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C-*erb*B-2/HER-2 Protein in Human Intracranial Tumours

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Normal and neoplastic human intracranial tissues were examined by immunohistochemistry for c-*erb*B-2/HER-2 protein expression. Positive staining was observed in 1/41 gliomas, 1/2 medulloblastomas, 1/1 germinoma, 11/16 meningiomas, 1/3 anaplastic meningiomas and 11/19 metastatic brain carcinomas. No positive staining was observed in normal intracranial tissues. Thus, the expression of the c-*erb*B-2/HER-2 protein is limited to intracranial tumour tissues, principally meningiomas and metastatic carcinomas to the brain.
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INTRODUCTION

THE PROTOONCOGENE c-*erb*B-2/HER-2 coding for a 185 kDa transmembrane receptor protein with structural similarities to epidermal growth factor receptor and endowed with tyrosine kinase activity, is overexpressed and amplified in various human tumours, most commonly in breast tumours [1]. Recently, a ligand for this receptor has been described [2].

In human intracranial tumours the c-*erb*B-2/HER-2 protein has been detected in meningiomas [3,4] whilst in gliomas conflicting results have emerged [3–7]. This study is an attempt to clarify this question, and a variety of human intracranial tumours were examined immunohistochemically with an anti-c-*erb*B-2 monoclonal antibody to identify tumours expressing this protein.

MATERIALS AND METHODS

Tumour samples from 84 human intracranial tumours of various histological types were obtained during surgery at the Department of Neurosurgery, University Hospital, Trondheim, Norway in the period 1986–1992. Normal and non-neoplastic intracranial tissues (brain and meningeal tissues from various pathological conditions including haemorrhages, infection, radiation and tumour infiltration) were obtained during surgery at the Department of Neurosurgery or during autopsy at the Department of Pathology, University Hospital, Trondheim, Norway. The tissue samples were immediately put in liquid nitrogen and stored frozen until analysis. In the immunohistochemical analyses the NCL-CB11 anti-c-*erb*B-2 monoclonal antibody (Novocastra Lab. Ltd., Newcastle upon Tyne, U.K.) [8] and an avidin-biotin immunoperoxidase technique (Vectastain ABC kit, Vector Lab., Burlingame, California, U.S.A.) were used. Frozen sections were fixed in acetone and incubated overnight at 4°C with the primary antibody at a 1:40 dilution. Frozen cell pellets of the SK-BR3 breast cancer cell line served as positive controls in each experiment [8]. In the negative controls the primary antibody was omitted or an irrelevant mouse monoclonal antibody of the same isotype was used. The specificity of staining was tested by pre-incubation of the primary antibody with protein extract of SK-BR3 cells. The intensity of positive staining was estimated as negative, weak, moderate or strong. The proportion of positive staining tumour cells and the proportion with strong intensity were estimated as < 25, 25–50 and $> 50\%$. The kappa statistic was used to test the intraobserver reproducibility of the immunohistochemistry [9].

RESULTS

The staining results are summarised in Tables 1 and 2. Positive staining was localised to the plasma membrane and to cytoplasm. Membrane staining was most prominent in the metastatic adenocarcinomas whereas granular cytoplasmic staining was most common in the other tumours. The positive staining intensity varied from tumour to tumour and within the same tumour but was, in general, moderate. In the majority of positive tumours more than 50% of the tumour cells were

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Table 1. Summary of c-erbB-2/HER-2 oncoprotein expression in human intracranial tumours

Tumour	Number of positive specimens/total number of specimens
Glioblastomas	1/21
Anaplastic astrocytomas	0/7
Astrocytomas	0/6
Oligodendrogliomas	0/3
Mixed gliomas	0/3
Ependymoma	0/1
Gangliocytoma	0/1
Medulloblastomas	1/2
Germinoma	1/1
Meningiomas	11/16
Anaplastic meningiomas	1/3
Metastatic carcinomas	11/19
Squamous cell carcinomas (lung)	2/2
Adenocarcinomas (lung, colon, uterus, unknown origin)	8/10
Large cell anaplastic carcinoma (lung)	0/1
Malignant melanomas	0/4
Undifferentiated carcinomas (lung)	1/2
Primary cerebral lymphoma	0/1

Table 2. Survey of c-erbB-2/HER-2 positive human intracranial tumours

No.	Tumour	Sex/ Age*	Intensity	% positive tumour cells	% positive tumour cells with strong intensity
1	Glioblastoma	F/46	Weak	< 25	0
2	Medulloblastoma	F/5	Weak	> 50	0
3	Germinoma	F/15	Weak-moderate	> 50	0
4	Meningioma	F/63	Weak-strong	> 50	25–50
5	Meningioma	F/63	Weak-strong	> 50	25–50
6	Meningioma	M/77	Weak-strong	> 50	25–50
7	Meningioma	F/51	Weak-strong	> 50	> 50
8	Meningioma	F/62	Weak-strong	> 50	25–50
9	Meningioma	F/70	Weak	> 50	0
10	Meningioma	M/75	Weak-moderate	> 50	0
11	Meningioma	F/62	Weak	> 50	0
12	Meningioma	F/76	Weak	> 50	0
13	Meningioma	F/75	Weak	> 50	0
14	Meningioma	F/69	Weak-moderate	> 50	0
15	Anaplastic meningioma	M/67	Weak-moderate	> 50	0
16	Metastasis (squamous cell carcinoma—lung)	M/70	Weak-strong	> 50	25–50
17	Metastasis (squamous cell carcinoma—lung)	M/38	Weak-strong	> 50	25–50
18	Metastasis (adenocarcinoma—lung)	M/65	Weak-moderate	> 50	0
19	Metastasis (adenocarcinoma—lung)	M/70	Weak-moderate	> 50	0
20	Metastasis (adenocarcinoma—lung)	M/52	Weak-moderate	> 50	0
21	Metastasis (adenocarcinoma—lung)	F/75	Weak-moderate	> 50	0
22	Metastasis (undiff. carcinoma—lung)	M/72	Weak-moderate	> 50	0
23	Metastasis (adenocarcinoma—colon)	F/67	Weak-strong	> 50	> 50
24	Metastasis (adenocarcinoma—uterus)†	F/69	Weak-strong	> 50	> 50
25	Metastasis (adenocarcinoma—uterus)†	F/69	Weak-strong	> 50	< 25
26	Metastasis (adenocarcinoma—unknown origin)	M/62	Weak-strong	> 50	> 50

F = female, M = male. * Age in years at diagnosis. † Same patient, reoperated because of recurrence of tumour.

regarded as positive. Of 44 neuroepithelial tumours only one glioblastoma and one medulloblastoma displayed positive staining whereas the rest of the gliomas were negative. Eleven of 16 benign meningiomas and one of three anaplastic meningiomas were positive. Of the metastatic brain tumours the two squamous cell carcinomas from the lung were positive. Eight of 10 adenocarcinomas from the lung, colon, uterus and of unknown origin were positive whereas one renal and one colon carcinoma were negative. One of two metastases from undifferentiated lung carcinomas and none of the four metastases from malignant melanomas were negative. Normal brain cortex, reactive astrocytes and normal and reactive meninges did not express detectable levels of the c-*erb*-B2/HER-2 protein.

No positive staining was observed when the primary antibody was omitted, an irrelevant antibody used or when the primary antibody was pre-incubated with SK-BR3 cell protein extract.

Kappa gave a score of 0.89 (S.E. =0.11) indicating a high degree of intraobserver reproducibility [9].

DISCUSSION

This study has shown that c-*erb*-B2/HER-2 protein was expressed in a large number of human meningiomas and metastatic carcinomas to the brain and rarely in neuroepithelial tumours. No protein expression was observed in normal intracranial tissues.

The frequent overexpression of the c-*erb*-B2/HER-2 protooncogene in human meningiomas is in agreement with others [3, 4]. Thus, this protein may be involved in the oncogenesis of many meningiomas. No differences in staining patterns between benign and anaplastic forms were noted suggesting that c-*erb*-B2/HER-2 plays a minor role in the progression of meningiomas. The immunoreactivity in the meningiomas was confined to the cytoplasm which was in contrast to the membrane staining in the metastatic adenocarcinomas, suggesting different cellular localisation of this protein from one type of tumour to another. This protein overexpression is not due to gene amplification as many of these meningiomas have demonstrated normal gene dosage [10].

The large number of positive staining brain metastases correlates well with the common overexpression of the c-*erb*B-2/HER-2 protooncogene in carcinomas of the lung, colon and uterus [1,6,11,12]. However, the metastatic malignant mela-

nomas did not demonstrate any positive immunoreactivity which corroborates other studies of primary malignant melanomas [6, 12].

Only one glioma displayed detectable c-*erb*B-2/HER2 immunoreactivity, and this uncommon expression of c-*erb*B-2/HER2 protooncogene in human glioma tissues is in agreement with some [6,7] but not with other reports [3,5]. These discrepancies may be related to different methods, tissue processing and/or antibodies used. The low levels of c-*erb*B-2/HER-2 protein in the examined gliomas are in contrast to their abundant epidermal growth factor receptor expression shown earlier [13].

In conclusion, we have shown that immunohistological cellular staining for c-*erb*-B2/HER-2 protein was limited to neoplastic intracranial tissues, principally meningiomas and metastatic carcinomas.

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Feature Articles

The Use of Immunotoxins for Cancer Therapy

Lee H. Pai and Ira Pastan

INTRODUCTION

IMMUNOTOXINS ARE a new class of cytotoxic agents composed of bacterial or plant toxins coupled to monoclonal antibodies or growth factors [1, 2]. Toxins are highly active protein molecules that enzymatically inactivate protein synthesis, leading to cell death. When coupled to a ligand (monoclonal antibody or growth factor), the resulting molecule can specifically target and kill cells that present the specific antigen or growth factor receptor on their surface.

The progress made in fields of immunology and molecular biology have let scientists select more specific and potent ligands and toxins for target therapy. In this article, we will discuss the structure and function of commonly used toxins, the clinical activity of the first generation immunotoxins and their problems, the production of recombinant toxins and prospects for the future.

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TOXINS

Several bacterial and plant proteins have been used to prepare immunotoxins. Of these, ricin, Pseudomonas exotoxin A (PE), and diphtheria toxin (DT) are the most extensively investigated. The bacterial toxins, Pseudomonas exotoxin A (PE) and diphtheria toxin (DT), ADP-ribosylate and thereby inactivate elongation factor 2, an enzyme necessary for protein synthesis. PE, a 66 kD protein secreted by *Pseudomonas aeruginosa*, is composed of three major structural domains [3, 4]: an amino-terminal cell-binding domain (domain I), a central translocation domain (domain II), and a carboxyl-terminal activity domain (domain III). Domain III catalyses the ADP ribosylation and inactivation of elongation factor 2, which inhibits protein synthesis and leads