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ORIGINAL ARTICLE

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Expression of type VII collagen, the major anchoring fibril component, in normal and neoplastic human nervous system

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Abstract The distribution of type VII collagen was examined in the normal human nervous system, in brain tumour biopsies and in glioma cell lines by immunohistochemistry and western blotting. In normal tissue, positivity was observed beneath choroid plexus epithelial cells and around pineal gland and pituitary gland cell nests, while other brain regions and peripheral nerves were negative. Expression was preserved in most related tumours (choroid plexus papilloma, pineoblastoma, pituitary adenoma). Scattered abnormal vessels showed neoexpression of type VII collagen in about half of the astrocytic and ependymal tumours. Glioma cells in situ were consistently negative for type VII collagen, whereas the glioblastoma cell lines were positive. Our results suggest that anchoring fibrils or at least epitopes of their major structural component are present in normal and pathological cerebral structures, indicating a unique distribution of type VII collagen in the nervous system.

Key words Choroid plexus · Extracellular matrix · Gliomas · Pituitary gland · Type VII collagen

Introduction

Type VII collagen represents the major structural protein of anchoring fibrils. These banded fibrils of 20–60 nm diameter and 200–800 nm length tether the lamina densa of some basement membranes (BM) to anchoring plaques of the underlying connective tissue matrix [9, 21]. Type VII collagen has been localized to epithelial BM zones of various tissues including epidermis, cutaneous glands, cornea, larynx, breast, prostate, cervix, vagina and umbilical cord, but it is absent from gastrointestinal, endothelial, muscle and fat BM, suggesting preferential expression beneath combined and stratified epithelia [11, 17, 22]. The expression pattern is retained in most tumours derived from these tissues [4, 22, 23]. The nervous system has not been systematically examined and so we studied the distribution of type VII collagen in brain and peripheral nervous system in normal and neoplastic states.

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Materials and methods

Normal human brain tissue was obtained from two neurologically asymptomatic subjects (ages, 65 and 74 years) within 6 h after death. Regions examined included frontal, temporal, parietal and occipital lobes (cortex, white matter and leptomeninges), basal ganglia, midbrain, pons, medulla oblongata, cerebellum, choroid plexus from lateral ventricle, hypophysis (anterior and posterior lobes) and pineal gland. Sural nerve biopsies from five adult patients with polyneuropathy showing no light microscopical abnormalities (three cases) or moderate loss of myelinated fibers (two cases) were also studied. The following 35 brain tumour biopsies were investigated: 12 glioblastomas, 5 fibrillary astrocytomas, 3 ependymomas, 3 medulloblastomas, 5 schwannomas, 3 pituitary adenomas, 2 choroid plexus papillomas and 2 pineoblastomas. The glioblastoma cell lines U-138MG and U-373MG were purchased from the American Type Culture Collection (Rockville, Md., USA).

Immunohistochemistry was performed using a standard alkaline phosphatase-anti alkaline phosphatase technique with neofuchsin development. Frozen sections 7 μm thick were fixed with acetone for 10 min at –20° C, blocked with normal serum, and incubated with the primary antibodies for 16 h at 4° C. Two antibodies to type VII collagen were used: the mouse monoclonal antibody LH7.2 (dilution 1:100) is directed to the amino-terminal, non-helical, NC-1 domain localized in the lamina densa [11], while the affinity-purified polyclonal rabbit antibody (diluted 1:2) recognizes the triple helical domain of type VII collagen that forms the banded anchoring fibrils [3]. Normal skin served as positive control, and omission of the primary antibodies as negative control.

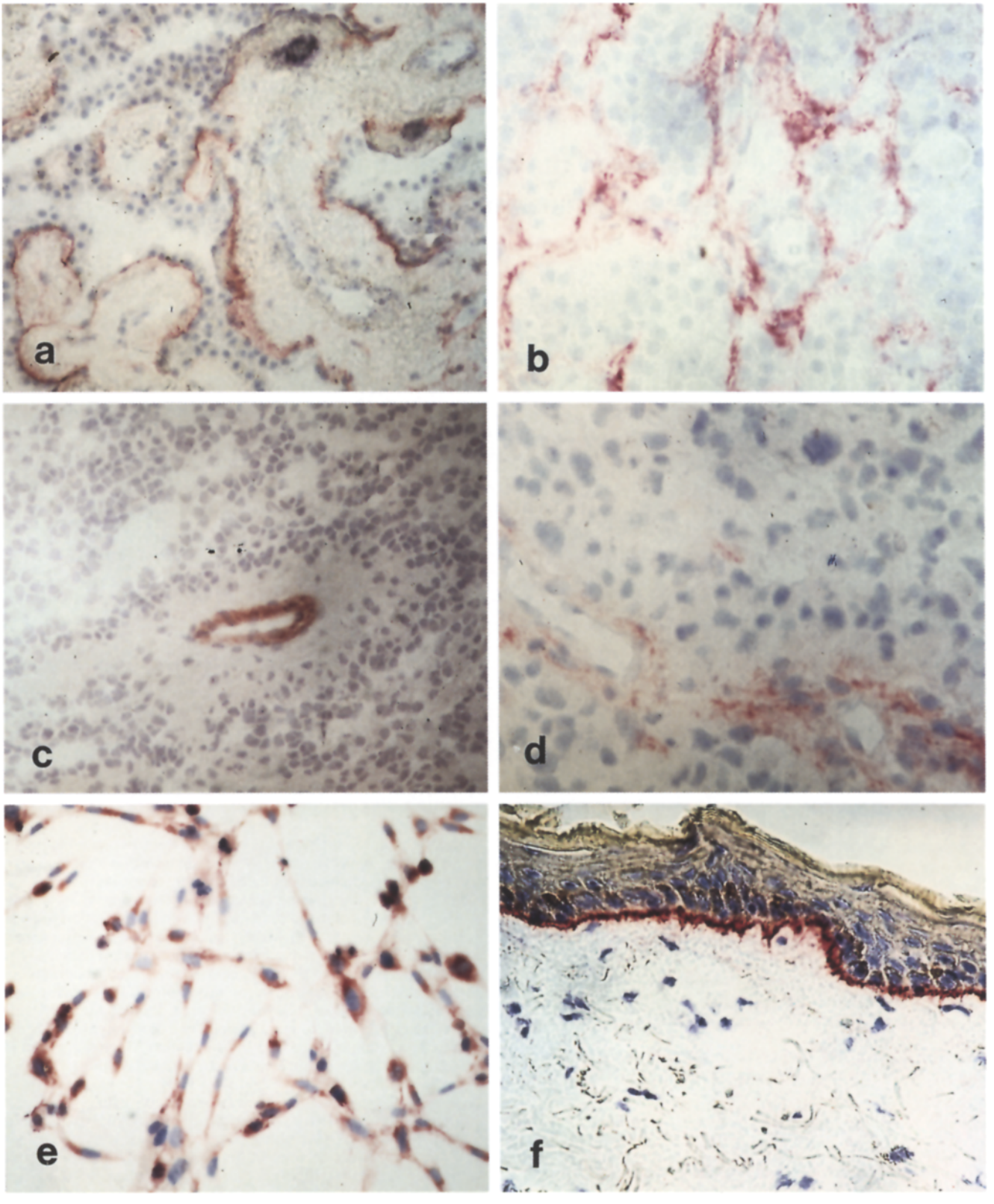

Fig. 1a–f Immunohistochemical distribution of type VII collagen. In normal brain, positivity is seen beneath choroid plexus epithelial cells (**a**) and around pituitary cell nests (**b**). Type VII collagen is present in glioma vessels, as demonstrated in a fibrosed ependymoma vessel wall (**c**) and weakly around glioblastoma endothelial proliferation (**d**). **e** Most cells of the glioblastoma cell line U-138MG express cytoplasmic type VII collagen. **f** In the normal skin, type VII collagen is restricted to the epidermal basement membrane area. **a**, **c** ×125; **b**, **d**, **e**, **f** ×250

For western blotting, the intact tissue form of type VII collagen was extracted from all brain areas of one patient and from five glioblastomas as described [3, 4]. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis was performed using 7.5% gels and a Mini-Protean II apparatus (Bio-Rad, Munich, Germany) under reducing conditions. Proteins were blotted to nitrocellulose at 100 V for 1 h. Sheets were blocked for 16 h with 3% defatted dried milk, incubated with LH7.2 (diluted 1:100) for 2 h at room temperature, and developed using a standard peroxidase antiperoxidase technique with diaminobenzidine development. Epidermal extracts from a normal skin biopsy specimen treated with epidermolysis buffer served as positive control [3]. Prestained molecular weight standards were purchased from Bio-Rad.

Results

The two anti-type VII collagen antibodies gave essentially the same immunohistochemical results. A strong linear subepidermal labelling was observed in the skin biopsy (Fig. 1f). The normal brains showed immunoreactivity at subepithelial BM areas of the choroid plexus (Fig. 1a) and around cell nests of the pineal and pituitary gland (Fig. 1b). When compared with the skin, the staining of these brain structures was weaker and focally absent. All other brain areas were consistently negative, including vascular BM and glia limitans. No staining was observed in the sural nerve.

Focal and weak immunoreactivity for type VII collagen was seen in some of the corresponding neoplastic tissues; in all pituitary adenomas, in one of two choroid plexus papillomas, and in one of two pineoblastomas. The distribution of the staining corresponded to that of the non-neoplastic tissues. While normal cerebral vessels were consistently negative, a distinct positivity for type VII collagen was seen in scattered abnormal vessels in seven glioblastomas, two astrocytomas and two ependymomas. The abnormal vessels showed either fibrosed walls (Fig. 1c) or endothelial proliferation (Fig. 1d). The other tumour biopsy specimens were negative. Immunoreactivity within or around tumour cell cytoplasm was absent in all biopsy specimens. In contrast, most U-138MG cells and about 30% of U-373MG glioma cells expressed cytoplasmic type VII collagen (Fig. 1e).

Immunoblotting revealed a single band of about 250 kDa, which was most prominent in the epidermis, but also clearly present in pituitary gland, pineal gland and choroid plexus (Fig. 2). A faint band was revealed in one of the five glioblastoma biopsies (Fig. 2, lane 4). Two glioblastoma biopsies were positive using immunohistochemistry but negative using western blotting, presumably due to low concentration of protein. The 250 kDa band detected in our samples corresponds to the molecular weight of the intact type VII collagen molecule extracted from various non-nervous tissues [3, 11].

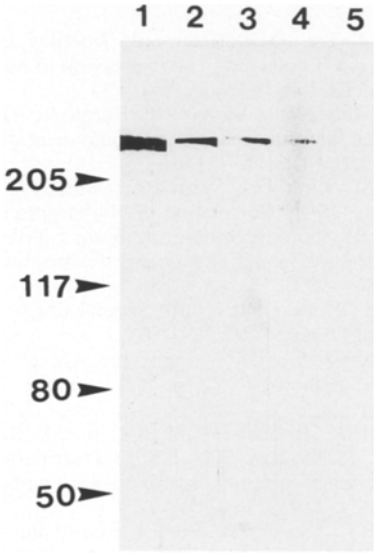

Fig. 2 Immunoblotting of type VII collagen extracts (intact tissue form) from epidermis (lane 1), pituitary gland (lane 2), pineal gland (lane 3) and glioblastoma biopsy specimen (lane 4). Lanes 1–4 were stained with monoclonal antibody LH7.2. Lane 5 is a negative control (incubation of epidermal extracts with normal mouse serum instead of LH7.2). All lanes were loaded with 5.9 µg protein. A band of about 250 kDa of variable intensity is present in lanes 1–4. Positions of molecular weight markers are indicated on the left

Discussion

Interaction of neuroectodermal cells with the extracellular matrix (ECM) is pivotal to various processes taking place in the normal and pathological nervous system, such as differentiation, migration, invasion and proliferation. In the normal brain and in gliomas, collagen types I and III–VI are usually restricted to mesenchymal elements [2, 13, 14], whereas other ECM components like tenascin and glycosaminoglycans may occur within and between individual glioma cells [8, 19]. The present data indicate that type VII collagen has a characteristic and unique distribution.

In the normal brain, type VII collagen expression was restricted to pituitary gland, pineal gland and choroid plexus. Although anchoring fibrils have not been demonstrated electron microscopically in the brain [6, 16], our data suggest that they probably exist in particular areas of the brain, because expression of type VII collagen in normal tissues usually indicates the presence of anchoring fibrils [18].

Absent or rudimentary subepidermal anchoring fibrils due to either mutations in the COL7A1 gene coding for the alpha(VII) chain or due to auto-antibodies to type VII collagen are the pathogenic basis for some subtypes of epidermolysis bullosa [5, 21]. Neither neurological complications attributable to pituitary gland, pineal gland or choroid plexus nor neuropathological abnormalities in these areas have been described in epidermolysis bullosa patients. Growth retardation of these patients is usually explained by chronic malnutrition due to oropharyngeal lesions, malabsorption and protein loss through skin lesions [7]. However, a hypothalamic disorder in secretion of growth hormone has been demonstrated in one patient [12]. It remains to be determined whether defec-

tive cerebral anchoring fibrils are related to endocrinological abnormalities in epidermolysis bullosa patients.

Type VII collagen was absent from normal vessels of the grey and white matter, but it was expressed by scattered abnormal tumour vessels. This finding resembles the distribution pattern of type VIII collagen, which is focally expressed by glioma vessels, but is lacking in normal cerebral vessels [15]. Most previous reports on the expression of type VII collagen described consistent negativity of blood vessels [22], but occasional studies found slight staining of some head, neck and umbilical cord blood vessels [17, 23]. A variety of stimuli may induce type VII collagen synthesis in vitro, including transforming growth factor-β and interactions of vascular cells with neuronal and other non-mesenchymal cells [1, 10]. Similar stimuli may trigger expression of type VII collagen in glioma vessels in vitro.

In accordance with previous immunohistochemical data [22], peripheral nerves did not express type VII collagen. It has been claimed that anchoring fibrils surround Schwann cell BM of unmyelinated nerve fibres [20]. However, the electron microscopical illustrations presented in this report are ambiguous and the results have not been confirmed [6]. Our immunohistochemical data provide further evidence for the absence of true anchoring fibrils in the human peripheral nervous system.

In conclusion, we have demonstrated that type VII collagen is restricted to distinct structures in the normal brain, is preserved in some, but not all tumours, and may be expressed by abnormal glioma vessels in situ and by glioma cell lines.

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