Virchows A Pathol Anat (1988) 413:325–332

Virchows Archiv A
Pathological Anatomy and Histopathology

Immunohistochemical investigation of collagen subtypes in human glioblastomas

W. Paulus[1], **W. Roggendorf**[1], **and D. Schuppan**[2]

[1] Institute of Brain Research, University of Tübingen, Calwer Strasse 3, D-7400 Tübingen, Federal Republic of Germany
[2] Steglitz Medical Center, Department of Medicine,
Free University of Berlin, D-1000 Berlin 41, Federal Republic of Germany

Summary. The immunohistochemical distribution of a spectrum of collagens and procollagens was studied in 16 glioblastomas. Anti-collagen IV antibodies frequently outlined thickened or disrupted basement membranes (BM) of tumour vessels. Glial BM were frequently penetrated by tumour cells; endothelial BM were not. Some proliferating vessels did not stain for extracellular collagen IV but were rimmed by collagen IV-positive cells, some of which expressed GFAP. Procollagen I was restricted to proliferating leptomeninges and pathological tumour vessels. Collagen III and procollagen III were codistributed in intratumoural and extratumoural interstitial connective tissue. Collagen VI was most pronounced in the adventitia of normal vessels and in spindle-cell proliferations of pathological vessels but not in the endothelial cell proliferations. On the basis of our findings, we conclude that glial cells play a major role in BM formation around tumour vessels, that procollagen I may serve as a marker for proliferation of interstitial connective tissue, and that the origin of spindle-cell proliferation is adventitial, rather than endothelial.

Key words: Glioblastoma – Collagen – Procollagen – Basement membrane – Extracellular matrix

Introduction

Collagens are the major components in most extracellular matrices (ECM). At least 11 genetically distinct collagen types have been characterized to date, each of which is endowed with distinct chemical, immunological and functional properties as well as a characteristic tissue localization (see Table 1). Glioblastomas, the most malignant gliomas, account for 13% to 20% of all intracranial tumours (Peiffer 1984). They express relatively large amounts of connective tissue, and are referred to as gliosarcomas in those cases with suspected neoplastic transformation of mesenchymal proliferation. Characterization of components of the extracellular matrix in gliomas is interesting since ECM's are involved in important cellular functions such as differentiation, proliferation, migration, and adhesion, in both the normal nervous system (Carbonetto 1984) and extracranial tumours (Liotta et al. 1983). Furthermore, Rutka et al. (1987a) demonstrated that the collagenous composition of ECM may influence the proliferation and differentiation of glial tumour cells in vitro. Moreover, ECM proteins appear to play an important

Table 1. Investigated collagen types

Type	Chain composition	Localization
I	$\alpha1\ (I)_2\ \alpha2(I)$, $\alpha1(I)_3$	ubiquitous major interstitial fibrils
II	$\alpha1(II)_3$	fibrils of hyaline cartilage, vitreous
III	$\alpha1(III)_3$	minor interstitial fibrils
IV	$\alpha1(IV)_2\ \alpha2(IV)$	basement membranes
VI	$\alpha1(VI)\ \alpha2(VI)\ \alpha3(VI)$	interstitial microfibrils

Eleven chemically and immunologically distinct collagen subtypes have been described (I–XI). Ultrastructural correlation or typical localization of the investigated collagen types are indicated. Each collagen molecule is composed of three α-chains. Type I, which is usually codistributed with type III collagen, constitutes 90% of the total collagen. Procollagens with additional propeptides are precursors of the respective collagen types (For further details, see Schuppan and Hahn 1987)

Offprint requests to: W. Paulus

role in the antigenicity of gliomas (Mc Comb and Bigner 1985a). Further, since gliomas rarely metastasize outside the CNS and since interruption of the basement membrane (BM), as in extracerebral tumours, indicates progressive infiltration, labelling of BM-specific proteins could help to clarify the presence or absence of extraneural metastases in malignant gliomas.

Finally, since some types of interstitial collagen show a certain localization, the presence of these types may allow histogenetic conclusions to be drawn regarding mesenchymal proliferations.

Whereas several investigations on the BM proteins collagen IV and laminin have been published (Schiffer et al. 1984; Bellon et al. 1985; Giordana et al. 1985; McComb and Bigner 1985b; Rutka et al. 1987b), few studies are available on interstitial collagen types in gliomas (collagens I, III, V: Bellon et al. 1985; collagen VI: Mc Comb et al. 1987). We used immunohistochemical methods with well-characterized affinity-purified or monoclonal antibodies to examine the distribution of collagens II, III, IV, and VI as well as procollagens I and III in human glioblastomas.

Materials and methods

16 formalin-fixed surgical biopsies of human glioblastomas with multiple areas of connective tissue proliferation were investigated. The paraffin blocks were stored for not more than 1 year. HE- and van Gieson-stained sections were subjected to histopathological examination.

For immunohistochemistry 4 µm-thick deparaffinized sections were pretreated with 0.05% trypsin in 0.1% $CaCl_2$ for 20 min at 37° C. Prior to incubation with the first antibody, the sections were incubated for 30 min with diluted normal serum of the species from which the second antibody was derived. The monoclonal or monospecific polyclonal anticollagen antibodies used in this study are listed in Table 2. Incubation was performed for 24 h at 4° C. The detection systems used were the standard peroxidase-anti-peroxidase (PAP) method (Sternberger 1986) and a modified alkaline phosphatase-anti-alkaline phosphatase (APAAP) technique (Cordell et al. 1984). The substrates used were diaminobenzidine (brown) for peroxidase and neofuchsin (red) for alkaline phosphatase. Endogenous peroxidase was blocked with 1% H_2O_2 in methanol for 15 min and intrinsic phosphatase with levamisol/tetramisol. In selected cases, successive double immunoenzymatic staining (Mason et al. 1983) was used to visualize two antigens in one cell of the same section (1. GFAP: monoclonal, Dako, 1:50, APAAP; 2. collagen IV or procollagen I: rabbit polyclonal, PAP). All sections were counterstained with hemalaun. For negative controls, the primary antibody was replaced by nonimmune serum of the respective species. Granulation tissue (procollagen I), costal cartilage (collagen II), cutis (procollagen III, collagen III) and aorta (collagen VI) served as positive controls.

Isolation, preparation and characterization of antigens, production of antisera as well as purification and characterization of antibodies have been described in detail elsewhere (Schuppan et al. 1985, 1986; Becker et al. 1986, 1987; see Table 2).

Table 2. Specificity and technical data of primary antibodies

Antigen	Antibody		
	species	concent.	dilution
Monkey procollagen I (PNIP)[4]*	rabbit	50 µg/ml	1:75
Bovine collagen II, nasal septum[1]	goat	1 mg/ml	1:50
Monkey procollagen III (PNIIIP)[4]**	rabbit	100 µg/ml	1:75
Collagen III[2]	mouse	2 mg/ml	1:500
Human collagen III, plac. villi[1]	goat	1 mg/ml	1:600
Human collagen IV (NC1-domain)[6]	rabbit	300 µg/ml	1:1000
Human collagen IV (7S-domain)[3]	goat	300 µg/ml	1:1200
Human collagen IV (NC1-domain)[6]	mouse	2 mg/ml	1:1500
Human collagen VI[5,3]	rabbit	60 µg/ml	1:100

[1] purchased from Bio-Nuclear Services Ltd., Reading, Great Britain
[2] donated by Dr. Bunte, Heyl-Pharma, Berlin, Federal Republic of Germany
[3] Becker et al. (1986)
[4] Becker et al. (1987)
[5] Schuppan et al. (1985)
[6] Schuppan et al. (1986)
Antibodies reactive only with amino-propeptide of *procollagen I or **procollagen III

Results

All investigated glioblastomas showed nuclear and cytoplasmic pleomorphism, pseudopalisading around areas of necroses, and pronounced vessel-associated mesenchymal proliferations. These proliferations, which are referred to as pathological vessels, appear either in the form of endothelial buds or fibroblast-like spindle cells. Variously extensive spindle-cell proliferations were observed in 13 tumours. These proliferations were not dependent on similar fibroblastic tissue reactions to necroses or to leptomeningeal spread of the tumour. Additional peritumoural parenchymal or non-tumoural leptomeningeal tissue was present in 11 cases. Immunohistochemical results are summarized in Table 3.

Basement membranes (BM) of extratumoural tissues and intratumoural capillaries as well as lacunar, proliferated, and necrotic tumour vessels were well delineated with each of the monoclonal and polyclonal antibodies against the N- and the C-terminal crosslinking domains of collagen IV (Fig. 1a). The number of vessels, especially capil-

Table 3. Distribution of collagen and procollagen types

	pI	II	pIII	III	IV	VI
Extratumoural arteries	−	−	++	++	++	++
Extratumoural capillaries	−	−	(+)	(+)	+	−
Intratumoural capillaries	(+)	−	(+)	(+)	++	(+)
Endothelial proliferations	+	−	+	+	++	−
Spindle-cell proliferations	++	−	++	++	(+)	++
Normal leptomeninges	−	−	+	+	+	++
Infiltrated leptomeninges	+	−	++	++	+	++
Glial cell cytoplasm	−	−	−	−	(+)	−

− = no staining. (+) = inconsistent staining or staining of few cases; + = weak staining of most or all examined structures; ++ = strong staining of most or all examined structures; pI = procollagen I; pIII = procollagen III

laries, was higher in the tumours than in normal brain. Frequently, staining for collagen IV was broadened. The endothelial BM was occasionally separated from the glial BM, which was sometimes accompanied by lymphocytic infiltrates in the space between endothelial and glial BM. Endothelial proliferations were embedded in abundant extracellular collagen IV. No extracellular collagen IV and only a few cells with a positive cytoplasmic reaction were found in the perivascular spindle-cell proliferations.

Scattered areas without linear marking of the BM were observed in proliferating endothelial buds of 5 tumours; most of these buds contained cells with intracellular collagen IV (Fig. 1b). Double staining revealed GFAP in the cytoplasm of some of those cells at the glial-endothelial border (Fig. 2a). Scattered endothelial proliferations were completely negative for collagen IV. A GFAP-collagen IV coexpression was also detected in several giant cells bordering extensive mesenchymal proliferations (Fig. 2b); GFAP-negative and collagen-IV-positive giant cells were also present. Frequently, focal disruptions of the glial BM with an outgrowth of glial tumour cells between the two membranes were observed (Fig. 2c, d). No penetration of the endothelial BM by glial tumour cells was detectable. Endothelial proliferations were especially pronounced at sites where they were apposed to infiltrating tumour cells, penetrating the glial BM.

No PNIP, the amino-propeptide of procollagen I, was detected in unaltered leptomeninges, meningeal vessels, or normal peritumoural tissue. It, however, was present in infiltrated leptomeningeal areas with marked fibrosis as well as in endothelial and spindle-cell proliferation (Fig. 3a, b). PNIP was usually localized intracellularly with weaker extracellular staining, especially in vessels with fibrosis. Double staining of selected cases with GFAP revealed the absence of PNIP in glial tumor cells.

No collagen II was found in any of the examined tissues.

As established by monoclonal as well as polyclonal antibodies, PNIIIP, the amino-propeptide of procollagen III, and collagen III, were always codistributed in both tumoural and extratumoural interstitial connective tissue. The labeled regions corresponded with those stained by conventional trichrome methods (e.g., van Gieson).

Collagen VI was seen in normal leptomeninges, but was more pronounced in the adventitial layer of arteries and venous walls, whereas extratumoural capillaries and intimal and medial layers of normal arteries showed no immunoreactivity. Collagen VI was also absent in endothelial proliferations proper but present adjacent to endothelial proliferations of small and large vessels in the adventitia (Fig. 4a, b). Abundant collagen VI was interwoven with spindle-cell proliferations (Fig. 4c).

Discussion

We studied the distribution of collagen types II, III, IV, and VI as well as procollagens I and III (aminoterminal propeptides) in glioblastomas abundant in connective tissue. A glioblastoma with presumed neoplastic transformation of connective tissue cells is referred to as a gliosarcoma or a glioblastoma with sarcomatous component. However, the histological criteria used to differentiate sarcomatous from hyperplastic proliferations (e.g. extent, cell type, temporal or topographic relation to glial portion), are specified rather differently by different authors (Rubinstein 1972; Jellinger 1978; Morantz et al. 1976; Schiffer et al. 1984). We, therefore, did not attempt to classify particular tumours either as glioblastoma or gliosarcoma.

Collagen IV, together with laminin and heparan sulfate proteoglycan, is a major component of all basement membranes (BM). In the normal central nervous system, BM are confined to vessels, epithelium of the choroid plexus, and glia limitans externa, which invests the entire cortical surface. BM were frequently thickened or disrupted in pathologic vessels and around proliferating endothelial cells of glioblastomas, which was already described previously (Schiffer et al. 1984, Bellon et al. 1985, Giordana et al. 1985, McComb and Bigner 1985b, Rutka et al. 1987b).

By double staining, we were able to locate collagen IV in the cytoplasm of GFAP-positive cells

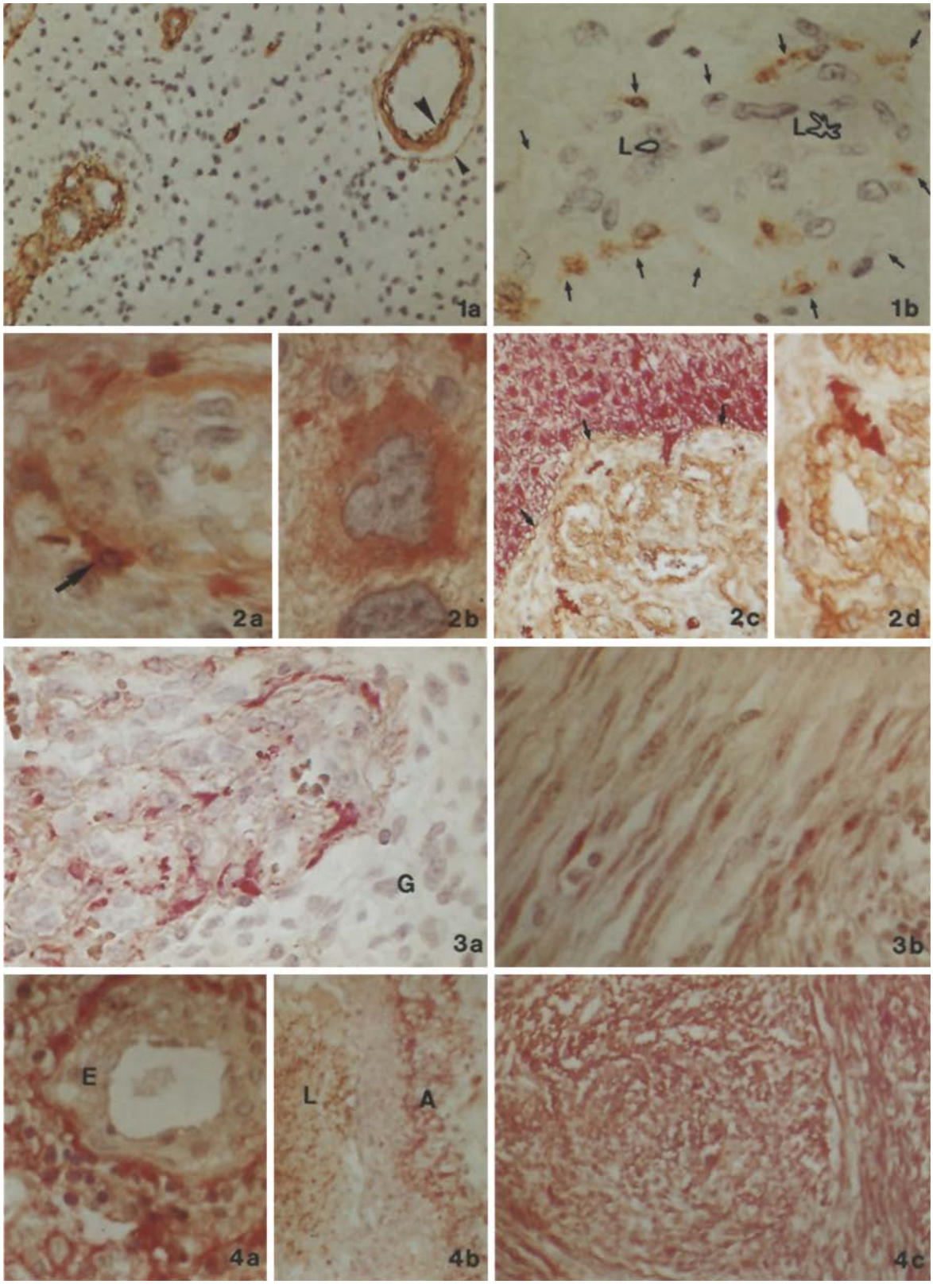

Fig. 1a, b. Collagen IV. **a** Basement membranes (BMs) of pathological vessels in glioblastoma outlined by collagen IV-antibodies. Note thickened endothelial BM (*large arrow*) separated from glial BM (*small arrow*), (rabbit anti-collagen IV, PAP, ×140). **b** Intracellular staining for collagen IV around a pathological vessel with endothelial proliferations. No extracellular BM collagen IV is visible. *L*=vessel lumen, indicated with indian ink, *Arrows*=extent of endothelial proliferation, (rabbit anti-collagen IV, PAP, ×350)

adjacent to endothelial proliferations and in GFAP-positive giant cells bordering mesenchymal tissues. These cells deposited no or, at best, little extracellular collagen IV. Given the identical results with each of the three applied anti-collagen IV antibodies, we consider this reaction to be specific and suggest that the periendothelial GFAP-positive cells are collagen IV-producing glial tumour cells. We, however, cannot exclude with certainty the phagocytosis of GFAP by mesenchymal giant cells. Although BM components in human astrocytes have not been described in vivo, some data indicate in vitro production of laminin and collagen IV by astrocyte and astrocytoma cell cultures (Liesi et al. 1983; Alitalo et al. 1983). Liesi et al. (1984) demonstrated that reactive rat astrocytes produce laminin in vivo. Astrocytes engaged in BM formation were observed in organotypic cultures of mouse spinal cord (Kusaka et al. 1985) and in "gliomas with adenoid components" (Kepes et al. 1985). Presumably, astrocytes can be induced to produce BM components on contact with mesenchymal tissues, e.g. during scar formation (Bernstein et al. 1985) or in glioblastomas (see above). Although BM have several other components in addition to collagen IV, the intracellular presence of collagen IV and GFAP emphasizes the important role played by glial cells in BM formation.

Electron microscopic data on vascular infiltration of glioma cells are contradictory. On the one hand, widely patent interendothelial junctions, connections between extracellular space and lumen of pathological vessels (Long 1970), and even free intravascular glial tumour cells (Kung et al. 1969) have been reported; while on the other hand investigators such as Hirano and Matsui (1975) and Weller et al. (1977), have observed no such abnormalities. Even though many theories have been proposed (Hoffman and Duffner 1985), the rare occurrence of metastazing gliomas remains unclarified. Our results (i.e., frequent penetration of glial BM but not endothelial BM, by tumour cells and the focal absence of any immunohistochemically detectable BM in some pathological vessels) tend to indicate that the endothelial cells, rather than the BM, play an important role as barriers against infiltrating tumour cells. It is noteworthy that tight junctions between endothelial cells and glial tumour cells which could prevent infiltration, were demonstrated by Tani and Ametani (1971).

We found extracellular codistribution of procollagen III and collagen III in reactively fibrosed and tumour tissue as well as all normal cerebral interstitial tissue compartments. Procollagen III and collagen III have been demonstrated in all vessel wall layers of various tissues (Shekhonin et al. 1985; Voss and Rauterberg 1986; Mayne 1986). While we found no procollagen I in normal vessels or unaltered leptomeninges, it was pronounced in extratumoural and intratumoural interstitial connective tissue proliferations. As previously demonstrated in bone (Becker et al. 1987), procollagen I may serve as an immunohistochemical marker of active de novo synthesis of collagen I, the primary component of interstitial collagen, also in gliomas. The different distribution of the procollagens may be explained by incomplete removal of the amino-propeptide from the surface of collagen III fibrils (Sato et al. 1986), whereas the amino-propeptide of procollagen I is released in an early phase of fibrillogenesis (Fleishmajer et al. 1981).

Collagen II has been localized in hyaline cartilage, nucleus pulposus of the vertebral disc, and vitreous body of the eye. Cartilage has occasionally been found in gliomas, originating either from connective tissue proliferations in glioblastomas ("gliochondrosarcomas," Tada et al. 1987) or metaplastic astrocytes (Kepes et al. 1984). Since during the morphogenesis of the cartilaginous neurocranium the appearance of collagen II precedes chondrogenesis (Thorogood et al. 1986), incomplete chondrogenesis may be detectable by positive staining for collagen II. Since no collagen II was

Fig. 2a–d. Collagen IV-GFAP double staining. **a** In addition to red GFAP-positive glial cells and brown collagen IV-positive BM, note auburn cells positive for both antigens (*arrow*). (PAP/APAAP, ×460). **b** Giant cell with GFAP-collagen IV coexpression. (PAP/APAAP, ×560). **c** Red, GFAP-positive, infiltrating glial tumor cells between brown endothelial and glial BM indicate disruption of glial BM but preservation of endothelial BM complex. *Arrows*=glial BM, (PAP/APAAP, ×90). **d** Infiltrating tumour cells not invested by glial BM; glial invaginations, therefore, excluded (magnification of *c*). (PAP/APAAP, ×220)

Fig. 3a, b. Procollagen I. **a** Weak extracellular and marked intracellular reaction for amino-propeptide of procollagen I in endothelial proliferations. Glial tumour portion (*G*) is devoid of procollagen I. (APAAP, ×350). **b** Spindle-cell proliferations expressing intracellular procollagen I. (APAAP, ×350)

Fig. 4a–c. Collagen VI. Collagen VI restricted to adventitial portion of small (**a**) and large (**b**) pathological tumour vessels as well as adjacent tissue; endothelial proliferations devoid of collagen VI. Collagen VI prominent between spindle-cell proliferations (**c**). *L*=lumen, *E*=endothelium, *A*=adventitia, (APAAP, a) ×350, b) ×90, c) ×90)

detected in mesenchymal proliferations or around glial cells in the examined tissue, chondrodifferentiation appears to be rare in gliomas.

Collagen VI is found in most, if not all, interstitial connective tissues (von der Mark et al. 1984; Hessle and Engvall 1984; Linsenmayer et al. 1986; Becker et al. 1987). Our findings substantiate its absence in the BM of glioblastomas and of normal cerebrum (Roggendorf et al. 1988). McComb et al. (1987), who applied a monoclonal antibody, however, reported the presence of collagen VI in the pial-glial BM of normal cerebrum and glioblastomas as well as in interstitial tissue. These contradictory results reported by McComb et al. could be due to the inability of the light microscope to differentiate the BM proper from the adjacent connective tissue (Martinez-Hernandez and Amenta 1983). Using immunoelectron microscopy, Von der Mark et al. (1984) demonstrated that collagen VI microfibrils often run perpendicular to and in between the major collagen fibrils, which are composed of collagens I and III. This finding suggests that collagen VI plays a role in fibril organization and stabilization.

In addition to endothelial proliferations, glioblastomas or gliosarcomas frequently harbour vessel-associated mesenchymal spindle-cell proliferations (SCP) of unknown histogenesis that resemble fibrosarcomas when they occupy large areas. Endothelial cells are usually considered to be the precursor cells (Feigin et al. 1958; Morantz et al. 1976; Schiffer et al. 1984; Slowik et al. 1985), but corroborating experimental evidence is lacking. Factor VIII-related antigen, a marker for endothelial cells, is absent from SCP (McComb et al. 1982; Slowik et al. 1985), whereas antiproteases ("monohistiocytic markers", Kochi and Budka 1987) and smooth muscle myosin (Kishikawa et al. 1986) have been detected. While endothelial cells may adopt a fusiform shape in vitro (Madri et al. 1983) and spindle cells in angiosarcomas like Kaposi's sarcoma may express endothelial cell markers (Hashimoto et al. 1987), extracerebral endothelial sarcomas with the histologic picture of pure fibrosarcomas have not been described. Our immunohistochemical results, that is a selective localization of collagen VI in the adventitia of normal vessels (Roggendorf et al. 1988) and in SCP versus the absence of collagen VI in proliferating buds of endothelial cells, argue strongly against an endothelial origin of SCP. This presumption is supported by cell culture studies demonstrating that endothelial cells synthesize collagen types I, III, IV, and V (Sage et al. 1981; Kramer et al. 1985) but, in contrast to collagen VI-containing cultures from fibroblasts (Bruns et al. 1986) and muscle cells (Engvall et al. 1986), lack collagen type VI (Hessle and Engvall 1984; Mayne 1986). We conclude that adventitial fibroblasts, myofibroblasts, or smooth muscle cells are precursor cells of SCP.

Our data indicate that immunohistochemical collagen typing of brain tumours may increase our knowledge of cell-matrix interactions and help us understand the histogenesis of intratumoral mesenchymal tissues. As more and more information is gathered on the selective distribution of collagen types, collagen typing may become a useful tool in the differential diagnosis of neoplastic lesions of the brain.

References

Alitalo K, Bornstein P, Vaheri A, Sage H (1983) Biosynthesis of an unusual collagen type by human astrocytoma cells in vitro. J Biol Chem 258:2653–2661

Becker J, Schuppan D, Hahn EG, Albert G, Reichart P (1986) The immunohistochemical distribution of collagens type IV, V, VI and of laminin in the human oral mucosa. Arch Oral Biol 31:179–186

Becker J, Schuppan D, Benzian H, Bals T, Hahn EG, Cantaluppi C, Reichart P (1987) Immunohistochemical distribution of collagens types IV, V, and VI and of pro-collagens types I and III in human alveolar bone and dentine. J Histochem Cytochem 34:1417–1429

Bellon G, Caulet T, Cam Y, Pluot M, Poulin G, Pytlinska M, Bernard MH (1985) Immunohistochemical localization of macromolecules of the basement membrane and extracellular matrix of human gliomas and meningiomas. Acta Neuropathol (Berl) 66:245–252

Bernstein JJ, Getz R, Jefferson M, Keleman M (1985) Astrocytes secrete basal lamina after hemisection of rat spinal cord. Brain Res 327:135–141

Bruns RR, Press W, Engvall E, Timpl R, Gross J (1986) Type VI collagen in extracellular, 100-nm periodic filaments and fibrils: identification by immunoelectron microscopy. J Cell Biol 103:393–404

Carbonetto S (1984) The extracellular matrix of the nervous system. TINS 7:382–387

Cordell JL, Falini B, Erber WN, Ghosh AK, Abdulaziz Z, MacDonald S, Pulford KAF, Stein H, Mason DY (1984) Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). J Histochem Cytochem 32:219–229

Engvall E, Hessle H, Klier G (1986) Molecular assembly, secretion, and matrix deposition of type VI collagen. J Cell Biol 102:703–710

Feigin I, Allen LB, Lipkin L, Gross SW (1958) The endothelial hyperplasia of the cerebral blood vessels with brain tumors, and its sarcomatous transformation. Cancer 11:264–277

Fleishmajer R, Timpl R, Tudermann L, Raisher L, Wiestner M, Perlish JS, Graves PN (1981) Ultrastructural identification of extension aminopropeptides of type I and III procollagen in human skin. Proc Natl Acad Sci (USA) 78:7360–7364

Giordana MT, Germano I, Giaccone G, Mauro A, Migheli A, Schiffer D (1985) The distribution of laminin in human brain tumors: an immunohistochemical study. Acta Neuropathol (Berl) 67:51–57

Hashimoto H, Müller H, Falk S, Stutte HJ (1987) Histogenesis of Kaposi's sarcoma associated with AIDS. A histologic, immunohistochemical and enzyme histochemical study. Path Res Pract 182:658–668

Hessle H, Engvall E (1984) Type VI collagen. Studies on its localization, structure, and biosynthetic form with monoclonal antibodies. J Biol Chem 259:3955–3961

Hirano A, Matsui T (1975) Vascular structures in brain tumors. Hum Pathol 6:611–621

Hoffman HJ, Duffner PK (1985) Extraneural metastases of central nervous system tumors. Cancer 56:1778–1782

Jellinger K (1978) Glioblastoma multiforme: morphology and biology. Acta Neurochi (Wien) 42:5–32

Kepes JJ, Rubinstein LJ, Chiang H (1984) The role of astrocytes in the formation of cartilage in gliomas. An immunohistochemical study of four cases. Am J Pathol 117:471–483

Kepes JJ, Sher J, Oliver MG (1985) Light and electron microscopic study of "adenoid" components in gliomas. Fibroblastic activity of neoplastic astrocytes. J Neuropathol Exp Neurol 44:359

Kishikawa M, Tsuda N, Fuji H, Nishimori I, Yokoyama H, Kihara M (1986) Glioblastoma with sarcomatous component associated with myxoid change. Acta Neuropathol (Berl) 70:44–52

Kochi N, Budka H (1987) Contribution of histiocytic cells to sarcomatous development of the gliosarcoma. Acta Neuropathol (Berl) 73:124–130

Kramer RH, Fuh G-M, Karasek MA (1985) Type IV collagen synthesis by cultured human microvascular endothelial cells and its deposition into the subendothelial basement membrane. Biochemistry 24:7423–7430

Kung PC, Lee JC, Bakay L (1969) Vascular invasion by glioma cells in man: an electron microscopic study. J Neurosurg 31:339–345

Kusaka H, Hirano A, Bornstein MB, Raine CS (1985) Basal lamina formation by astrocytes in organotypic cultures of mouse spinal cord tissue. J Neuropathol Exp Neurol 44:295–303

Liesi P, Dahl D, Vaheri A (1983) Laminin is produced by early rat astrocytes in primary culture. J Cell Biol 96:920–924

Liesi P, Kaakkola S, Dahl D, Vaheri A (1984) Laminin is induced in astrocytes of adult brain by injury. EMBO J 3:683–686

Linsenmayer TF, Mentzer A, Irwin MH, Waldrep NK, Mayne R (1986) Avian type VI collagen. Monoclonal antibody production and immunohistochemical identification as a major connective tissue component of cornea and skeletal muscle. Exp Cell Res 165:518–529

Liotta LA, Rao CN, Barsky SH (1983) Tumor invasion and the extracellular matrix. Lab Invest 49:636–649

Long DM (1970) Capillary ultrastructure and the blood-brain barrier in human malignant brain tumors. J Neurosurg 32:127–144

Madri JA, Williams SK, Wyatt T, Mezzio C (1983) Capillary endothelial cell cultures: phenotypic modulation by matrix components. J Cell Biol 97:153–165

Mark H von der, Aumailley M, Wick G, Fleischmajer R, Timpl R (1984) Immunohistochemistry, genuine size and tissue localization of collagen VI. Eur J Biochem 142:493–502

Martinez-Hernandez A, Amenta PS (1983) The basement membrane in pathology. Lab Invest 48:656–677

Mason DY, Abdulaziz Z, Falini B, Stein H (1983) Double immunoenzymatic labelling. In: Polak JM, van Noorden S (eds) Immunocytochemistry. Practical applications in pathology and biology. Wright PSG, Bristol, pp 113–128

Mayne R (1986) Collagenous proteins of blood vessels. Arteriosclerosis 6:585–593

McComb RD, Jones TR, Pizzo SV, Bigner DD (1982) Immunohistochemical detection of factor VIII/von Willebrand factor in hyperplastic endothelial cells in glioblastoma multiforme and mixed glioma-sarcoma. J Neuropathol Exp Neurol 41:479–489

McComb RD, Bigner DD (1985a) Immunolocalization of monoclonal antibody-defined extracellular matrix antigens in human brain tumors. J Neurooncol 3:181–186

McComb RD, Bigner DD (1985b) Immunolocalization of laminin in neoplasms of the central and peripheral nervous systems. J Neuropathol Exp Neurol 44:242–253

McComb RD, Moul JM, Bigner DD (1987) Distribution of type VI collagen in human gliomas: comparison with fibronectin and glioma-mesenchymal matrix glycoprotein. J Neuropathol Exp Neurol 46:623–633

Morantz RA, Feigin I, Ransohoff J (1976) Clinical and pathological study of 24 cases of gliosarcoma. J Neurosurg 45:398–408

Peiffer J (1984) Neuropathologie. In: Remmele W (ed) Pathologie. Vol 4. Springer Berlin Heidelberg New York Tokio, p 253

Roggendorf W, Opitz H, Schuppan D (1988) Altered expression of collagen type VI in brain vessels of patients with chronic hypertension. A comparison with the distribution of collagen IV and procollagen III. Acta Neuropathol (Berl), in press

Rubinstein LJ (1972) Tumors of the central nervous system. Armed Forces Institute of Pathology, Washington, DC, pp 74–78, 198–199

Rutka JT, Giblin JR, Apodaca G, DeArmond SJ, Stern R, Rosenblum ML (1987a) Inhibition of growth and induction of differentiation in a malignant human glioma cell line by normal leptomeningeal extracellular matrix proteins. Cancer Res 47:3515–3522

Rutka JT, Myatt CA, Giblin JR, Davis RL, Rosenblum ML (1987b) Distribution of extracellular matrix proteins in primary human brain tumours: an immunohistochemical analysis. Can J Neurol Sci 14:25–30

Sage H, Pritzl P, Bornstein P (1981) Secretory phenotypes of endothelial cells in culture: comparison of aortic, venous, capillary, and corneal endothelium. Arteriosclerosis 1:427–442

Sato S, Leo MA, Lieber CS (1986) Ultrastructural localization of type III procollagen in baboon liver. Am J Pathol 122:212–217

Schiffer D, Giordana MT, Mauro A, Migheli A (1984) GFAP, FVIII/RAg, laminin, and fibronectin in gliosarcomas: an immunohistochemical study. Acta Neuropathol (Berl) 63:108–116

Schuppan D, Rühlmann T, Hahn EG (1985) Radioimmunoassay for human type VI collagen and its application to tissue and body fluids. Analyt Biochem 149:238–247

Schuppan D, Besser M, Schwarting R, Hahn EG (1986) Radioimmunoassay for the carboxy-terminal cross-linking domain of type IV (basement membrane) procollagen in body fluids. J Clin Invest 78:241–248

Schuppan D, Hahn EG (1987) Components of the extracellular matrix (collagens, elastin, glycoproteins and proteoglycans). In: Wolff JR et al. (eds.) Mesenchymal-epithelial interactions in neural development. NATO ASI series, vol. H5. Springer, Berlin Heidelberg New York Tokyo, pp 3–29

Shekhonin BV, Domogatsky SP, Muzykantov VR, Idelson GL, Rukosuev VS (1985) Distribution of type I, III, IV and V collagen in normal and atherosclerotic human arterial wall: immunomorphological characteristics. Coll Rel Res 5:355–368

Slowik F, Jellinger K, Gaszó L, Fischer J (1985) Gliosarcomas:

histological, immunohistochemical, ultrastructural, and tissue culture studies. Acta Neuropathol (Berl) 67:201–210

Sternberger LA (1986) Immunocytochemistry. Wiley, New York Chichester Brisbane Toronto. 3rd edition. pp 90–209

Tada T, Katsuyama T, Aoki T, Kobayashi S, Shigematsu H (1987) Mixed glioblastoma and sarcoma with osteo-chondral tissue. Clin Neuropathol 6:160–163

Tani E, Ametani T (1971) Intercellular contacts of human gliomas. In: Zimmerman HM (ed) Progress in neuropathology. Vol 1. Grune And Stratton, New York London, pp 218–231

Thorogood P, Bee J, von der Mark K (1986) Transient expression of collagen II at epitheliomesenchymal interfaces during morphogenesis of the cartilaginous neurocranium. Dev Biol 116:497–509

Voss B, Rauterberg J (1986) Localization of collagen types I, III, IV and V, fibronectin and laminin in human arteries by the indirect immunofluorescence method. Path Res Pract 181:568–575

Weller RO, Foy M, Cox S (1977) The development and ultrastructure of the microvasculature in malignant gliomas. Neuropathol Appl Neurobiol 3:307–322

Accepted May 25, 1988