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Keratin expression in astrocytomas: an immunofluorescent and biochemical reassessment

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Abstract Several studies have shown that immunoenzymatic staining of formalin-fixed, paraffin-embedded astrocytomas with keratin antibodies frequently yields positive labelling, but no biochemical evidence of keratin expression in astrocytomas has been reported. We have investigated the presence of keratin in astrocytoma and normal brain tissues both by immunofluorescence on frozen sections and by 1D and 2D immunoblotting using seven monoclonal antibodies that, collectively, recognize most keratin polypeptides. Four of these antibodies did not stain neural tissues by immunofluorescence and were also negative by immunoblotting. The remaining three keratin antibodies stained normal brain and/or a high proportion of astrocytomas. Two of these three antibodies only stained glial fibrillary acidic protein (GFAP)-positive cells, while the third only stained GFAP-negative cells. 1D and 2D immunoblotting analysis showed that positive immunofluorescence staining of normal brain and/or astrocytomas seen with these three keratin antibodies was due to cross-reactivity with non-keratin proteins, such as GFAP. These results demonstrate that, contrary to earlier suggestions, keratin polypeptides are not frequently expressed in astrocytomas. Our studies also emphasize that keratin antibodies should be used cautiously for the differential diagnosis of undifferentiated gliomas from tumours of non-glial origin.

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Introduction

The constituent proteins of intermediate filaments (IF) belong to a multigene family composed of about 40 polypeptides, which have been subdivided into six major types based on sequence homologies (for reviews see [16, 27, 51]). While type I–IV and VI IF proteins form cytoplasmic filamentous networks, type V IF proteins, the lamins, are the principal components of the nuclear lamina. Numerous studies have established that cytoplasmic IF proteins are expressed in a tissue- and cell-type specific pattern. Epithelia express type I and type II IF proteins, the keratins, which include 20 polypeptides referred to as K1 to K20 [36] (for references on K20 see [6, 37]). Type III IF proteins have a distribution limited to certain cell types, such as astrocytes for GFAP (glial fibrillary acidic protein), muscle cells for desmin, and various mesenchymal cells for vimentin. Type IV IF proteins are specifically expressed in differentiated neurons of the central nervous system. Finally, nestin, the only known type VI IF protein, is found primarily in neuroectodermal stem cells and in embryonic muscle cells [30, 49].

As a general rule, neoplastic cells retain the expression of the cytoplasmic IF proteins found in their progenitor cell type [42, 50]. However, in certain cases, neoplastic cells express additional IF proteins not present in their non-malignant counterparts (for reviews see [35, 53]). This extraneous expression of IF proteins may have important functional consequences for the malignant properties of tumour cells. For instance, K8 and K18 are normally found only in simple epithelia, but the presence of these keratins in tumours derived from other tissues appears to correlate with increased metastatic potential [18, 19, 32, 34, 48, 58]. Furthermore, compelling evidence for a role of K8 and K18 in the progression of tumours not derived from simple epithelia has been provid-

Table 1 Specificity of our panel of keratin antibodies for the 20 keratin polypeptides. Numbers in brackets refer to studies cited in the References and describing the specificity of each antibodies

Antibody	Specificity for keratin polypeptide no.																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
AE1 [8, 9, 56]										+				+	+	+			+	
AE3 [8, 9, 56]	+	+	+	+	+	+	+	+												
AE5 [8, 9]			+																	
M20 [55]								+												
KS-1A3 [31]													+							
CK-E3 [54]																	+			
KS-B17.2 [31]																		+		

ed by the finding that transfection of K8 and K18 genes into sarcoma cells increases the migratory and invasive activities of these cells [7].

Astrocytes normally express GFAP alone or GFAP in addition to vimentin, and usually maintain the expression of these two proteins during neoplastic transformation. Several immunohistochemical observations have suggested that astrocytomas may also express keratin [4, 10–12, 20, 39]. These observations are intriguing since the expression of keratin polypeptides by astrocytomas could have a bearing on the malignant properties of these tumours. However, the presence of keratin polypeptides in astrocytomas has not been confirmed systematically by biochemical or molecular biological methods. Such data would be important because it has been shown that unexpected immunohistochemical staining of gliomas with antibodies against IF proteins can be artefactual [2, 14].

Using a panel of seven monoclonal antibodies, which together are directed against most keratin polypeptides, we have systematically re-examined the presence of keratin in normal brain and astrocytoma tissues both by immunofluorescence on frozen, acetone-fixed sections and by 1D and 2D immunoblotting. While we find that some keratin monoclonal antibodies stain a large percentage of astrocytomas, our biochemical results indicate that this staining is not due to the presence of keratin polypeptides but to cross-reactivity with other proteins such as GFAP. This demonstrates that, contrary to earlier suggestions, keratin polypeptides are not widely expressed in astrocytomas. In addition, our findings underscore the limitations of some routinely used keratin monoclonal antibodies for the differential diagnosis of undifferentiated gliomas from tumours of non-glial origin.

Materials and methods

Normal, human frontal cortex was obtained from VA Wadsworth Medical Center (Los Angeles, Calif.). Human astrocytomas were obtained after surgical resection from Columbus Hospital Neurosurgery Department (Chicago, Ill.). The specimens were frozen shortly after resection and consisted of 1 grade II (case no. 1), 1 grade III (case no. 2) and 7 grade IV (cases no. 3–9) astrocytomas. The tumours were graded according to the WHO classification [26]. Bovine cornea was dissected from an eye obtained from a local slaughterhouse. A431 human epidermoid carcinoma cells were obtained from the American Type Culture Collection (Rockville, Md.) and were grown in DMEM containing 10% fetal calf serum, 100 U/ml penicillin and 100 µg/ml streptomycin.

The following mouse monoclonal keratin antibodies were used: AE1 and AE3 (both purchased from Biomeda, Foster City, Calif.), AE5 (ICN Biologicals, Costa Mesa, Calif.), M20, CK-E3, KS-1A3 and KS-B17.2 (these latter four antibodies were purchased from Sigma, St Louis, Mo.). The specificity of each of these monoclonal antibodies for the different keratin polypeptides is summarized in Table I. A rabbit antiserum raised against bovine brain GFAP was also used (Incstar, Stillwater, Minn.).

As secondary antibodies we used affinity purified, human serum adsorbed goat anti-mouse or rabbit IgGs (Kirkegaard & Perry, Gaithersburg, Md.) which were conjugated to peroxidase for immunoblotting and to either fluorescein or rhodamine for immunofluorescence.

Immunofluorescence on tissues was performed on 4-µm-thick cryostat sections fixed for 5 min in methanol at –20°C and air-dried at room temperature for 1 h. All antibodies were diluted at 1:20 in PBS (6 mM phosphate buffer, 170 mM NaCl, 3 mM KCl, pH 7.4). Each incubation of tissue sections with antibodies was performed for 60 min at 37°C and was followed by three 5-min washes in PBS. After the final wash, the tissue sections were mounted in Fluoromount G (Southern Biotechnology Associates, Birmingham, Ala.).

Microscopic observations were carried out with an Axiophot microscope (Carl Zeiss, Oberkochen, Germany) equipped with epi-illumination and specific filters for rhodamine and fluorescein. Photographs were taken on Tmax 400 black and white films (Kodak, Rochester, N.Y.).

For one-dimensional gel electrophoresis (1D-GE), tissue or cultured cell samples were lysed in 3% sodium dodecyl sulfate (SDS) and 3% β-mercaptoethanol sample buffer [29] containing 1 mM phenylmethylsulfonylfluoride (PMSF) and 1 mM *N-p*-tosyl-L-arginine methylester (TAME) as protease inhibitors. For 2D-GE tissue samples were lysed in 1% SDS containing 20 mM dithiothreitol, 1 mM PMSF and 1 mM TAME. Samples for both 1D-GE and 2D-GE were sonicated and boiled for 3 min. The protein concentration of each sample was determined by the method of Bradford [5].

For 1D-GE samples, 50 µg of protein was separated on 7.5% SDS-polyacrylamide gel [29]. At the end of the electrophoresis, the proteins were either stained with 0.05% Coomassie brilliant blue in 50% methanol and 10% acetic acid or blotted onto nitrocellulose paper.

For 2D-GE samples, 5 µg of protein was separated first by isoelectric focusing according to the method of O'Farell [41]. Subsequent separation of the proteins according to their molecular weight was performed on 7.5% SDS-polyacrylamide gels. At the end of the electrophoresis, the proteins were either silver stained following the method of Wray et al. [57] or blotted onto nitrocellulose paper.

The proteins separated by 1D-GE or 2D-GE were transferred electrophoretically onto nitrocellulose membranes for 2 h at 110 V and at 4°C using a buffer tank and a mini Trans-blot module (Bio-Rad, Hercules, Calif.) [52]. Under these conditions the efficiency of the transfer was excellent as determined by staining of the nitro-

Table 2 Results of immunofluorescence staining of normal brain and astrocytoma tissues with keratin monoclonal antibodies and with GFAP antibodies

Case no.	Astrocytoma grade	Antibody							
		AE1	AE3	AE5	M20	KS-1A3	CK-E3	KS-17.2	GFAP
Normal brain		–	+	+	–	–	–	–	+
1	II	–	+	+	–	+	–	–	+
2	III	–	–	+	–	–	–	–	+
3	IV	–	+	+	–	+	–	–	+
4	IV	–	+	+	–	–	–	–	+
5	IV	–	+	+	–	+	–	–	+
6	IV	–	+	+	–	–	–	–	+
7	IV	–	+	+	–	+	–	–	+
8	IV	–	+	+	–	–	–	–	+
9	IV	–	–	–	–	–	–	–	–

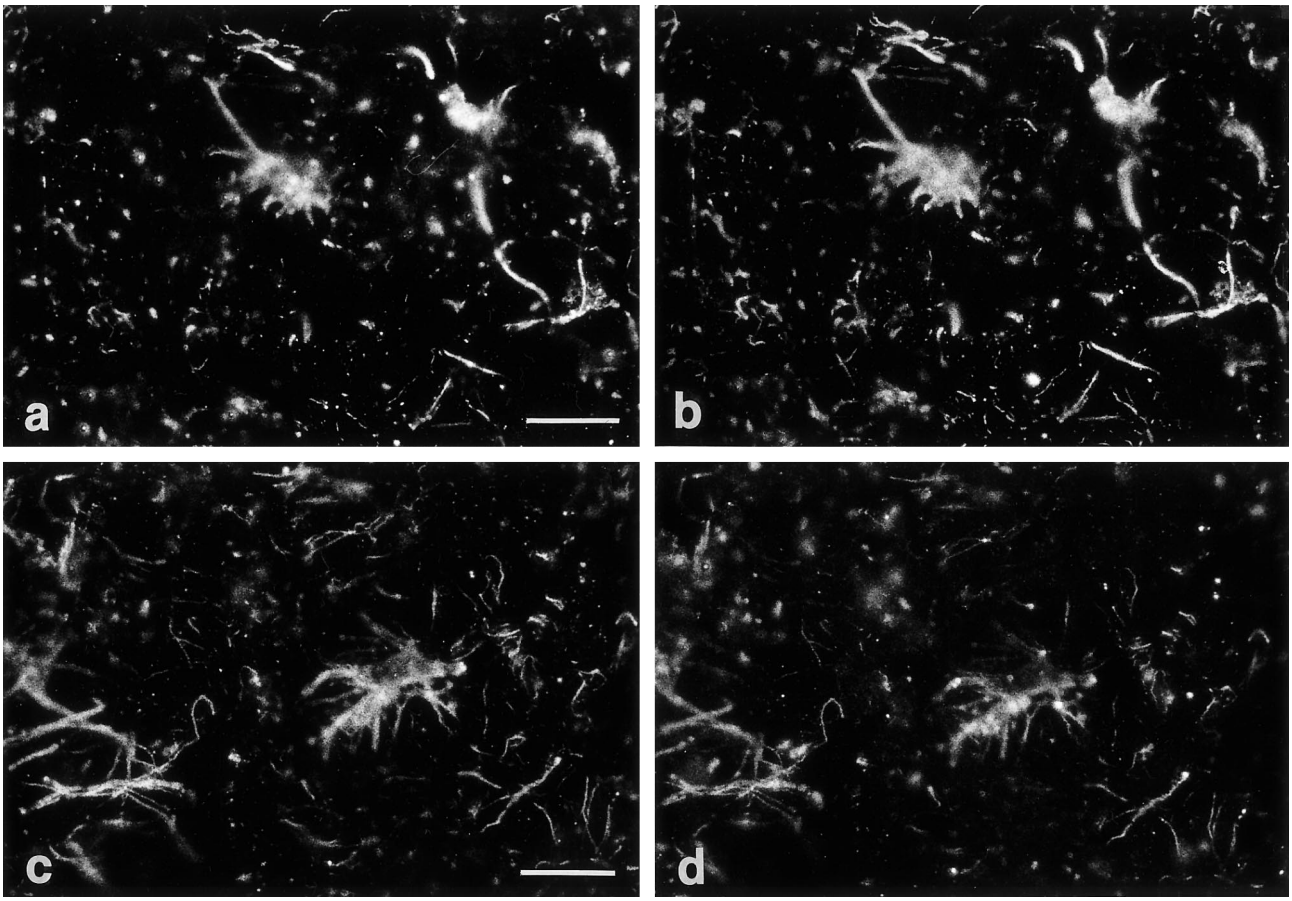

Fig. 1 Double immunofluorescent staining of normal human brain with either **a** AE3 or **c** AE5 monoclonal keratin antibodies and **b, d** GFAP antibodies. Note that AE3 and AE5 stain cellular profiles and processes, and that their staining pattern is identical to that of GFAP antibodies. *Bars* 20 μm

cellulose membranes with Ponceau S as previously described [47]. Non-specific protein binding sites on the nitrocellulose blots were blocked with PBS containing 5% non-fat milk (PBS-milk). The primary antibodies were diluted in PBS-milk at the following dilutions: 1:250 for AE1, AE3 and AE5, 1:100 for M20, CK-E3, KS-1A3 and KS-B17.2, and 1:1000 for GFAP antibodies. Incubation of the nitrocellulose blots with these antibodies was performed overnight at room temperature. The blots were then washed with PBS and incubated for 4 h at room temperature with the appropriate peroxidase-conjugated antibodies diluted at 1:1000 in PBS-milk. Following PBS washes, the peroxidase activity of the nitrocellulose-bound secondary antibodies was detected either with the ECL chemiluminescent reagents (Amersham, Arlington Heights, Ill.) and Kodak Biomax MR films (Kodak, Rochester, N.Y.), or with a chromogenic substrate consisting of 0.06% (w/v) 4-chloro-1-naphthol, 20% (v/v) methanol, and 0.01% (v/v) H_2O_2 in PBS.

Results

Table 1 lists the seven keratin monoclonal antibodies used in our study and demonstrates that, collectively,

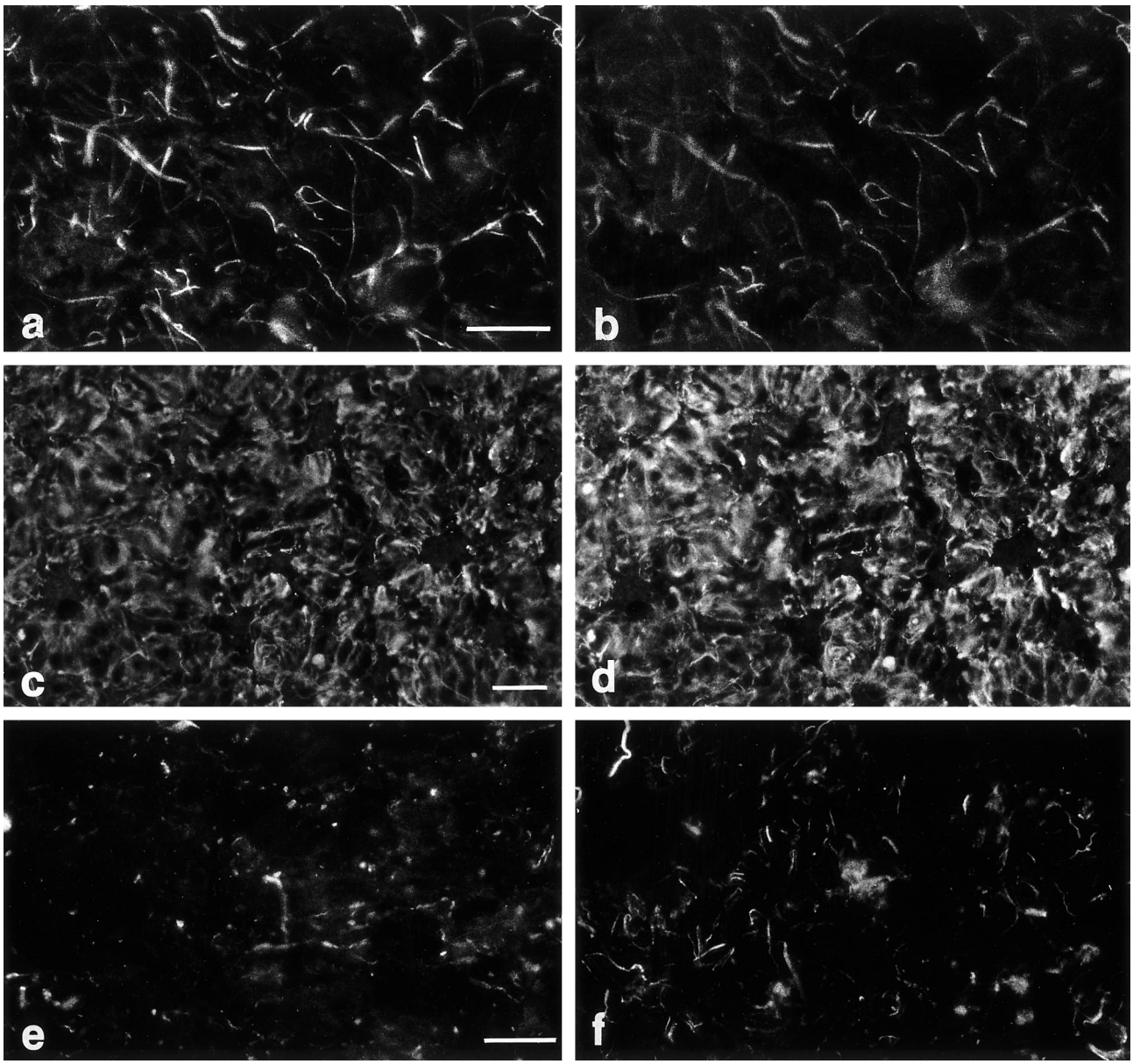

Fig. 2 Double immunofluorescent staining of astrocytomas with **a** AE3, **c** AE5, or **e** KS-1A3 monoclonal keratin antibodies and **b, d, f** GFAP antibodies. **a, b** Case 7; **c, d** case 6; **e, f** case 5. Note that the staining patterns of GFAP antibodies and AE3 or AE5 are identical, but that the staining patterns of GFAP antibodies and KS-1A3 are different. *Bars* 20 μm

they recognize 16 of the 20 keratin polypeptides. Table 2 summarizes the results of immunofluorescent staining of normal brain and of astrocytomas with these antibodies, as well as with GFAP antibodies. While four of the seven keratin antibodies, AE1, M20, CK-E3, and KS-B17.2, were negative on all tissue samples examined, the remaining three antibodies, AE3, AE5 and KS-1A3, stained normal brain and/or a variable proportion of astrocytomas.

Single-label immunofluorescence of normal brain sections with either AE3 or AE5 monoclonal antibodies revealed numerous brightly stained cellular profiles and processes. Double-label immunofluorescence showed that, in normal brain, the staining pattern of these two antibodies was indistinguishable from that given by GFAP antibodies (Fig. 1). AE3 also stained seven out of nine (78%) astrocytomas, while AE5 stained eight out of nine (88%) astrocytomas. In these tumours, the staining obtained with both antibodies was mostly fibrillar, and, as in normal brain, on double-label immunofluorescence it appeared to be identical to that obtained with antibodies directed against GFAP (Fig. 2).

Monoclonal keratin antibody KS-1A3 did not stain normal brain, but did stain four out of nine (44%) astrocytomas. The staining consisted of a variable number of slender processes heterogeneously distributed within tissue sections. Double-label immunofluorescence revealed that these processes were not recognized by antibodies against GFAP (Fig. 2).

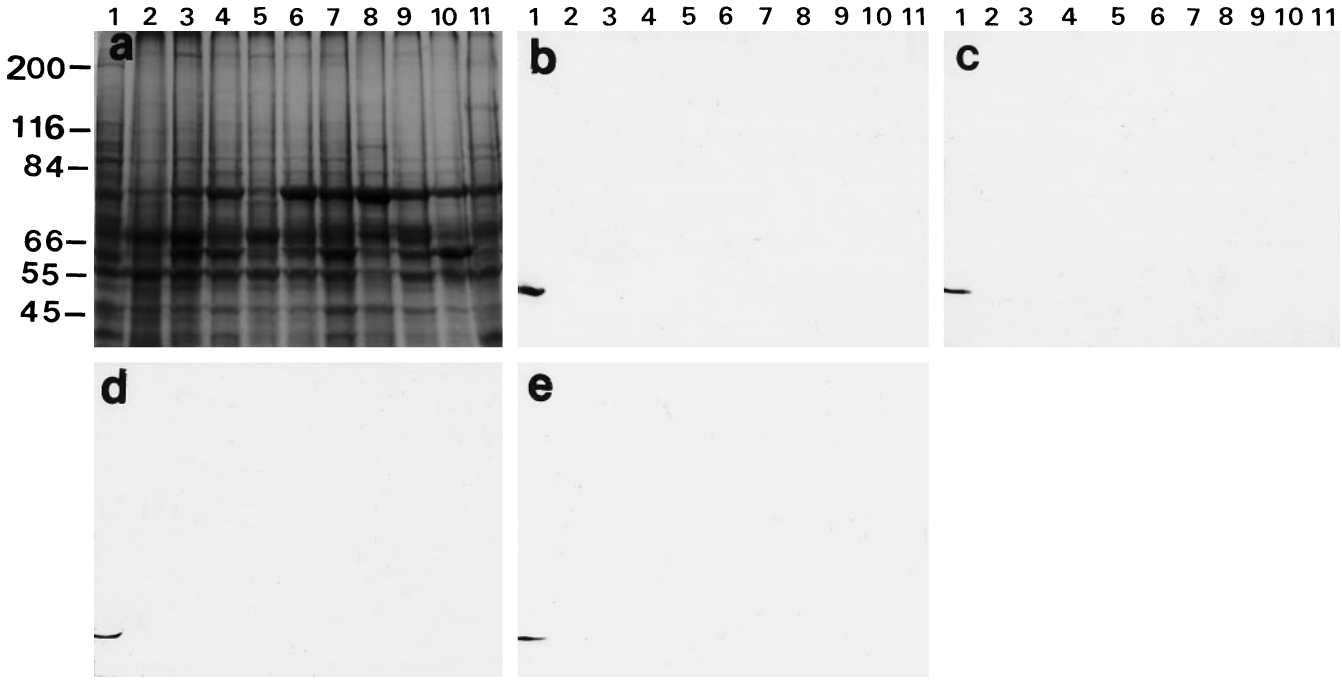

Fig. 3 **a** Coomassie blue-stained SDS-polyacrylamide gel of: A431 epidermoid carcinoma cells (*lane 1*), normal brain (*lane 2*), and astrocytoma cases 1–9 (*lanes 3–11*); molecular mass of protein standards are indicated in kilodaltons at the left of the gel. **b–e** Blots of gels similar to that in **a** were incubated with the following keratin monoclonal antibodies: **b** AE1, **c** M20, **d** CK-E3, and **e** KS-B17.2. Note that these antibodies do not react with proteins present in either normal brain or astrocytoma samples (*lanes 2–11* of each blot), but that they recognize in A431 epidermoid carcinoma cells (*lane 1* of each blot) proteins of **b** 50 kDa for AE1, **c** 52 kDa for M20, **d** 45 kDa for CK-E3, and **e** 46 kDa for KS-B17.2

Monoclonal antibodies AE1, M20, CK-E3, and KS-B17.2 that did not stain normal brain or astrocytomas by immunofluorescence were also negative in immunoblots of these tissues (Fig. 3). A431 epidermoid carcinoma cells were used as positive control for these experiments, as they contain the keratin polypeptides recognized by these antibodies [36]. Immunoblots of A431 cells revealed that monoclonal antibodies AE1, M20, CK-E3, and KS-B17.2 recognized proteins of the expected molecular mass, including a 50-kDa protein corresponding to K14 and K15 for AE1, a 52-kDa protein corresponding to K8 for M20, a 45-kDa protein corresponding to K18 for KS-B17.2, and a 46-kDa protein corresponding to K17 for CK-E3 (Fig. 3).

Tissues that stained positive by immunofluorescence with monoclonal antibody AE5 were also positive by immunoblotting for a 50-kDa protein (Fig. 4). This protein did not comigrate with the 64-kDa corneal keratin polypeptide K3 (Fig. 4), which is the keratin polypeptide for which AE5 is specific in epithelial tissues [8, 9]. Instead, the electrophoretic mobility of the protein recognized by AE5 in normal brain and in astrocytomas was similar to that of GFAP (Fig. 4). This prompted us to determine, by 2D-gel immunoblotting assays, whether or not monoclonal antibody AE5 cross-reacted with GFAP. On immunoblots of 2D gels of astrocytoma tissues, antibodies against GFAP recognized three closely related spots, with a molecular mass of 50 kDa and isoelectric points between 5.6 and 5.8 (Fig. 5). The migration pattern of these spots was identical to that previously reported for GFAP and its phosphorylation variants [3]. Three closely related spots of 50 kDa with isoelectric points between 5.6 and 5.8 were also recognized by AE5 on immunoblots of 2D gels of astrocytoma tissues (Fig. 5). These spots were identical to those recognized by GFAP antibodies, as demonstrated by the fact that the incubation of immunoblots of 2D-gels of astrocytoma tissues with a mixture of monoclonal antibody AE5 and GFAP antibodies reveals only three spots (Fig. 5).

In some immunoblotting experiments, the AE3 monoclonal antibody recognized two proteins of approximately 63 and 65 kDa. When present, this doublet was found even in parts of the blot corresponding to the molecular mass standards or to lanes loaded with plain sample buffer. Previous studies have shown that this staining pattern is characteristic of contamination with human stratum corneum keratins [1, 9, 40], which are recognized by AE3 [9]. Appropriate precautions during sample preparation and gel electrophoresis [40] allowed us to eliminate contamination with exogenous keratins. Under these conditions, AE3 did not label any protein in either normal brain or astrocytomas. However, in A431 cells, this antibody recognized three proteins, which had a molecular mass corresponding to keratin polypeptides expressed by these cells and which have been previously shown to react with this antibody; these include K5 (58 kDa), K6 and K7 (54 kDa) and K8 (52 kDa) (Fig. 4).

Monoclonal antibody KS-1A3 did not react with any protein in our series of normal and neoplastic brain tissues on immunoblotting (Fig. 4), even in samples that stained positive with this antibody in immunofluores-

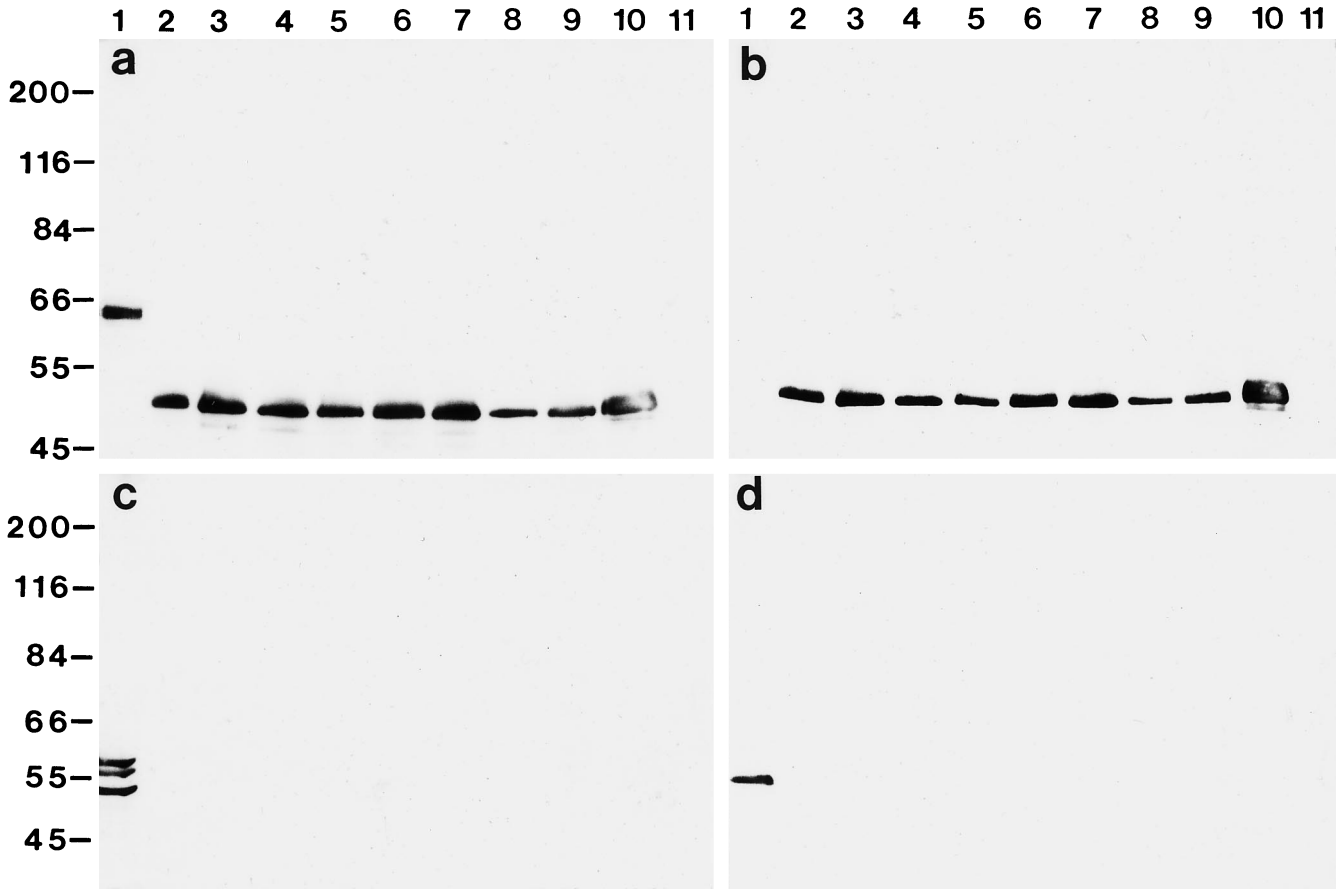

Fig. 4 Immunoblotting with **a** AE5, **b** GFAP antibodies, **c** AE3, and **d** KS-1A3 of bovine cornea (*lane 1* in **a** and **b**), A431 epidermoid carcinoma cells (*lane 1* in **c** and **d**), normal human brain (*lane 2* in **a–d**), and astrocytoma cases 1–9 (*lanes 3–11* in **a–d**); molecular mass of protein standards are indicated in kilodaltons at the left of the immunoblots. **a** AE5 reacts with a 64-kDa protein in bovine cornea (*lane 1*), and with a 50-kDa protein in normal brain (*lane 2*) and in astrocytomas 1–8 (*lanes 2–10*). **b** GFAP antibodies react with a 50-kDa protein in normal brain and astrocytomas 1–8 (*lanes 2–10*); note that this antibody does not react with any corneal protein (*lane 1*). **c** AE3 and **d** KS-1A3 do not react with any protein in normal brain (*lane 2*) or astrocytoma samples (*lanes 3–11*). In A431 epidermoid carcinoma cells, AE3 recognizes proteins of 58, 56 and 52 kDa (**c** *lane 1*), and KS-1A3 recognizes a 54-kDa protein (**d** *lane 1*)

cence experiments. However, as expected from the keratin polypeptide composition of A431 cells [36], KS-1A3 recognized a 54-kDa protein corresponding to K13 in these cells (Fig. 4). In some immunoblot experiments, the amount of protein loaded for A431 cells was 20 times less than that loaded for normal brain and astrocytoma samples. Under these condition, AE3 and KS-1A3 still labelled proteins of the appropriate molecular mass in A431 cells, but did not react with normal brain and astrocytoma samples.

Discussion

Our findings demonstrate that, contrary to what has been previously suggested by several studies based on immunoenzymatic staining of tissue sections [4, 10, 11, 20, 39], keratin polypeptides are not frequently expressed by astrocytomas. This conclusion is based on the results of our immunoblotting experiments, which show that the immunofluorescence staining seen with different keratin monoclonal antibodies in a large proportion of astrocytomas is not due to the presence of keratin polypeptides.

Immunoenzymatic staining of formaldehyde-fixed, paraffin-embedded gliomas with antibodies against IF proteins has been shown to yield artefactual results in some cases [14]. These have been attributed to cross-reactivities of the primary and/or secondary antibody, and also to contaminating antibodies present in the ascitic fluids used for staining [14]. A recent study has also demonstrated that the staining of gliomas with various keratin antibodies, such as 35 β H11 and AE1/AE3, depends on the fixation method and on the pretreatment of the tissue sections with proteases [2]. This has lent further credence to the suspicion that the positive labelling of gliomas by some keratin antibodies could be artefactual.

Interestingly, our immunofluorescence results show that the positive immunoenzymatic staining of a high percentage of astrocytomas with the keratin antibodies AE3 and AE5 seen in previous studies [2, 4, 10, 11, 20,

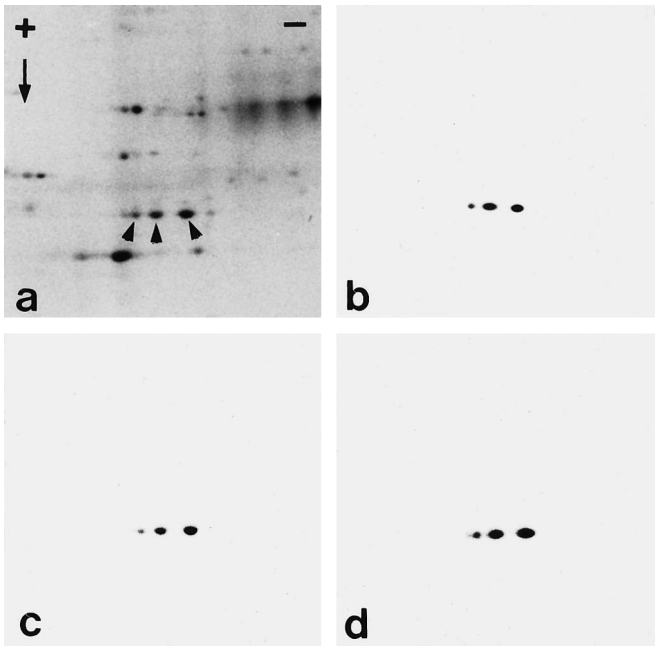

Fig. 5 **a** Silver-stained 2D gel of astrocytoma case 3; + and – indicate respectively the position of the anode and cathode during isoelectric focusing, and the *arrow* indicates the direction of protein migration during SDS-PAGE. **b–d** Blots of 2D gels similar to that in **a** and incubated with: **b** monoclonal keratin antibody AE5, **c** GFAP antibodies, and **d** a mixture of AE5 and GFAP antibodies. After incubation with either AE5 (**b**) or GFAP antibodies (**c**) three spots are seen in the same region of the immunoblots. The three spots stained by AE5 and GFAP antibodies are identical, as shown after incubation of the blots with a mixture of AE5 and GFAP antibodies (**d**); *arrowheads* in **a** indicate the position of these spots on the silver-stained gel

39] is reproducible on astrocytoma tissue sections that have been neither fixed with formalin nor treated with proteases. Additionally, we have found that acetone-fixed, frozen sections of normal brain tissue stain with AE3 and AE5, and that both antibodies label normal and neoplastic astrocytes, as shown by double-label immunofluorescence with GFAP antibodies. Of all the other keratin antibodies we have tested, the only other one that also stains astrocytoma tissue sections is KS-1A3. This antibody, however, does not stain normal brain. Double-label immunofluorescence of astrocytoma tissue sections shows that the staining pattern of KS-1A3 is clearly different from that obtained with GFAP antibodies, suggesting that KS-1A3 labels tumour cells devoid of GFAP or neuronal remnants trapped within the tumour.

We have used Western blotting to determine whether or not the staining of normal brain and astrocytomas with the keratin antibodies AE3, AE5 and KS-1A3 is due to presence of keratin polypeptides in these tissues. The AE5 keratin monoclonal antibody has been characterized by immunofluorescence and immunoblotting in epithelial tissues, where it has been found to be specific for K3, the 64-kDa cornea-specific keratin polypeptide [8, 9, 28]. However, this antibody has not been extensively tested in non-epithelial tissues. In either normal brain or astrocytoma samples, AE5 labels a 50 kDa protein that comigrates with GFAP, as determined by 1D and 2D immunoblotting analysis. These results reveal the existence of an epitope common to GFAP and K3, and demonstrate that the immunofluorescent staining of normal and neoplastic astrocytes with AE5 is due its binding to GFAP, but not to K3. The occurrence of epitopes shared by different IF proteins is relatively frequent [8, 13, 44, 45], and is due to the sequence similarities that exist among IF proteins [51].

Despite the positive immunofluorescent staining of normal brain and astrocytomas with AE3 and KS-1A3, both antibodies failed to react with proteins in immunoblots of these tissues. This is similar to what has been previously reported for the monoclonal keratin antibody CAM5.2 [2]. The discrepancy between the immunofluorescence and Western blotting results is not likely to be due to a limiting amount of protein for the immunoblotting procedure. This is suggested by the fact that the immunofluorescent staining seen with AE3 and KS-1A3 was usually bright and present in a large number of cellular processes, a result which typically indicates that the amount of antigen present in the tissue is sufficient for detection with the highly sensitive immunoblotting method. Furthermore, in A431 human epidermoid carcinoma cells, AE3 and KS-1A3 labelled the keratin polypeptides for which they are specific, even when the amount of protein loaded for A431 cells was 20 times less than that present on the same blot for the astrocytoma and normal brain samples. These results indicate that the keratin polypeptides recognized by AE3 and KS-1A3 in epithelial tissues are not present in the normal brain and astrocytoma samples that these antibodies stain by immunofluorescence. It is likely that, in these samples, AE3 and KS-1A3 recognize conformational epitopes created by the interaction of two or more proteins and mimicking the keratin epitopes recognized by these antibodies in epithelial tissues. Such conformational epitopes would not be recognized by AE3 and KS-1A3 in immunoblotting experiments, since they would be destroyed upon treatment of protein complexes with SDS and β-mercaptoethanol, as occurs during sample preparation for gel electrophoresis.

Taken together, the results obtained with our panel of keratin monoclonal antibodies rule out the expression of 16 of the 20 keratin polypeptides in normal brain and in our series of astrocytomas. The presence of K9, K11, K12 and K20 was not tested in these tissues, owing to the lack of commercially available antibodies. However, it is unlikely that normal or neoplastic astrocytes express these keratin polypeptides, as their expression is limited to terminally differentiated epithelia, such as the suprabasal layers of the epidermis for K9 and K11 [36], the cornea for K12 [8, 9, 28, 36] and epithelial cells of the gastrointestinal tract for K20 [6, 37].

It should be stressed that our results do not exclude the possibility that, in rare cases, astrocytomas may express keratin polypeptides. For instance, keratin polypeptides could be present in astrocytomas displaying a pat-

tern of epithelial metaplasia [25, 38] or in astrocytomas that have reversed to a phenotype similar to that of neuroectodermal cells, as this cell type expresses keratin until the completion of neural tube formation [22, 43]. Alternatively, keratin polypeptides could be expressed by astrocytomas derived only from certain regions of the brain, as suggested by the detection of a keratin-like protein in a subclass of cerebellar astrocytes in the hamster [15]. Interestingly, in various lower vertebrates, the presence of keratin polypeptides related to mammalian K8 and K18 has been reported in specific regions of the brain or spinal cord [17, 21, 33, 46]. The possibility that some gliomas may contain keratin polypeptides is also raised by the finding that A172 astrocytoma cells contain K18 mRNA and protein [11] and that two oligodendroglioma cell lines express K7 and K8 mRNA, but not protein [23, 24]. It is not clear, however, whether these cells are derived from gliomas that were already expressing keratin, or whether keratin expression appeared after the establishment of these cells as permanent lines.

Finally, a practical consequence of our study is that a positive immunohistochemical staining of normal brain or glioma tissues with keratin antibodies is not necessarily proof of keratin expression. It is important that this be remembered when keratin antibodies are used for the recognition of poorly differentiated carcinomas metastasizing to the brain or for discrimination between undifferentiated gliomas and tumours of non-glial origin. Clearly, it is necessary to use a combination of morphological and biochemical techniques for conclusive identification of keratin expression in normal and pathological brain tissues and, more generally, for the study of the IF protein composition of gliomas.

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