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E-Cadherin in human brain tumours: loss of immunoreactivity in malignant meningiomas

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Abstract Cadherins are a family of glycoproteins that are associated with cell adhesion mechanisms. They are divided into subclasses. The E- and P-cadherins are regarded as the epithelial subtype. Their expression has been demonstrated in many different carcinoma types. Using immunomorphological techniques, we studied the expression of E-cadherin in a series of 145 human brain tumours with the monoclonal antibody 5H9. Western blot analysis was used to confirm the immunohistochemical data. The tumour types represented were astrocytoma WHO I (*n* = 7), astrocytoma WHO II (*n* = 6), astrocytoma WHO III (*n* = 14), glioblastoma WHO IV (*n* = 8), oligodendroglioma WHO II (*n* = 5), ependymoma WHO II (*n* = 5), choroid plexus papilloma WHO I (*n* = 5), pineoblastoma WHO IV (*n* = 5), medulloblastoma WHO IV (*n* = 5), neurinoma WHO I (*n* = 5), meningioma WHO I and WHO III (*n* = 75) and pituitary adenoma WHO I (*n* = 5). Only choroid plexus papillomas (5/5) and meningiomas showed E-cadherin expression. In benign meningiomas (*n* = 45; 100%), positive E-cadherin immunoreactivity was found regardless of the histomorphological subtype. E-Cadherin was also expressed in 21 WHO I meningiomas (100%) invading dura, bone, brain, and muscle. In contrast, E-cadherin was absent from the majority of morphologically malignant meningiomas (6/9, 66.6%). In addition, in recurrent meningiomas (*n* = 9), E-cadherin expression in the recurrent tumours was identical to that in the primary neoplasm except in cases with malignant progression, where the malignant recurrent tumour was E-cadherin negative. In 2 cases of metastasizing meningiomas, no E-cadherin immunoreactivity was found in the primary tumours or their metastases.

Key words E-Cadherin · Brain tumours · Meningiomas

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Introduction

Cadherins are a family of glycoproteins involved in the Ca^{2+}-dependent cell–cell adhesion mechanism detected in most types of tissues. Inhibition of the cadherin activity with antibodies induces dissociation of cell layers, indicating the fundamental importance of these polypeptides in maintaining the multicellular structure. Cadherins are divided into subclasses, including E-, N-, and P-cadherins [21]

E- and P-cadherins are regarded as beloning to the epithelial subtype, whose expression has been demonstrated in many different carcinoma types [1–4, 9, 12–14, 18–21, 24]. In addition, E-cadherin expression has been correlated with differentiation, invasiveness, and metastases. In carcinomas of the lung [1], the stomach [13, 21] the liver [20], the colon and the rectum [4], the female genital tract [9], head and neck [18], and the thyroid gland [2] E-cadherin was lost or reduced in dedifferentiated tumours. In infiltrating carcinomas of the breast, E-cadherin immunoreactivity was positive in ductal and negative in lobular variants [14]. In ductal carcinomas of the breast, there was significantly more expression of E-cadherin in well-differentiated ductal carcinoma in situ (DCIS) than in poorly differentiated DCIS [7]. In prostate cancer, decreased expression of E-cadherin was correlated with poor prognosis [24]. Among tumours of the central nervous system, E-cadherin expression has been studied exclusively in meningiomas; the reported data, however, are not congruent [5, 23]. The aim of our immunocytochemical and immunochemical study was the examination of E-cadherin expression in a large series of brain tumours, and in particular, in meningiomas. In meningiomas, the immunoreaction in histological subtypes, malignant neoplasms, invasive and recurrent tumours and in metastatic meningiomas was examined.

Materials and methods

Arachnoid villi were prepared from post-mortem brains and fixed in 4% formaldehyde. Processing of the tissue followed standard procedures.

Table 1 E-cadherin expression in human brain tumours

Tumour entity	Grade	*n*	E-Cadherin immunoreactivity	
			Positive	Negative
Astrocytoma	WHO I	7	0	7
Astrocytoma	WHO II	6	0	6
Astrocytoma	WHO III	14	0	14
Glioblastoma	WHO IV	8	0	8
Oligodendroglioma	WHO II	5	0	5
Ependymoma	WHO II	5	0	5
Choroid plexus papilloma	WHO I	5	5	0
Pineoblastoma	WHO IV	5	0	5
Medulloblastoma	WHO IV	5	0	5
Neurinoma	WHO I	5	0	5
Meningioma	WHO I/III	75	69	6
Pituitary adenoma	WHO I	5	0	5
Total		145		

Table 2 E-Cadherin expression in meningioma subtypes

Subtypes	WHO	*n*	E-Cadherin immunoreactivity[a]			
			–	+	++	+++
Meningothelial	I	10	0	1	3	6
Fibroblastic	I	10	0	2	4	4
Transitional	I	10	0	2	6	2
Psammomatous	I	6	0	0	2	4
Papillary	I	1	0	0	1	0
Angiomatous	I	2	0	1	0	1
Secretory	I	3	0	0	3	0
Microcystic	I	3	0	1	2	0
Malignant	III	9	6	2	1	0
Invasive						
Dura	I	6	0	0	2	4
Bone	I	6	0	0	3	3
Brain	I	4	0	1	3	0
Muscle	I	5	0	0	5	0

[a] Positive cells per slide: – negative, + less than 30%, ++ 30–70%, +++ more than 70%

Table 3 E-cadherin expression in recurrent meningiomas (*m* male, *f* female, *men* meningothelial meningioma, *trs* transitional meningioma)

No.	Age	Sex	Primary tumour				First recurrence			Second recurrence		
			Year	Type	WHO	5H9	Year	WHO	5H9	Year	WHO	5H9
1	44	f	1982	men	I	+++	1986	I	+++	1992	I	+++
2	39	m	1982	men	I	++	1986	I	++	1993	III	–
3	20	f	1984	men	I	–	1991	I	–	1993	I	–
4	56	m	1985	men	I	+++	1993	I	+++			
5	70	m	1986	trs	I	+++	1990	I	++	1992	III	–
6	42	m	1987	men	I	+++	1993	I	+++			
7	49	f	1991	men	I	++	1992	III	–			
8	72	f	1992	trs	I	–	1993	I	–			
9	69	f	1992	trs	I	++	1993	III	–	1993	III	–

Tumour samples were collected from the files of the Institute of Neuropathology, University of Essen, Germany. They were received immediately after surgery and fixed in 4% formaldehyde. After fixation, 4- to 6-µm paraffin sections were cut and stained with haematoxylin and eosin. Tumour diagnoses were made following the guidelines of the World Health Organization for histological typing of tumours of the central nervous system [11]. Tumour entities are quoted in Tables 1–3. The only therapy for patients suffering from recurrent meningiomas (Table 3) was surgery.

The monoclonal antibody 5H9 (IgG1) is an E-cadherin-specific antibody, which was received after immunization of BALB/c mice with 3 µg per animal and immunization of the 80 kDa tryptic fragment of E-cadherin from human A-431 vulvar carcinoma cells. Spleen cells from immunized mice were fused with P3-X63-Ag 8.653 mouse myeloma cells [6].

For immunocytochemistry, 4- to 6-µm paraffin sections were cut on polylysine coated slides, dewaxed in fresh xylene for 2 × 10 min, rehydrated in ethanol and incubated in 1% H_2O_2/methanol (30%) for 20 min to block the endogenous peroxidase activity. After a brief washing in 0.05 M TRIS buffer, pH 7.6, the slides were predigested with 0.1% trypsin containing 0.1% Ca^{2+}, pH 7.4, for 10 min, rinsed again in Tris-HCl (pH 7.6) and in-

cubated with 5% normal swine serum (Dako, Hamburg, Germany) for 30 min to reduce background staining.

Afterwards 100 µl of the undiluted supernatant monoclonal antibody 5H9 was applied to each slide for 60 min in a moist chamber at room temperature. After washing for 2 × 5 min in Tris-HCl, pH 7.6, the slides were incubated with biotinylated rabbit anti-mouse immunoglobulins (1:200, 30 min, room temperature, moist chamber), and finally streptavidin–horseradish peroxidase (1:300; 20 min, room temperature, moist chamber) was applied. Both antibodies were purchased from DAKO, Hamburg, Germany. The reaction product was visualized using amino-ethylcarbazole. Nuclear counterstaining was done with haematoxylin.

For Western blot analysis, a frozen tumour sample of about 50 mg was reduced to small pieces and lysed in SDS-PAGE sample buffer by brief sonication and heating to 95°C. Protein lysates were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (50 µg total proteins/lane) on 7.5–15% gradient gels (Bio-Rad Laboratories, Munich, Germany) and transferred to nitrocellulose membranes. The filters were incubated with mab 5H9 for 2 h at room temperature. Binding of the antibody was visualized using the streptavidin–biotin complex method as described above. Western blots were performed in five cases: meningiothelial, transitional, fibroblastic, recurrent invasive, and malignant meningiomas.

Paraffin slides of a human breast adenocarcinoma were used as positive controls. A positive immunoreaction was seen as a reddish-brown reaction product, occurring preferentially at the cell borders and more weakly in the cytoplasm of tumour cells. Negative controls were done by omitting the first antibody or by using an irrelevant primary antibody; they always gave negative results.

Results

In our series of 145 primary human brain tumours, E-cadherin immunoreactivity was found in choroid plexus papillomas and meningiomas only. Different types of neuroepithelial tumours, such as astrocytomas, glioblastomas, oligodendrogliomas, ependymomas, pineoblastomas, medulloblastomas, neurinomas and pituitary adenomas were negative (Table 1). In meningiomas and plexus papillomas, strong positive immunoreactions were concentrated at the tumour cell borders. A weaker intracytoplasmic immunoreactivity was also seen. In meningiomas of different histological subtypes, including meningothelial, transitional, fibroblastic, psammomatous, papillary, angiomatous and microcystic variants, a positive immunoreaction was observed (Table 2, Fig. 1 a–c). The positive reaction was seen in all 45 meningiomas classed as WHO I and also in case of papillary meningioma classed as WHO III. The same type of positive immunoreaction was also found in arachnoidal villi. In meningiomas, the number of positive tumour cells varied from case to case and is shown in Table 2.

According to the WHO classification of brain tumours malignant meningiomas have enhanced cellularity, cellular polymorphism and a high number of mitoses. In morphologically malignant meningiomas E-cadherin expression was absent in the majority of the cases (6/9; Table 2; Fig. 1d). All benign meningiomas exhibiting invasion of the surrounding tissues, such as dura, bone, brain, and muscle, demonstrated E-cadherin expression (Table 2; Fig. 1e). These tumours were the first manifestation of the meningioma. In benign recurring meningiomas WHO I, E-cadherin immunoreactivity was identical to that in the primary tumour (Table 3). It was positive in the primary tumour and in the recurrence in 5 cases (5/9, 33.3%; Fig 1f). In 2 WHO I meningiomas, both the primary tumour and the recurrent tumour were negative. Positive E-cadherin immunoreaction in the primary benign meningioma was lost in the recurrent tumour in 4 cases. In these examples, the recurrent tumour showed histomorphological signs of malignancy (4/9; 44.4%).

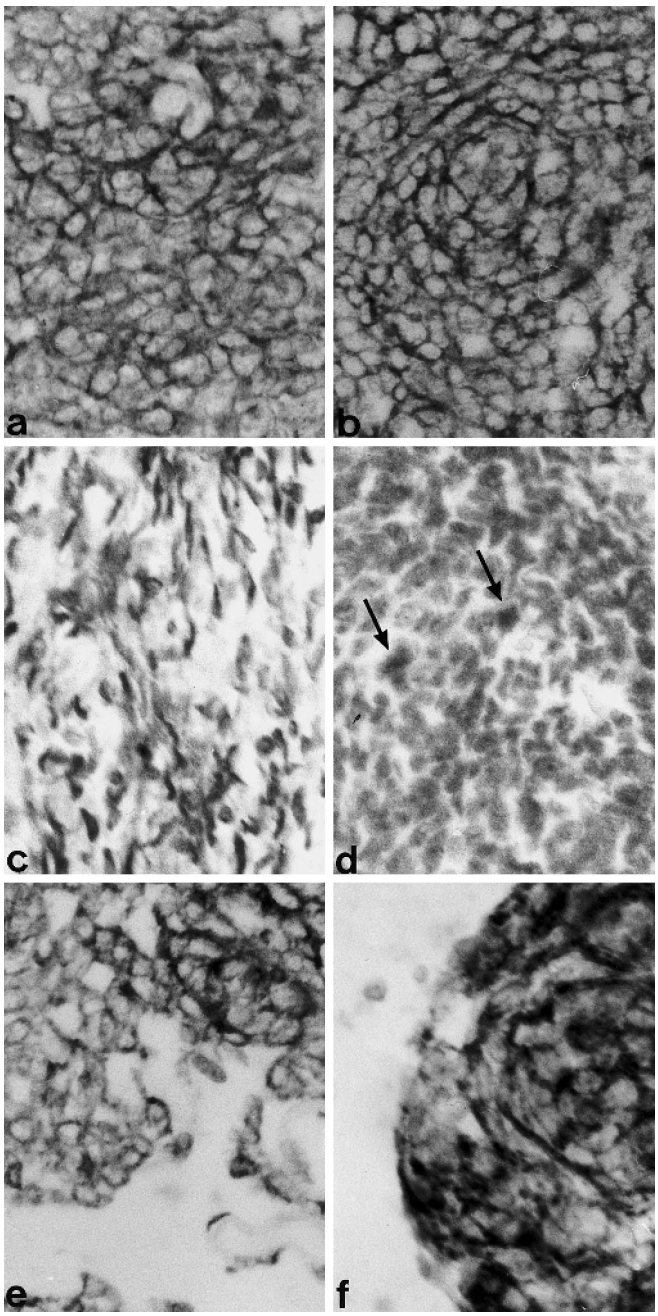

Fig. 1 Positive E-cadherin immunoreaction in **a** a meningothelial, **b** transitional, and **c** fibroblastic meningioma. **d** Loss of E-cadherin immunoreactivity in a malignant meningioma. E-Cadherin is positive **e** in a meningioma invading the muscle and **f** in recurrent meningiomas. Paraffin sections, streptavidin–biotin method, hematoxylin counterstain, original magnification ×25. (*Arrows* indicate mitotic figures.)

Table 4 E-cadherin expression in metastasizing meningiomas (*fibr* fibroblastic meningioma, *malig* malignant meningioma)

No	Age	Sex	Primary tumour				Local recurrence			Metastasis			
			Year	Type	WHO	5H9	Year	WHO	5H9	Year	WHO	5H9	Organ
1	47	m	1990	fibr	I	–	1993	I	–	1994	I	–	Lung
2	57	f	1993	malig	III	–	1994	III	–	1994	III	–	Spine Lung

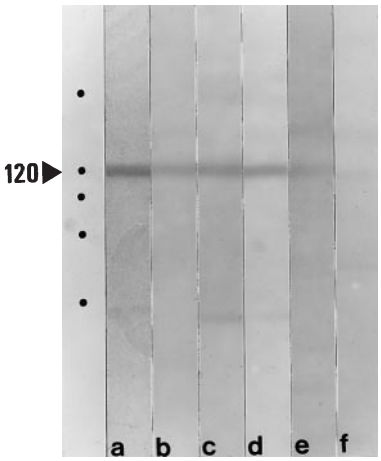

Fig. 2 Western blots demonstrating E-cadherin expression in **a** liver, **b** meningothelial, **c** transitional, **d** fibroblastic, and **e** invasive meningiomas. **f** Loss of E-cadherin in a malignant meningioma

Two metastasizing meningiomas (Table 4) lacked E-cadherin immunoreaction both in the primary tumour and in the metastases. In one case, lung metastases and the primary fibroblastic meningiomas WHO I were E-cadherin negative. In the other case, the patient suffered from a primary malignant meningioma class WHO III, which showed a negative E-cadherin immunoreaction, as did the metastases in spine and lungs.

The immunocytochemical findings were confirmed by Western blot experiments in every case studied (Fig. 2). Five examples of meningothelial, transitional, fibroblastic recurrent and invasive meningiomas, but not the malignant meningiomas, exhibited an immunoreaction corresponding to a molecular weight of about 120 kDa, which correlates to the position of E-cadherin.

Discussion

In our series of tumours of the central nervous system, E-cadherin expression has been found in plexus papillomas and in meningiomas. As E-cadherin is the epithelial type member of the cadherin family, expression in an epithelial brain tumour such as choroid plexus papilloma was expected. E-Cadherin expression in human meningiomas has previously been described by Figarella-Branger et al. [5] and Tohma et al. [23]. As in our series, Figarella-Branger et al. [5] found E-cadherin expression in all types of meningiomas studied. Tohma et al. [23] observed E-cadherin in syncytial and transitional, but not in fibroblastic meningiomas, although they used the same monoclonal antibody HECD-1 as Figarella-Branger [5]. In our series, E-cadherin immunoreactivity has been uniformly demonstrated in the most common histological subtypes of human meningiomas. The amount of positive cells varied from case to case. E-Cadherin was also found in arachnoid villi. The differences observed by other groups could be due to the different methods used.

The occurrence of E-cadherin as the epithelial type of the cadherin family is not surprising. It is well known from ultrastructural studies that meningiomas exhibit desmosomes, the epithelial type of cell contact [8]. Immunomorphologically it has been demonstrated that most types of meningiomas show desmoplakin immunoreactivity [10, 19].

In malignant meningiomas E-cadherin expression was absent in 6 of 9 cases. These data contrast with those of others [5]. Three positive malignant meningiomas showed only a weak immunoreaction. These data correlate very well with findings in human carcinomas, where a decrease or loss of E-cadherin expression has been correlated with tumour dedifferentiation. Examples of this phenomenon have been described in carcinomas of the lung [1], the stomach [12, 13, 21], the liver [20], the colon and rectum [14], the female genital tract [9], the head and neck [18] and the thyroid gland [2]. Our data fit in very well with recent observations in carcinomas of the endometrium and the ovary [16]. Risinger et al. found four mutations of the E-cadherin gene-coding region (on chromosome 16q22) and concluded that E-cadherin can be regarded as a tumour suppressor gene. An association between morphologically malignant meningiomas and loss of heterozygosity (LOH) for loci on chromosome 10 came from the study of Rempel et al. [15], who found LOH for loci on chromosome 10 in 2 of 4 morphologically and invasively malignant meningiomas and in 2 of 4 only morphologically malignant meningiomas.

Invasion is a common and frequent feature of meningiomas [17]. Meningiomas can invade surrounding tissues such as dura, bone, muscle and brain. In our series, E-cadherin was expressed in all meningiomas invading surrounding tissues. The morphological phenotype of these meningiomas correlated to benign meningiomas in WHO class I. Reduced levels of E-cadherin expression have been observed in infiltrating tumour cells of gastric cancers [12]. These differences could be explained by variations in the invasion process between carcinoma and meningioma cells at the molecular level. Frixen et al. [6] have demonstrated that E-cadherin can act as a suppressor of tumour invasion. It is not clear whether the lack of

E-cadherin expression in gastric cancer is the result of tumour dedifferentiation or whether infiltration process occurs as a consequence.

Recurrence is often found in meningiomas [17]. It can be due to incomplete surgical resection of the primary tumour, multifocal manifestation, and other reasons. In most cases of recurrent meningiomas, E-cadherin expression in the recurrent tumour was identical that in the primary tumour. E-Cadherin is present in recurrent tumours if the morphology is benign, but if the recurrent tumour shows morphological signs of malignancy (WHO III), E-cadherin expression was lost. This phenomenon again indicates that E-cadherin expression in meningiomas is associated with the differentiation level of the tumour. Dedifferentiation of meningiomas, as shown in morphologically malignant meningiomas, is often manifest as loss of E-cadherin immunoreaction.

Metastases from meningiomas are rare. However, meningiomas can metastasize even in the absence of morphological signs of malignancy [17]. In 1 case, E-cadherin expression was lost in the primary malignant meningioma and in the spine and lung metastases. In an other case, E-cadherin immunoreactivity could be found neither in the benign primary tumour nor in the metastasis. It is interesting to note that an E-cadherin-negative benign meningioma (WHO I), which usually is E-cadherin positive, exhibited a metastatic potential. From the clinical point of view, it would be interesting to follow up a series of E-cadherin-negative meningiomas classed as WHO I. Given the restrictions of the small sample number, whether loss of E-cadherin is a prerequisite for metastases from meningiomas can only be a subject of speculation.

Our results show that E-cadherin is expressed in differentiated (benign) meningiomas and that its expression is lost in dedifferentiated (malignant) tumours. In the light of this interpretation, the expression of E-cadherin in benign invading and recurrent meningiomas is not surprising.

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