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Epidermal growth factor receptor expression and growth fraction in human tumours of the nervous system*

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Summary. 100 tumours of the human nervous system were investigated by means of immunohistochemistry in order to determine the expression of epidermal growth factor receptor (EGFr) and the proliferative activity as evaluated by demonstration of the proliferation-associated Ki-67 antigen. Epidermal growth factor receptor immunoreactivity was present in 79% (23/29) of the high-grade malignant gliomas examined but in only 9% (2/22) of the low-grade gliomas. Besides the gliomas, EGFr-expression was detectable in smaller amounts in most (13/15) meningiomas, in one anaplastic neurinoma and in individual tumour cells of one medulloblastoma. In addition, EGFr-expression was found in 50% (6/12) of metastatic carcinomas. Seven of eight medulloblastomas, two cerebral primitive neuroectodermal tumours (PNETs), three benign neurinomas, one ganglioneuroma, one metastatic intracerebral malignant melanoma, three spinal plasmocytomas and one immunocytoma showed no detectable EGFr-expression. Our results indicate that (1) the expression of EGFr in human tumours of the nervous system depends on the histological tumour type and (2) in the glioma group is related to the grade of malignancy. A close correlation between EGFr-expression and proliferative activity as evaluated by Ki-67 staining could not, however, be established.

Key words: Brain neoplasms – Growth fraction – Ki-67 – Epidermal growth factor receptor – Immunohistochemistry

Introduction

Epidermal growth factor receptor (EGFr) is a transmembrane glycoprotein of about 170 kD molecular weight with close homology to the v-erb-B oncogene product (Downward 1984) and with binding specifity for epidermal growth factor (EGF) (Adamson and Rees 1981), transforming growth factor alpha (Massague 1983; Pike et al. 1982), and virus vaccinia growth factor (Stroobant et al. 1985). EGFr is present in human tissues derived from all germ layers showing its major expression in the proliferating cell compartments of skin, breast, and gastrointestinal and genitourinary tract epithelia (Damjanov et al. 1986; Green et al. 1983; Gusterson et al. 1984). Numerous investigators have reported the expression of EGFr in human carcinomas, especially of the squamous cell type originating in the upper respiratory tract (Eisbruch et al. 1987), lung (Berger et al. 1987a; Veale et al. 1987; Haeder et al. 1988), breast (Sainsbury et al. 1985; Wrba et al. 1988), gastrointestinal tract (Yasui et al. 1988) and genitourinary tract (Gullick et al. 1986; Berger et al. 1987b). In addition, biochemical studies have demonstrated the presence of EGFr in human meningiomas (Weismann et al. 1987; Westphal and Herrman 1986; Liberman et al. 1984) and anaplastic gliomas (Liberman et al. 1984). In the latter tumour group, EGFr gene amplification and possible rearrangement have been demonstrated in a number of instances (Liberman et al. 1985; Wong et al. 1987) and evidence has been accumulated suggesting a relation between alterations of chromosome 7 and EGFr overexpression (Bell et al. 1986; Henn et al. 1986).

In order to determine the expression of EGFr in a variety of human tumours of the nervous system, we examined a large series of different benign and malignant neoplasms by means of immunohistochemistry, using a specific monoclonal antibody against EGFr. All tumours were also stained for the proliferation-associated antigen Ki-67 to determine a possible relationship between growth fraction and EGFr-expression. Thus, the major objec-

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Table 1. EGFr immunoreactivity and Ki-67 labeling index (LI) in tumours of the human nervous system

Diagnosis and grading	No.	EGFr-staining intensity					Ki-67-LI (%)		
		0	1	2	3	4	$\bar{x}$	SD	range
Astrocytoma, pilocytic (I)	3	3	–	–	–	–	<1	<1	0 –<1
Recurrent astrocytoma, pilocytic (I)	1	1	–	–	–	–	<1	–	–
Astrocytoma (II)	10	8	–	2	–	–	0.5	0.7	0 – 1.9
Recurrent astrocytoma, gemistocytic (II)	2	2	–	–	–	–	2.5	2.5	0 – 5.0
Astrocytoma, anaplastic (III)	5	1	–	–	2	2	8.6	7.3	2.0– 21.2
Recurrent astrocytoma, anaplastic (III)	1	–	–	–	–	1	10.0	–	–
Recurrent astrocytoma, anaplastic (IV)	2	–	1	1	–	–	48.5	4.5	44.0– 53.0
Oligodendroglioma (II)	2	2	–	–	–	–	2.6	0.1	2.5– 2.7
Oligodendroglioma, anaplastic (III)	1	–	–	–	–	1	11.0	–	–
Recurrent oligodendroglioma, anaplastic (III)	4	1	–	–	2	1	12.8	3.1	10.4– 18.0
Mixed glioma (II)	1	1	–	–	–	–	3.3	–	–
Mixed glioma, anaplastic (III)	6	1	–	2	–	3	16.0	8.2	5.0– 29.0
Ependymoma (II)	3	3	–	–	–	–	1.3	1.4	0 – 3.2
Glioblastoma (IV)	8	2	1	1	4	–	6.5	7.1	<1 – 22.1
Recurrent glioblastoma (IV)	2	1	–	–	1	–	2.0	2.0	<1 – 4.0
Medulloblastoma (IV)	8	7	1	–	–	–	14.5	11.3	5.0– 36.4
Primitive neuroectodermal tumor (IV)	2	2	–	–	–	–	13.4(x)	–	–
Meningioma (I)	14	2	1	7	4	–	1.0	1.1	0 – 3.8
Recurrent meningioma (III)	1	–	–	1	–	–	10.0	–	–
Neurinoma (I)	3	3	–	–	–	–	<1	<1	0 –<1
Neurinoma, anaplastic (III)	1	0	–	–	–	1	22.0	–	–
Ganglioneuroma (I)	1	1	–	–	–	–	0	–	–
Ganglioneuroblastoma (III)	1	1	–	–	–	–	7.7	–	–
Metastatic carcinoma	12	6	–	–	5	1	14.9	15.7	<1 – 46.0
Malignant melanoma	1	1	–	–	–	–	<1	–	–
Immunocytoma (*)	1	1	–	–	–	–	n.d.	–	–
Plasmocytoma (*)	3	3	–	–	–	–	7.7	7.4	0.9– 18.0
Eosinophilic granuloma	1	1	–	–	–	–	<1	–	–

The fraction of EGFr-positive tumour cells in each tumour was estimated on a rating scale ranging from 0 = no positive cell, 1 = single positive cells, 2 = moderate fraction of positive cells (< 50%), 3 = high fraction of positive cells (> 50%), 4 = very high fraction of positive cells (> 90%). Ki-67 staining is expressed in the percentage of positive cell nuclei given as mean value ($\bar{x}$) with standard deviation (SD, n-weighted) and range (minimal–maximal value). Grade I–IV refers to WHO-grade of malignancy

(*) = spinal
(x) = only one tumour evaluated
n.d. = not determined

tive of this study was to find out whether EGFr-expression in human nervous system tumours is correlated with specific types or with the degree of malignancy assessed by conventional methods according to the WHO classification (Zülch 1979), and whether this expression, together with determination of the Ki-67 growth fraction, might be of relevance for tumour diagnosis and tumour grading in neuro-oncology.

Materials and methods

100 tumours of the human nervous system were investigated (Table 1). All were classified using the criteria of the WHO classification of tumours of the central nervous system (Zülch 1979). Diagnoses were made on the basis of formalin-fixed paraffin-sections stained by conventional methods comprising haematoxylin-eosin, cresyl-violet, Masson's trichrome stain, and silver impregnation according to Tibor-Pap. In addition, diagnoses of individual tumours were confirmed by immunohistochemical staining using antibodies against differentiation antigens such as intermediate filaments and other neuronal- and glial-associated antigens on frozen and paraffin sections (see Kleihues et al. 1987; Perentes and Rubinstein 1987; Reifenberger et al. 1987 for reviews). The immunoreactivity for EGFr in normal human brain tissue was tested using frozen sections from various regions of two neuropathologically normal autopsy brains shock-frozen at a postmortem interval of less than 12 h.

The mouse monoclonal IgG2b-antibody EGFR1 was produced by immunization of balb/c-mice with trypsinized A431-cells and immunoprecipitates an approximately 170 kD-protein from lysates of A431-cells (Waterfield et al. 1982). EGFR1 recognizes an antigenic determinant located on the polypeptide chain of the extracellular receptor domain (Mayes and Waterfield 1984). The antibody does not compete with EGF for the ligand binding site (Gullick et al. 1984). EGFR1 (obtained from Amersham Buchler, Braunschweig, FRG) was used in a concentration of 2 µg/ml for 12 h at room temperature. The mouse monoclonal IgG1-antibody Ki-67 was produced by immuniza-

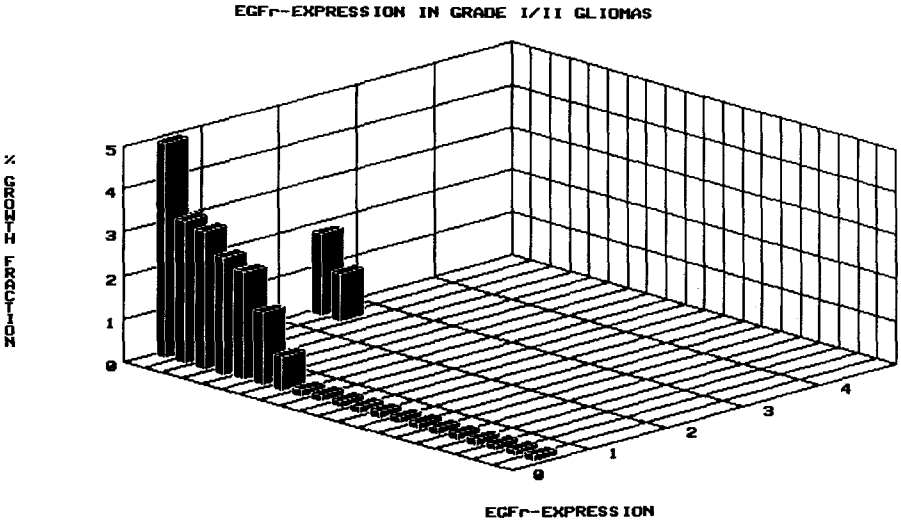

Fig. 1. Schematic representation of EGFr-expression in human gliomas of WHO-grade I and II. Immunoreactivity for EGFr was graded semiquantitatively on a rating scale ranging from 0 = no positive cell, 1 = single positive cells, 2 = moderate fraction of positive cells (< 50%), 3 = high fraction of positive cells (> 50%), 4 = very high fraction of positive cells (> 90%). Tumour growth fraction is given in % of Ki-67 positive nuclei. Each *bar* represents one tumour. Only 2 of 22 WHO-grade I and II gliomas demonstrated moderate EGFr-expression. Tumour growth fractions in this tumor group were generally low with values of less than 5%

tion of balb/c-mice with a crude nuclear fraction of L428-cells (Gerdes et al. 1983). In human cells Ki-67 recognizes a nuclear antigen present in all proliferating cells, but absent in resting (G_0) cells (Gerdes et al. 1984). We used the Ki-67 antibody (obtained from Dakopatts, Hamburg, FRG) diluted 1:20 for 12 h at room temperature.

Since EGFR1 and Ki-67 do not work on formalin-fixed paraffin-sections, immunohistochemical staining was performed on frozen sections using the avidin-biotin-peroxidase method according to Hsu et al. (1981). Tissue samples from surgically removed brain tumours were divided, one part being fixed in 4% buffered formalin for routine tumour diagnosis, the other being shock-frozen in isopentane at −150° C for 30 s. The frozen samples were sectioned at 5 µm on a cryostat microtome, mounted on poly-1-lysine coated glass slides and air-dried for 1 h. The sections were then fixed in acetone at −20° C for 10 min and dried again. Endogenous peroxidase activity was blocked by 0.3% H_2O_2 in phosphate-buffered saline (PBS) for 20 min. To reduce nonspecific binding the sections were preincubated for 1 h in PBS containing 5% bovine serum albumin and 10% normal horse serum. Thereafter they were incubated with the primary antibody diluted in PBS plus 5% bovine serum albumin. This incubation was followed by biotinylated horse anti-mouse IgG antibodies (Vectastain, via Camon, Wiesbaden, FRG) for 30 min and avidin-biotin peroxidase complex (Vectastain) for 45 min. We used 0.05% 3,3-diaminobenzidine-tetrahydrochloride (Sigma, Deisenhofen, FRG) plus 0.01% hydrogen peroxide in PBS as chromogen for the peroxidase reaction. After each step the sections were gently rinsed in PBS. All incubations were performed at room temperature. All sections were counterstained with haematoxylin. Negative controls were performed by omitting the primary antibody or by applying an irrelevant mouse monoclonal IgG-antibody instead of the primary antibody.

The Ki-67 labelling indices were determined as described by Deckert et al. (1988) by counting about 200 to 4000 cells in five representative tumour areas at a magnification of 500 ×.

Results

We found no immunoreactivity for EGFr or Ki-67 in the cerebral cortex and white matter, the hippocampus, the basal ganglia, the hypothalamus, the midbrain or the cerebellum of neuropathologically normal adult human brain. Reactive astrocytes forming the fibrillary gliosis in the tumour periphery and within the peritumour oedematous zones of all the intracerebral neoplasms were also consistently negative with EGFR1.

Growth fractions and intensity of EGFr-expression in the tumours investigated are summarized in Table 1. Among 22 low-grade gliomas, comprising 16 astrocytomas (grade I and II), 2 oligodendrogliomas (grade II), 1 mixed glioma (grade II), and 3 ependymomas (grade II) only two grade II astrocytomas showed detectable amounts of EGFr (Fig. 1). In most tumours of this group immunostaining for Ki-67 was absent or less than 1%. Only the oligodendrogliomas, one mixed glioma, one ependymoma and one recurrent gemistocytic astrocytoma showed slightly higher values reaching a maximum of 5% Ki-67-positive cell nuclei. However, the majority (23/29) of grade III or IV gliomas, comprising 8 anaplastic astrocytomas (grade III and IV), 5 anaplastic oligodendrogliomas (grade III), 6 anaplastic mixed gliomas (grade III), and 10 glioblastomas (grade IV) showed intense immunoreactivity for EGFr (Fig. 2). Immunoreactivity appeared as a fine granular reaction product located on the cell surface as well as in the cytoplasm of the tumour cells (Fig. 3a, b, c, e). In most cases, the distribution of immunostained tumour cells appeared diffuse and homogeneous in intensity (Fig. 3a), although we observed individual cases which demonstrated varying intensity in EGFr-expression by the cells (Fig. 3b, c, e). Intratumour blood vessels, vascular proliferations and areas of necrosis were always negative for EGFr (Fig. 3a, c). EGFr-positive gliomas frequently showed high cellularity due to the

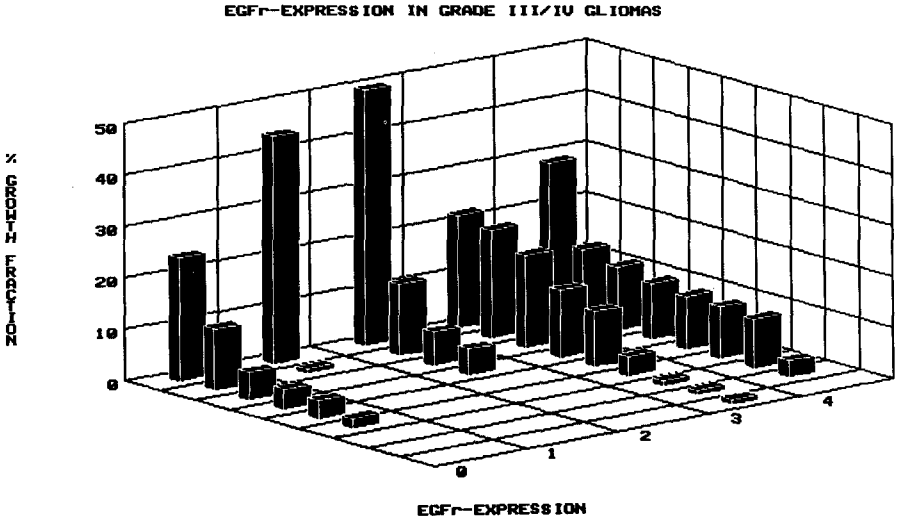

Fig. 2. Schematic representation of EGFr-expression in human gliomas of WHO-grade III and IV. Immunoreactivity for EGFr was semiquantitatively graded on a rating scale ranging from 0 = no positive cell, 1 = single positive cells, 2 = moderate fraction of positive cells (< 50%), 3 = high fraction of positive cells (> 50%), 4 = very high fraction of positive cells (> 90%). Tumour growth fraction is given in % of Ki-67 positive nuclei. Each *bar* represents one tumour. The majority of tumours showed moderate to strong immunoreactivity for EGFr. There is, however, no close correlation between tumour growth fraction and intensity of EGFr-staining

presence of many poorly differentiated so-called small anaplastic glioma cells. These anaplastic glioma cells also showed a particularly high labelling index for the Ki-67 antigen, pointing to high proliferative activity (Fig. 3d). In contrast, multinucleated giant cells in glioblastomas and anaplastic gliomas demonstrated heterogeneous immunoreactivity for both EGFr and Ki-67 (Fig. 3e, f).

There was no close correlation between EGFr-expression and a high Ki-67 staining index since, particularly in the group of glioblastomas, there were individual tumours with low proliferative indices but strong EGFr-immunostaining in contrast to other glioblastomas and anaplastic gliomas presenting with a high percentage of Ki-67 positive cells and negative or only slight EGFr-immunostaining (Fig. 2).

Seven of eight medulloblastomas and two primitive neuroectodermal tumours were negative for EGFr. Only one desmoplastic medulloblastoma demonstrated weak EGFr-immunoreactivity which was restricted to tumour areas with glial cell differentation as indicated by positive immunostaining for glial fibrillary acidic protein. Ki-67 labelling indices in medulloblastomas were generally high ranging from 5% to near 40%. Among 14 benign meningiomas (10 of the endotheliomatous and 4 of the endotheliomatous-fibromatous type) and one recurrent malignant meningioma only two tumours showed no immunoreactivity for EGFr. All other meningiomas presented with moderate EGFr-staining (Fig. 4c). There was no obvious difference between the histological subtypes, although staining of fibrous areas was slightly weaker. In accordance with their benign nature, proliferative activity in the meningiomas was generally low (0 to 3.8%). Only the recurrent malignant meningioma had a Ki-67 labelling index of 10%, while EGFr-expression was also moderate. As in the glioma group, no obvious positive correlation could be established between tumour growth fraction and degree of EGFr-expression in meningiomas. Three benign neurinomas and one ganglioneuroma were found to be negative for Ki-67 and EGFr, whereas one anaplastic neurinoma with a labelling index of 22% showed generalized strong EGFr-expression (Fig. 4a, b). One ganglioneuroblastoma of the adrenal medulla demonstrated a proliferation index of 7.7% while EGFr was not detectable. Six of twelve intracerebral and spinal metastatic carcinomas presented with strong EGFr-immunoreactivity (Fig. 4d). In contrast, three undifferentiated metastases, one small cell carcinoma, one adenocarcinoma and one metastatic thyroid carcinoma of the follicular type were EGFr-negative. Ki-67 indices in the metastatic carcinoma group ranged from less than 1% to 46% with no apparent correlation to the degree of EGFr-expression. One intracerebral metastatic malignant melanoma, three vertebral plasmocytomas, one spinal epidural immunocytoma and one eosinophilic granuloma of the skull were EGFr-negative.

Discussion

Since the first description 25 years ago (Cohen 1962), epidermal growth factor (EGF) has been extensively investigated and many effects of EGF on cultured cells including cytoskeletal changes (Schlessinger and Geiger 1981), protein synthesis (Nilsen-Hamilton and Holley 1983), induction of

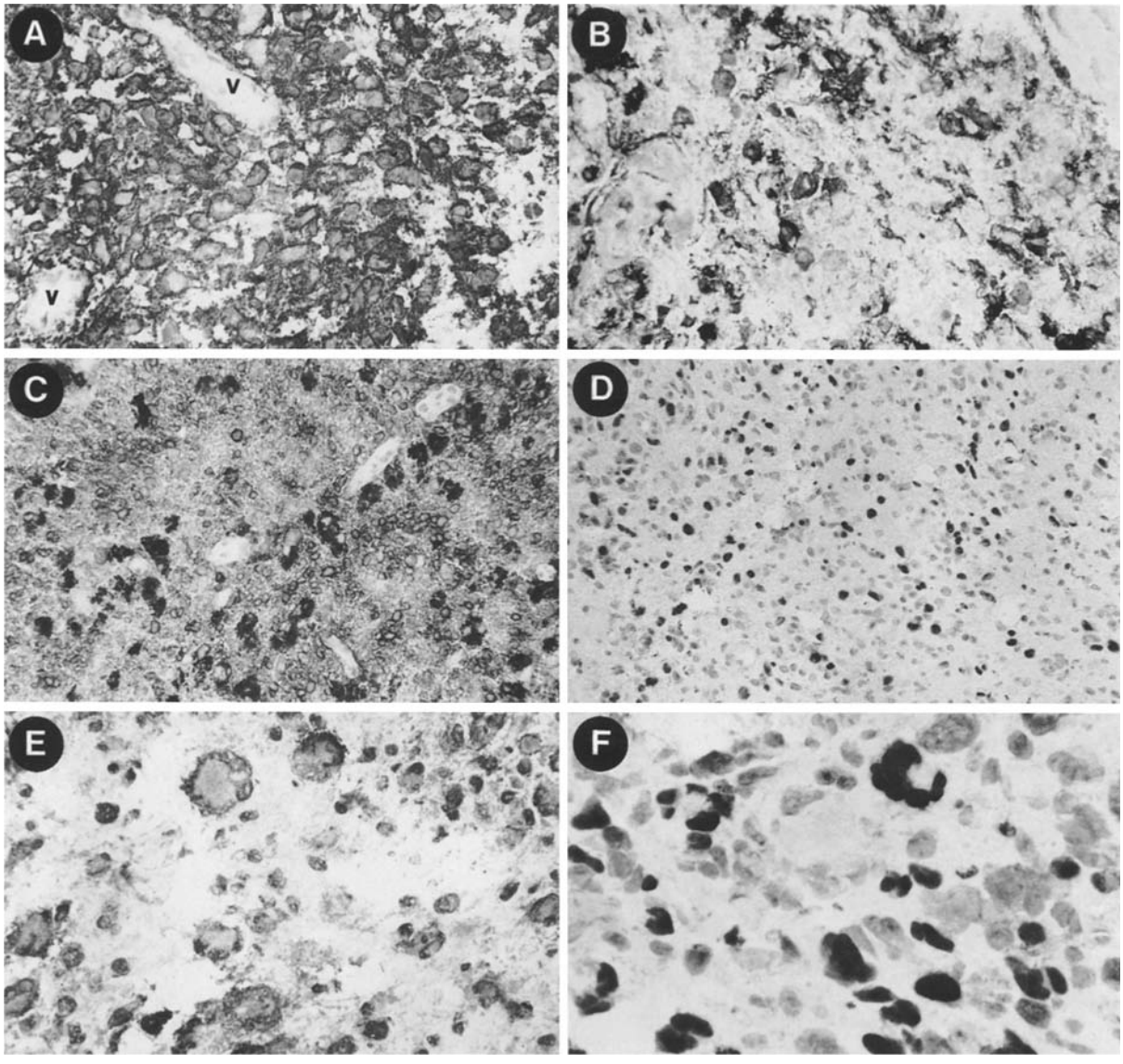

Fig. 3. (**A–B**) Anaplastic mixed glioma (WHO-grade III). (**A**) Marked expression of granular EGFr immunoreactivity in all tumour cells. Note completely unstained tumour vessels (v). (**B**) Partially necrotic area in the same tumour with residual vital cells stained strongly for EGFr. NP 33/88, 400 ×. (**C–F**) Glioblastoma (WHO-grade IV). (**C**) Tumour area with heterogeneous EGFr-immunoreactivity of cells. (**D**) The same area stained for Ki-67 demonstrating high proliferative activity. The average labelling index of this tumour was 22%. (**E**) Larger magnification of a tumour area with large multinucleated giant cells exhibiting variable EGFr staining and (**F**) Ki-67 immunoreactivity. NP 741/87, 150 × (C, D); 400 × (E, F). All sections slightly counterstained with haematoxylin

c-fos and c-myc proto-oncogenes (Müller et al. 1984; Bravo et al. 1983) and stimulation of cell proliferation (Carpenter and Cohen 1975; Westermark 1976) have been reported. All cellular effects of EGF are probably mediated by the well characterized EGF receptor (EGFr). This approximately 170-kD transmembrane glycoprotein consists of an extracellular domain with binding capacity for EGF (Adamson and Rees 1981) transforming growth factor alpha (Massague 1983; Pike et al. 1982) and virus vaccinia growth factor (Stroobant et al. 1985), a transmembrane region, and an intracellular portion with tyrosine kinase activity (Ushiro and Cohen 1980) and close homology to the erb-B oncogene product of the avian erythroblastosis virus (Downward 1984). Binding of the ligand to the extracellular domain of EGFr is followed by stimulation of the intrinsic receptor tyrosine kinase which, by phosphorylation of specific substrates, initiates a complex and poorly understood

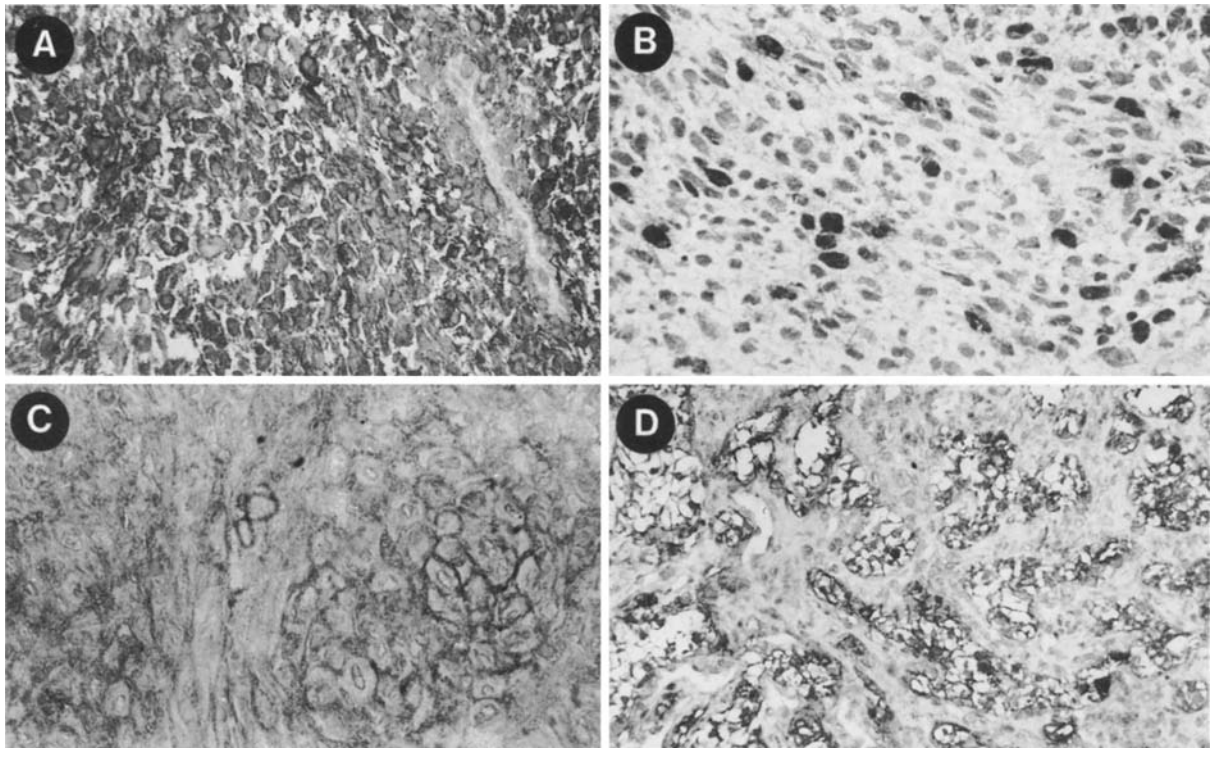

Fig. 4. (**A–B**) Malignant neurinoma (WHO-grade III). (**A**) Strong generalized expression of EGFr immunoreactivity in the tumour cells. (**B**) The same tumour demonstrating high proliferative activity with a Ki-67 labelling index of 22%. NP 344/88, 400×. (**C**) Meningioma, endotheliomatous (WHO-grade I). Tumour area with relatively strong immunoreactivity for EGFr. NP 721/87, 400×. (**D**) Intracerebral metastatic hypernephroma. EGFr-immunoreactivity is prominent on the carcinoma cells whereas the mesenchymal stroma remains unstained. NP 24/88, 150×. All sections slightly counterstained with haematoxylin

cascade of events finally leading to the physiological changes already noted (Hunter 1984). Various investigators have described the expression of EGFr in human tumours of different histogenesis including carcinomas (Berger et al. 1987a; Berger et al. 1987b; Eisbruch et al. 1987; Gullick et al. 1986; Haeder et al. 1988; Sainsbury et al. 1985; Veale et al. 1987; Wrba et al. 1988; Yasui et al. 1988), meningiomas (Liberman et al. 1984; Weismann et al. 1987; Westphal and Herrmann 1986) and gliomas (Liberman et al. 1984, 1985; Wong et al. 1987; Bigner et al. 1988; Collins et al. 1988). This suggests involvement of EGFr-mediated growth regulation in the pathophysiological processes underlying malignancy.

In the present study we investigated a variety of primary and metastatic tumours of the human nervous system for the presence of immunohistochemically detectable EGF receptors. In the glioma group we observed a close association between EGFr-expression and WHO-grade of malignancy. Only 9% of low-grade (WHO-grade I and II) gliomas demonstrated moderate immunoreactivity, whereas 79% of the high-grade (WHO-grade III and IV) gliomas showed mostly strong staining for EGFr. Our results are in line with those reported by Liberman et al. (1984, 1985) who found amplification of the EGFr gene in 4 of 10 glioblastomas and high levels of EGFr in 12/15 samples from glioblastoma multiforme by biochemical and molecular biological analysis. In a more recent study, Bigner et al. (1988) demonstrated immunoreactivity for EGFr in 25 of 31 high-grade gliomas, among them 14 gliomas with amplification of the EGFr gene. Taken together, these data indicate that EGFr-expression is a common feature of human malignant gliomas, while according to our series the majority of low-grade gliomas appear to be EGFr-negative. EGFr-expression in the glioma group therefore seems to be correlated to anaplastic tumour growth as indicated by the WHO-grades. In high-grade gliomas there was, however, no close relation to proliferative activity as determined by the amount of Ki-67 immunostained tumour cells. We observed tumours with strong EGFr-immunoreactivity and low Ki-67 labeling indices and vice versa. We may speculate that those malignant gliomas with high

Ki-67 indices and low or absent EGFr-staining belong to a group of tumours demonstrating amplification of the cytoplasmic domain of EGFr only, as recently described by Malden et al. (1988). In this case we would have failed to demonstrate immunoreactivity because EGFR1 reacts with an epitope of the external receptor domain. However, another explanation might be that these gliomas have completely EGFr-independent mechanisms of growth promotion, via other growth factors or receptors such as PDGF and its receptor (Pantazis et al. 1985; Heldin et al. 1987; Nister et al. 1988). The occasional occurrence of highly EGFr-immunoreactive glioblastomas with low or absent Ki-67 staining could be explained by the expression of an altered non-functional EGF-receptor protein which is still recognized by the monoclonal antibody used (see also Steck and Yung 1987). However, sampling problems due to the pronounced regional heterogeneity of proliferative activity in glioblastomas might also contribute to these findings (Deckert et al. 1988). In addition to the glial neoplasms it has already been shown by various investigators that EGFr-expression is associated with advanced stages of tumour disease in carcinomas of the breast (Sainsbury et al. 1985), lung (Veale et al. 1987), gastrointestinal tract (Yasui et al. 1988) and urinary bladder (Berger et al. 1987a). Only Wrba et al. (1988) have found no correlation with proliferative activity or tumour stage in an immunohistochemical study of human breast carcinomas.

The crucial question of whether the overexpression of EGFr in human malignant gliomas is causally involved in malignant transformation or at least in tumor growth promotion remains debatable. Recent work by Velu et al. (1987) has provided evidence that increased numbers of EGF receptors can transform non-neoplastic cells directly in vitro. Furthermore, Samuels et al. (1987) were able to demonstrate large amounts of transforming growth factor alpha in high-grade gliomas, indicating a putative autocrine stimulatory mechanism in the evolution of malignant gliomas. Investigating the expression of messenger RNAs for transforming growth factor alpha and EGFr in human malignant glioma cell lines, Nister et al. (1988) found positive expression of both in the majority of cell lines studied. Since the same authors also demonstrated messenger RNAs for platelet-derived growth factor and its receptor, they suggested the existence of two possible autocrine loops in human malignant glioma lines. In addition, numerous cytogenetic studies have found that malignant human gliomas frequently have structural alterations or numerical aberrations of chromosome 7, the chromosome carrying the EGFr gene (Bell et al. 1986; Henn et al. 1986). Thus, evidence for the involvement of EGFr in the growth promotion of glial neoplasms is accumulating.

Apart from the interesting implications for tumor biology, our findings might also be of considerable value for histopathological tumour classification, in particular the problem of tumour grading of gliomas. Since most low-grade gliomas appear to lack immunoreactivity for EGFr while a large percentage of high-grade tumours react strongly, immunohistochemistry with the EGFR1 monoclonal antibody might well facilitate assessment of the grade of malignancy. Another interesting aspect of EGFr overexpression in malignant gliomas is the possibility of using EGFr as target for antibody-guided imaging and therapy (Epenetos et al. 1985; Takahashi et al. 1987). Clinically, the occurrence of high molecular weight growth factor resembling high molecular weight EGF in the urine of patients with malignant brain tumours might be of relevance as an additional diagnostic tool for the monitoring of such patients (Stromberg et al. 1987).

We also observed EGFr-like immunoreactivity in moderate amounts in most meningiomas. This immunohistochemical result is in line with previous biochemical studies reporting the presence of EGFr in meningioma cells in vitro (Westphal and Herrmann 1986) and in vivo (Liberman et al. 1984; Weisman et al. 1987). We found no association of EGFr expression in meningiomas with particular histological features, the WHO-grade of malignancy, or the Ki-67 growth fraction. The absence of immunohistochemical staining for EGFr in 3 benign neurinomas and the strong immunoreactivity observed in one case of malignant neurinoma parallels the results obtained in the glioma group. This finding should be substantiated by further investigations of a larger number of nerve sheath tumours. Despite their high degree of malignancy, medulloblastomas and primitive neuroectodermal tumours (PNETs) generally had no detectable amounts of EGFr. These results are consistent with those of Libermann et al. (1984, 1985) who reported only very low EGFr-levels in two central neuroblastomas and no EGFr gene amplification in one PNET. In our series only one desmoplastic medulloblastoma showed weak EGFr-expression which was restricted to tumour areas with glial differentiation as indicated by positive GFAP-immunostaining. In the group of metastatic carcinomas included in our series we found varying immunoreactivity for EGFr with no obvious correlation

to the type of carcinoma or the degree of anaplasia. There was no apparent relation between the Ki-67 growth fraction and EGFr-expression. However, the group of metastases investigated here is too small and heterogeneous to permit a conclusive statement.

In summary, the expression of EGFr in tumours of the human nervous system is heterogeneous with respect to tumor type. In the glioma group and probably also the nerve sheath tumour group, EGFr-expression appears to be related to the WHO-grade of malignancy. A convincing correlation to the proliferative activity as determined by Ki-67 immunostaining could not, however, be established. Further studies employing biochemical, molecular biological and cell culture techniques will be needed to elucidate the possible functional implications of EGFr-expression in human cerebral neoplasms. Immunohistochemical demonstration of EGFr with the monoclonal antibody used in the present study can, however, contribute to histopathological tumour classification as a useful aid for the grading of gliomas.

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