylated-peroxidase complex were supplied by Vectastain via Camon (Wiesbaden, FRG). Protease digestion was carried out prior to incubation with primary antibodies such as factor VIII-related antigen (pronase), EMA (pronase) and type IV collagen (pepsin). Positive and negative controls were simultaneously done fo all antibodies used.

Immunohistochemical design of antibodies listed in table 2

Representative sections were cut into 5 µm sections and processed for immunohistochemistry.

PCNA

Sections were incubated overnight without protease digestion. Immunostaining was performed using the APAAP technique (see above). Incubation with secondary antibody and APAAP-complex was repeated once and sections subsequently counterstained with haematoxylin. Negative controls were carried out using non-immune serum rather than primary antibody. Sections of a peripheral lymph node served as positive controls. Staining always remained localized to the cell nuclei; there was never non-specific cytoplasmic staining.

Progesterone receptor

As detection system a modified APAAP technique was used. After incubation with the primary antibody overnight, a mouse anti-rat antibody (Dianova) was added as first bridge-antibody and subsequently a rabbit anti-mouse antibody (Dakopatts) was given as secondary bridge-antibody. Nitro blue tetrazolium (NBT) and bromo-chloro-indolyl-phosphate (both from Sigma) were employed as chromogenes. Labelling was restricted to the cell nucleus.

Sections of breast carcinoma served as positive controls.

EGF-R

APAAP technique (see PCNA staining) with prior protease digestion (pronase). Sections of epidermis and gliomas served as positive controls.

C-erbB-2

APAAP technique (see PCNA staining) without prior protease digestion. Sections of breast carcinoma served as positive controls.

Cathepsin D

APAAP technique without prior protease digestion, mouse anti-rabbit (Dianova) as primary and rabbit anti-mouse (Dianova) as secondary bridging-agent. Positive staining of macrophages within meningiomas was not evaluated. Macrophages of chronic inflammatory tissue served as positive controls.

Gamma-gamma-Enolase

APAAP technique (see PCNA staining) without prior protease digestion. Neurons of the cerebral cortex, gangliogliomas and paragangliomas served as positive controls.

Quantitation of labelling

PCNA labelling index (LI) and mitotic index (MI)

The fraction of proliferating cells was determined by counting tumor cell staining with PC 10 in maximally labelled fields of the section at a magnification of 400. Only unequivocally labelled nuclei were taken into consideration. The PCNA labelling index was calculated as the percentage of labelled cells of 300 counted tumor cells. The mitotic index of each tumor was determined in the same way (here 1000 tumor cells were counted); within the individual section the area with the highest density of mitotic cells was chosen on Nissl-stained sections.

EGF-R, c-erbB-2, cathepsin D and gg-E

Immunoreactivity for these antibodies was graded semiquantitatively on a rating scale ranging from 0=no positive cells, 1=few positive cells (<10%), 2=moderate fraction of positive cells (<50%) and 3=high fraction of positive cells (>50%). Solely tumor cells were estimated.

Progesterone receptor

Immunoreactivity for this antibody was graded

Table 2. List of antibodies used in this study.

Antibody	Type	Specificity	Source	Working dilution
PC 10	m, IgG2a	proliferation cell nuclear antigen	Dakopatts	1:25
PgR-ICA	m, rat-antibody	progesterone receptor	Abbott	1:50
EGF-R >P<	m, IgG1	human epidermal growth factor receptor	Merck	1:100
c-erbB-2	m, IgG1	c-erbB-2 oncoprotein	Medac	1:200
Cathepsin D	p, rabbit antibody	human cathepsin D	Medac	1:1000
NSE, H-14	m, IgG1	human gamma-gamma enolase	Dakopatts	undiluted

m= monoclonal antibody; p= polyclonal antiserum.

semiquantitatively as so-called immunoreactive score (IRS) according to the method performed in breast carcinoma (Remmele and Stegner, 1987). The IRS was calculated as the percentage of positive cells (PPxSI; PP, O=no positive cells, 1=<10%, 2=10-50%, 3=51-80%, 4>80%) and staining intensity, (SI, 0=negative, 1=weak, 2=moderate, 3=strong). To control staining intensity, sections of PgR-positive and -negative breast carcinomas were always stained along with the test slides.

AgNOR staining and image analysis

Sections were cut at 4 µm using a rotation microtome. The Feulgen reaction was performed prior to AgNOR silver staining in all specimens. For silver staining the sections were prepared in the following manner: sections were incubated in the dark for 14 minutes in a solution of 50% silver nitrate and 2% gelatin in 1% aqueous formic acid with a volumetric

Table 3. Summary of mitotic index (MI). labelling index (LI) of PCNA, PgR, EGF-R, c-erbB-2, Cathepsin D (CD), gamma-gamma-Enolase (gg-E) and silver-stained nucleolar organizer region protein (AgNOR) counts.

Case No.	MI %	PCNA LI %	PgR IRS*	EGF-R LI	c-erbB-2 LI	CD LI	gg-E LI	AgNOR counts
Grade I								
1	0.0	3.3	1	2	0	1	1	n.t
2	0.0	9.0	6	2	3	3	1	1.48
3	0.0	1.0	6	3	2	1	2	1.87
4	0.0	3.0	1	2	2	3	2	1.37
5	0.0	17.7	0	2	2	2	0	1.87
6	0.0	19.3	12	3	2	1	3	1.30
7	0.0	11.7	9	2	2	2	2	n.t.
8	0.0	16.7	0	3	3	1	3	1.56
9	0.1	0.7	0	2	1	1	1	1.48
10	0.0	8.3	9	2	2	1	2	1.36
11	0.0	4.0	6	3	3	1	2	1.26
12	0.0	19.7	9	2	1	2	3	1.99
13	0.1	14.3	12	3	3	1	3	2.13
14	0.2	16.7	1	0	1	2	3	1.24
15	0.0	1.0	0	3	1	3	3	1.75
16	0.2	3.3	4	3	2	1	3	1.03
17	0.0	12.0	6	3	2	1	2	1.60
18	0.0	24.0	0	2	2	2	2	2.49
Grade II								
19	0.0	24.0	6	1	0	2	2	n.t.
20	0.5	20.3	4	2	0	1	2	1.60
21	0.4	4.3	1	2	0	2	2	2.00
22	0.4	13.3	2	3	3	1	3	1.40
23	0.6	25.0	9	2	0	2	2	1.30
24	0.2	34.0	1	3	3	1	3	1.95
25	0.2	13.3	1	3	2	1	3	2.65
Grade III								
26	0.4	5.0	0	0	3	3	1	1.39
27	0.7	40.0	0	3	0	1	2	2.38
28	0.3	1.7	0	0	2	1	1	1.56
29	0.0	7.0	2	3	0	3	3	1.26
30	0.4	1.7	0	3	0	2	1	3.52
31	0.5	9.3	0	0	3	3	3	3.27
32	1.0	16.3	0	3	2	2	1	2.18
33	0.3	1.0	0	1	0	2	2	2.47
34	0.2	11.0	0	3	3	3	1	1.94
35	0.2	6.7	0	3	3	2	3	1.31
36	0.2	14.7	1	3	2	2	2	n.t.
37	0.2	25.0	1	3	0	3	2	1.63
Grade IV								
38	0.2	1.7	0	2	1	2	2	2.04
39	0.5	16.7	0	1	0	1	2	1.44
40	1.3	26.0	0	3	2	2	2	2.35
41	11.0	10.0	0	0	3	1	1	1.56
42	0.3	13.0	2	1	3	3	3	n.t.

*IRS= immunoreactive score (see materials and methods). n.t.= not tested.

Table 4. Comparison of 10 different parameters by linear regression analysis.

	grade	Mi %	PCNA LI %	PgR IRS	EGF-R LI	c-erbB-2 LI	CD LI	gg-E LI	AgNOR counts
Grade	1	0.64	0.14	-0.49	-0.33	-0.17	0.16	-0.18	0.39
MI %	0.64	1	0.38	-0.32	-0.06	-0.16	0.06	-0.12	0.35
PCNA LI %	0.14	0.38	1	0.05	0.15	-0.02	-0.15	0.16	0.08
PgR IRS	-0.49	-0.32	0.05	1	0.20	0.14	-0.26	0.28	-0.31
EGF-R LI	-0.33	-0.06	0.15	0.20	1	0.01	-0.22	0.19	0.03
c-erbB-2 LI	-0.17	-0.16	-0.02	0.14	0.01	1	-0.10	0.05	0.01
CD LI	0.16	0.06	-0.15	-0.26	-0.22	-0.10	1	-0.16	0.14
gg-E LI	-0.18	-0.12	0.16	0.28	0.19	0.05	-0.16	1	0.13
AgNOR counts	0.39	0.35	0.08	-0.28	0.03	0.01	0.14	0.13	1
Dexa. Days	-0.05	-0.29	-0,46	0.11	-0.36	0.10	-0.12	-0.03	-0.16

LI= labelling index; IRS= immunoreactive score; Dexa. Days= preoperative dexamethasone therapy; AgNOR counts= mean number of AgNOR/nucleus.

proportion of 2:1; the solution having been prepared immediately before use. After this process, sections were washed thoroughly in distilled water, dehydrated, cleared in tetrachlor-ethylene and mounted onto slides (for methodological details see Korek et al., 1991; Martin et al., 1991). Tissue sections were scanned by light microscopy using a x100 oil magnification. Measurement was performed on a BVS A 6471 image processing system (Robotron, Berlin, FRG). Nuclei were scanned automatically with interactive control (for details see Martin et al., 1991, 1992). AgNORs were counted in 100 cells of each specimen. The mean number of AgNORs per nucleus was calculated for each case.

Statistical analysis

The correlation coefficient r was estimated by a linear correlation analysis (see Table 4). PCNA expression in both treatment groups was compared using the Wilcoxon-Whitney-Mann test.

Results

The results of MI, PCNA LI, PgR IRS, EGF-R LI, c-erbB-2 LI, cathepsin-D LI, gg-E LI and AgNOR counts are given in Table 3.

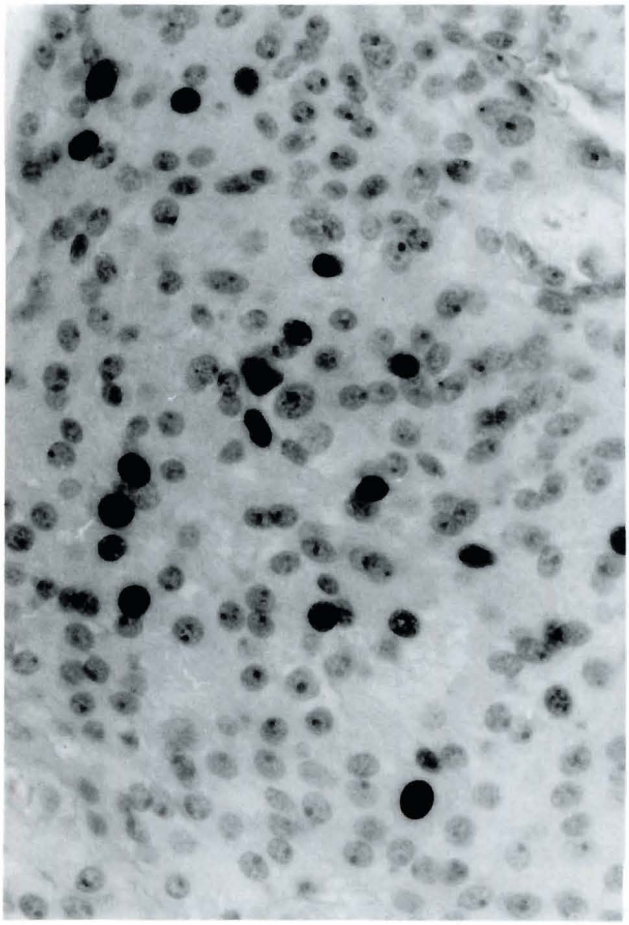

Fig. 1. PCNA nuclear staining of a benign meningioma. APAAP technique. x 400

PCNA

Positive immunoreaction was restricted to the cell nuclei (Fig. 1). The staining pattern differed between various tumor regions; there were focal centres of pronounced labelling, though a correlation to distinct histological structures could not be detected. The percentage of stained nuclei ranged from 0.1% in simple meningiomas to 40% in a malignant meningioma.

Progesterone receptor

Only cell nuclei were labelled whereas the cytoplasm always remained negative (Fig. 2). No background staining was observed. As staining intensity varied, semiquantitative evaluation could be performed. Meningioma slides were adequately comparable to control slides of the breast carcinoma. Tumors with an IRS of 0 were detected among benign and malignant meningiomas, while an IRS score greater than 2 only occured in grade I and II meningiomas. Distribution of labelled nuclei was not associated with characteristic histological structures.

EGF-R

Membranous staining of the cytoplasm was the predominant feature, whereas endothelial cells of the vessels remained negative. Distribution and staining intensity varied from tumor to tumor, though there was no distinct correlation to histological structures or grade of malignancy. Background staining was never found.

c-erbB-2

At first sight, immunohistochemical results closely resembled those of EGF-R, but direct comparison disclosed that distribution was not alike. No background staining was found.

Cathepsin D

Within the cytoplasm fine granular marks were detected; these were investigated separately with the monoclonal antibody KP1 (CD 68) to outrate infiltrates

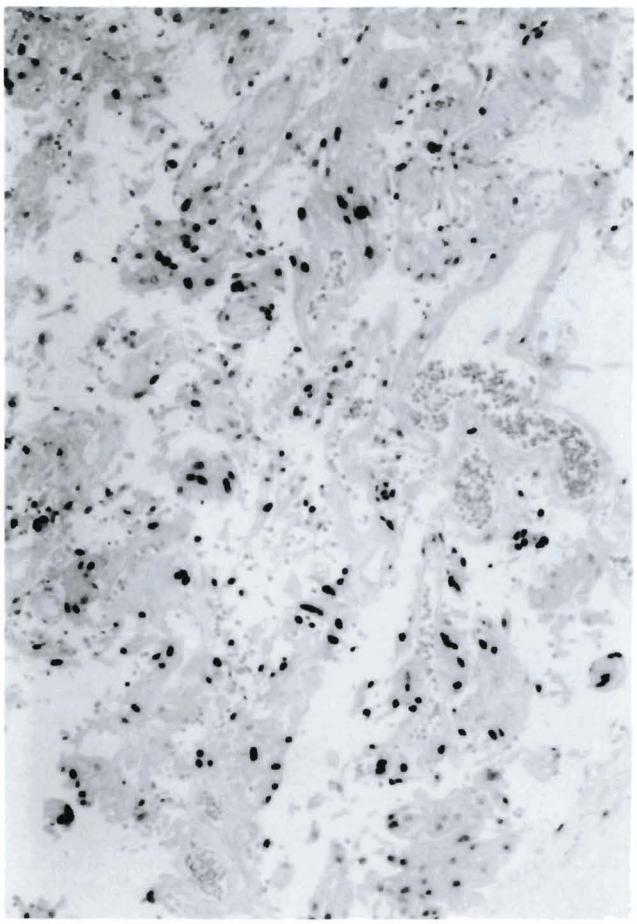

Fig. 2. Immunostaining of progesterone receptors in nuclei of an angiomatous meningioma. Modified APAAP technique. x 200

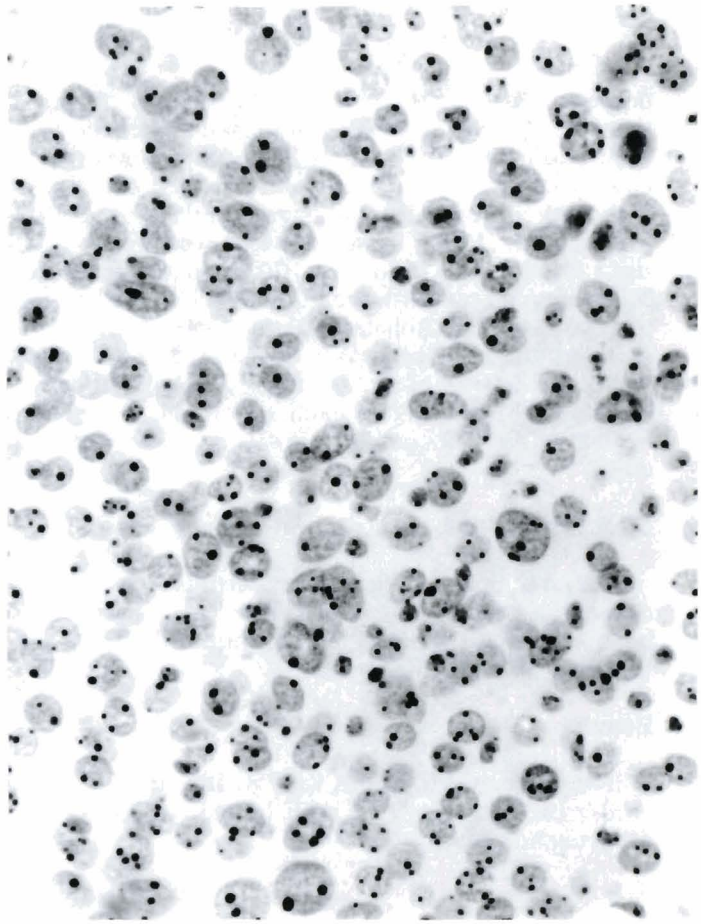

Fig. 3. Nucleolar argyrophilic bodies (AgNORs) in nuclei of malignant meningioma cells. AgNOR method. x 473

of macrophages. Only tumor cells were recorded.

Gamma-gamma-Enolase

In most cases there was a focal pattern of distribution, though sometimes the size of positively-stained areas was extensive. These positive cell clusters were often associated with whirls, fissures, areas of loosely arranged cells or areas where cells tended to be in parallel arrays. There was no clear cut distinction between meningiomas of different grades of malignancy.

AgNOR counts

AgNORs appeared as black spots and were found either within karyoplasm or within nuclei, sometimes constituting aggregates, which complicated identification of individual AgNORs (Fig. 3). Apart from 2 exceptions, all simple meningiomas had scores below 2, whereas atypical and malignant meningiomas scored markedly higher.

Correlations

Correlations between preoperative dexamethasone therapy, PCNA LI and the other parameters with listing of linear regression coefficients are given in table 4.

Days of preoperative dexamethasone therapy (Dexa. Days)

The grade of malignancy and the days of preoperative dexamathosone therapy did not correlate. The linear regression coefficient, r=-0.05, confirmed the lack of direct relationship. In contrast, comparison of duration of steroid medication to PCNA-expression hinted at a possible dependence, regardless of the grade of malignancy. Average labelling indices of PCNA were higher in those specimens that had been treated with glucocorticoids for less than 2 days. On the other hand, the average PCNA LI declined with increasing duration of treatment (r= -0.46). The difference of PCNA LI between group I (n=13) and II (n=29) was statistically significant ($p<0.05$) as shown by the Wilcoxon-test. It should be pointed out that indices above 20% were only observed in tumors after dexamethasone therapy for 2 or fewer days. The single tumor with a labelling index of 26% and 2 days glucocorticoid application was a malignant meningioma with foal sarcomatous components.

Reviewing the remaining parameters, only EGF-R is worth mentioning as it was weakly correlated to length of treatment (r= -0.36).

Grade of malignancy and mitotic index

As anticipated, grade of malignancy and mitotic index were highly correlated (r=0.64). Furthermore, a negative correlation between grade of malignancy and progesterone receptor status (r=-0.49) could be demonstrated. Table 3 visualizes that while malignancy increased, IRS decreased. AgNOR counts (r=0.39) and EGF-R (r=0.33) were the only other parameters which were weakly correlated to the grade of malignancy. Mitotic index, PCNA LI (r=0.38), AgNOR counts (r=0.35) and PgR IRS (r=-0.32) showed a weak correlation.

Relationship between the other parameters

Besides the above-mentioned results, there was no further relevant relation demonstrable, all correlation coefficients being below r=0.32.

Discussion

Appropriate and effective treatment of brain edema caused by brain tumors includes glucocorticoid therapy. In an attempt to explain the physiological basis of this finding, Yu et al. (1981) measured the content of glucocorticoid receptors in the cytosol of cells of various brain tumors. Meningiomas had the most pronounced receptor density within primary intracranial tumors, regardless of their grade of malignancy. A comparison of receptor concentration and clinical response to glucocorticoid therapy revealed a striking correlation.

PCNA is expressed as part of the DNA-synthesizing machinery at the G1/S boundary of the cell cycle (Baserga et al., 1988). Expression of PCNA increases during the G1-phase, reaches a climax in S-phase and declines in G2/M-phase (Kurki et al., 1988). Analysis of nuclear staining, most likely associated with increased proliferation, with the monoclonal antibody to 19A2 to PCNA in cultured breast cancer cells revealed two distinct patterns within the S-phase, allowing further subdivision of the cell cycle (van Dierendonck et al., 1991). According to Kurki et al. (1987) dexamethasone restricts the approach of cells into the G1-phase of the cell cycle. By using a polyclonal antibody to PCNA, the authors demonstrated an inhibition of PCNA-expression in stimulated T-lymphocyte cell cultures by dexamethasone (Kurki et al., 1987). This finding may in part be explained by our observation of a correlation between PCNA staining and preoperative dexamethasone therapy rather than PCNA-staining and malignancy. Taking into account that a fairly high degree of correspondence between the expression of PCNA and the proliferating fraction of cells may exist, the observed labelling index might have been obscured by selective inhibition of PCNA expression by dexamethasone. If the potential cross-reactivity of preoperative dexamethasone therapy could be avoided, a positive correlation between PCNA labelling index and the degree of malignancy is not entirely theoretical. Likewise, cyclosporin, an agent known to interfere with the G1-phase of the cell cycle as well, inhibited PCNA-expression in stimulated T-lymphocyte cultures (Kurki et al., 1987). The authors also found that PCNA-expression in cells incubated with

hydroxyurea and cytarabine (ara-C), substances which affect the S-phase and prevent DNA synthesis, was not affected. Lambert and Borek (1988) studied the effect of X-rays and bleomycin, and alkylating agent, on normal rat cell lines by using protein analytical techniques and found a striking suppression of PCNA-contents. In contrast, methotrexate and tamoxifen, two drugs that are known to reduce the growth fraction of proliferating cells dramatically, did not influence PCNA-staining in cell cultures of human breast cancer (van Dierendonck et al., 1991). Growth factors, platelet-derived growth factor (PDGF) and epidermal growth factor (EGF), are other regulators of PCNA-expression in cell cultures (Jakulski et al., 1988). Though the majority of information has been gained by in vitro experiments, these and further observations indicate an intricate relationship between pharmaceutical agents and PCNA expression.

The preponderance of women among patients with meningiomas (in our study group: 28F vs 14M) could be the consequence of a possible influence of endogenous steroids on meningioma development. Immunohistochemical studies of sex steroid receptors in meningiomas found conflicting results. There is considerable agreement that estrogen receptors are virtually absent in meningioma cells (Halper et al., 1989; Schrell et al., 1990). Progesterone receptors, however, were encountered by Halper et al. (1989) in 90% of the meningioma cells by immunohistochemistry. Nuclear binding, an indicator of functional activity, was observed in 60% of the cells (57% in our study). These results contradict the findings of Schrell et al. (1990) who identified progesterone receptors in the nuclear compartment of only 10% of their meningioma cells. It is noteworthy that the contents of progesterone receptors declined with increasing malignancy in our material. The biological significance of these receptors remains doubtful. In vitro experiments with meningioma cells demonstrated either an unchanged growth pattern (Adams et al., 1990) or marked suppression (Maiuri et al., 1989) after progesterone application, while preoperative dexamethasone therapy does not seem to influence the growing fraction at all.

Our findings with monoclonal anti-EGF-R antibody on paraffin-embedded sections confirm previous studies on frozen material which did not find a significant relation between the pattern of distribution of EGF-R and the grade of malignancy in meningiomas (Jones et al., 1990). Preoperative dexamethasone therapy might alter EGF-R pattern, though our material does not justify a definite statement (r=-0.36). Our data suggest no impact of preoperative dexamethasone therapy on mitotic index, AgNOR counts, c-erbB-2 oncoprotein, cathepsin D and gg-E.

In the light of the complexities associated with dexamethasone treatment, the interpretation of labelling indices of proliferation markers such as PCNA or Ki-67 to assess the degree of malignancy in neoplasms should be handled with great care. Equally important or more so, data determined in brain tumors (high frequency of preoperative glucocorticoid therapy), haematopoietic malignancies (previous cytotoxic therapy) or chronic inflammatory processes (prolonged steroid treatment) and therefore susceptible to cross-reactions. Another relevant fact to be considered is the evaluation of continued medical treatment for coexisting diseases. In neuroepithelial tumors, a significant stimulation of the cell growth was observed in glucocorticoid receptor-positive cultures when dexamethasone was added to the culture (Paoletti et al., 1990) in doses ranging from 0.016 to 2 µg/ml, while receptor-negative cultures showed no modulation of growth index at the same dosage. However, increased dexamethasone doses induced a significant decrease in the growth fraction independent of glucocorticoid receptor status. Further research is necessary to elucidate these interactions, so that the connotation of proliferation markers can be correctly assessed in routine examination of the proliferative potential of individual neoplasms.

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