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Immunostaining of a heterodimeric dermatan sulphate proteoglycan is correlated with smooth muscles and some basement membranes

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Abstract A heterodimeric 760-kDa dermatan sulphate proteoglycan tentatively named PG-760 was characterized as a product of keratinocytes, endothelial cells, and fibroblasts. The two core proteins of 460 kDa and 300 kDa are linked by disulphide bridges, and both carry one or only very few dermatan sulphate chains. Different antisera against PG-760 were used in the present study to investigate the distribution in selected murine tissues by light and electron microscopy. PG-760 immunostaining was observed in cornea (epithelium including basement membrane, stroma, and Descemet's membrane), skin, mucosa of the small intestine, Engelbreth-Holm-Swarm (EHS)-tumour (matrix and cells), and the smooth muscle layers of uterus, small intestine, and blood vessels. No staining was observed in capillaries, striated muscles, and liver parenchyma including the central vein. The expression of PG-760 in EHS-tumour was also demonstrated after extraction with 4 *M* guanidine and partial purification by diethylaminoethyl (DEAE)-chromatography. We conclude that this novel proteoglycan exhibits a unique tissue distribution being a constituent of some but not all basement membranes, of some other extracellular matrices, and additionally, of all investigated smooth muscle layers.

Introduction

Proteoglycans are defined as glycoproteins carrying at least one glycosaminoglycan chain, such as chondroitin sulphate, dermatan sulphate, or heparan sulphate. This definition is fulfilled by a wide variety of structurally and functionally diverse macromolecules (for reviews see Hardingham and Fosang 1992; Upholt et al. 1993; Kresse et al. 1993; Timpl 1993; Esko 1991). Proteoglycans are components of pericellular and extracellular matrices and of intracellular storage granules, and may be divided into several different families. (1) Large space-filling proteoglycans e.g. aggrecan and versican, which provide tissues such as cartilage with resistance to compressive forces (Doege et al. 1990; Krusius et al. 1987; Zimmermann and Ruoslahti 1989). (2) Small interstitial proteoglycans such as biglycan and decorin, which interact with a variety of extracellular matrix components and growth factors (Fisher et al. 1989). (3) Lipid-linked and intercalated membrane proteins, respectively such as syndecans containing binding sites for growth factors and other matrix components (David 1993). (4) Intracellular proteoglycans such as serglycins, which form an intravesicular matrix in storage granules (Stevens et al. 1988). (5) Constituents of basement membranes such as perlecan which is an essential component of all basement membranes and is involved in the ionic control of filtration through basement membranes (Noonan et al. 1988; Timpl 1989). In addition to these proteoglycan families quite diverse macromolecules including CD44 (Goldstein et al. 1989; Stamenkovic et al. 1991), fibril-associated collagens such as collagens type IX (Ninomiya and Olsen 1984; Noro et al. 1983; Vaughan et al. 1985), type XII (Koch et al. 1992), type XIV (Watt et al. 1992) and growth factors such as colony stimulating factor-1 CSF-1 (Price et al. 1992) have been shown to occasionally be linked with glycosaminoglycan chains, thus representing "part-time" proteoglycans.

Recently the number of extracellular proteoglycans was enriched by a novel 760-kDa heterodimeric dermatan sulphate proteoglycan (PG-760). The proteoglycan consists of two distinct disulphide cross-linked core proteins of 460 kDa and 300 kDa, respectively. Both subunits carry one or very few dermatan sulphate chains of ~20 kDa exhibiting a similar chemical composition regardless of the subunit from which each was obtained (Breuer et al. 1991). PG-760 is one of the principal proteoglycans of fetal fibroblasts, representing 10–20%

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of [^{35}S]methionine and *c.* 5–10% of [^{35}S]sulphate incorporated into the pool of secreted proteoglycans. Most of it is secreted as heterodimers, but small amounts of monomers were also found (Breuer et al. 1991). Handling of the proteoglycan in solution appeared to be rather difficult. Even in the presence of 0.1% sodium dodecyl sulphate (SDS), the non-reduced proteoglycan forms aggregates in a concentration dependent process (Faber et al. 1992).

In cultures of fetal fibroblasts about two thirds of the extracellular PG-760 pool is bound to the cell surface, whereas the remaining third is found in the medium. The cell surface bound proteoglycan appears as a component of fibrillar structures on the cell body and is present on cellular extensions (Faber et al. 1992). Immunofluorescence studies of cultured fetal fibroblasts, keratinocytes, and endothelial cells revealed a fibrillar and punctuated staining pattern and a remarkable co-localization of PG-760 with fibronectin, laminin, perlecan, and collagen IV (Faber et al. 1992). In an investigation of rat cornea immunofluorescence staining of PG-760 was detected in the epithelium, the stroma, and in Descemet's membrane. Due to the strong fluorescence of the epithelium, whether PG-760 is a component of the epithelial basement membrane could not be ascertained. In Descemet's membrane predominately the junction with the endothelium (inner corneal epithelium) was PG-positive (Faber et al. 1992). In the present study, PG-760 is shown by immunohistochemistry to represent a component of some but not all basement membranes, of some other extracellular matrices, and of all the investigated smooth muscle layers.

Materials and methods

Immunochemical reagents

The production of antisera against PG-760 core proteins has been described previously (Breuer et al. 1991; Faber et al. 1992). Antiserum 1 was raised in a rabbit using the unreduced heterodimeric core protein. The core protein was obtained by digestion of PG-760 with chondroitin ABC lyase, and final purification by SDS-agarose gel electrophoresis. In addition to epitopes on both core proteins this antiserum was highly reactive against the unsaturated polysaccharide protein linkage region which is created by the action of the enzyme (Breuer et al. 1991). Two additional antisera were raised in rabbits against the proteoglycan forms of the 300-kDa (antiserum 2) and 460-kDa subunits (antiserum 3), respectively. The 300-kDa and 460-kDa subunits were obtained after SDS-agarose gel electrophoresis, as previously described for the preparation of the 300-kDa subunit (Faber et al. 1992). As tested by immunoprecipitation followed by SDS-polyacrylamide gel electrophoresis (PAGE) and fluorography of [^{35}S]methionine-labelled fibroblasts, antisera 2 and 3 did recognize both core proteins. Occasionally, the immunoprecipitate contained small amounts of fibronectin, although the antisera did not recognize fibronectin in enzyme-linked immunosorbent assays (ELISA). Most likely fibronectin was precipitated as a "cold insoluble protein". Therefore, antisera 2 and 3 were purified by affinity chromatography on a fibronectin-Sepharose column in order to exclude any possible reactivity towards fibronectin (Faber et al. 1992). The purified antisera recognizes neither fibronectin nor laminin in a dot blot assay (Breuer et al. 1991; Faber et al. 1992). Small quantities of affinity purified polyclonal antibodies were also obtained by affinity purification of antiserum 3 on the antigen immobilized in situ in SDS-polyacrylamide gels (Faber et al. 1992; Madara et al. 1990). Immunoelectron microscopy was performed using antiserum 3. Anti-laminin serum was kindly provided by Dr. Mats Paulsson (M.E. Müller Institute for Biomechanics, University of Berne, Switzerland).

Immunofluorescence

With the exception of Engelbreth-Holm-Swarm (EHS) tumour (Timpl et al. 1979) all tissues were obtained from adult SIV-Z-50 rats. EHS-tumour was obtained from tumour growing mice as described in Schittny and Yurchenco (1990). After the animals were killed with an overdose of anaesthetics, the tissues were immediately removed, briefly rinsed in phosphate buffered saline (PBS, 10 m*M* sodium phosphate, 127 m*M* sodium chloride, pH 7.4), and frozen in liquid nitrogen. Cryosections of 8 to 10 µm in thickness were cut on a Leica AG (Zürich, Switzerland) cryostat-microtome 1720 at –18°C, transferred onto siliconized (aminopropyl-trimethoxy-silane) micro slides, and air dried overnight at room temperature. The sections were rehydrated in PBS containing 5 mg/ml bovine serum albumin (PBS/BSA) for 5–10 min and incubated with the antisera for 1–2 h. Subsequent treatment of three changes of PBS was followed by BSA (15 min) further incubation for 30–60 min with rhodamine labelled second antibody (goat anti-rabbit IgG, Cappel Research Products, Organon Teknika Co., Durham, N.C., USA; 1 mg/ml diluted 1:60 in PBS/BSA). After three more changes of PBS/BSA (30 min) sections were embedded in modified polyvinyl alcohol (Lennette 1978), or in Mowiol (PBS, containing 15% Hoechst 4.88 and 50 mg/ml 1,4-diazabicyclo[2.2.2]octane), respectively. All steps were carried out in a moist chamber at room temperature. The stained sections were examined and photographed with filters appropriate for rhodamine staining, or interference contrast, respectively.

Ultrastructural localization

Corneas were removed from adult Balb/C mice, cut into pieces of less than 1 mm, and frozen with a high pressure freezing apparatus HPM 010 (Balzers, Liechtenstein) according to Moor et al. (1980). Samples were stored in liquid nitrogen, substituted in pure acetone for 96 h at –85°C and for 7 h at –60°C. The acetone was exchanged with Lowicryl K11M as a graded series at –60°C for 38 h. The resin was polymerized with UV-light at –60°C and at room temperature for 2 days each (Schittny 1987; Schittny et al. 1988). Labelling followed the protocol of Roth (1984). Nonspecific binding of the antisera was blocked by treating for 5 min with 2% milk powder in PBS. The grids were incubated with antisera diluted 1:15 in PBS containing 0.1% Tween 20, 0.1% Triton X-100 and 2% milk powder for 2 h. After washing six times for 1 min on drops of PBS, a second incubation was performed with a solution of 10- to 14-nm-diameter protein A-gold (reduced with ascorbate according to Roth (1984)) in PBS containing 0.1% Tween 20, 0.1% Triton X-100, 1% gelatin and 1% BSA. Finally, grids were washed six times for 1 min with PBS and three times for 30 s with distilled water before air drying. Counter-staining was done by incubating the grids three times on a drop of 5% uranyl acetate for 2 min each, twice on a drop of CO_2-free distilled water for 30 s each, once on a drop of 2% lead-citrate (Reynolds 1963) for 90 s, and finally washed with CO_2-free distilled water. Micrographs were taken with a Philips EM-400 (Philips, Eindhoven, The Netherlands).

Proteoglycan extraction and dot-blot assay

Homogenized (EHS)-tumour was extracted and a proteoglycan fraction was isolated as previously described (Gloessl et al. 1984). Briefly, the extraction was done with 4 *M* guanidine hydrochloride

in 50 m*M* sodium acetate, pH 5.8, containing T+I (0.1% Triton X-100 and the protease inhibitors 6-amino-*n*-hexanoic acid (0.1 M), ethylene diamine tetraacetic acid (10 m*M*), *N*-ethylmaleimide (10 m*M*), and benzamidine-HCl (5 m*M*; all from Fluka Chemie AG, Buchs, Switzerland] during 4 h at 4°C. After centrifugation at ~9000 *g* for 20 min at 4°C the supernatant was dialysed against buffer A (7 *M* urea, 0.15 *M* NaCl, 20 m*M* TRIS/HCl, pH 7.4, T+I) and loaded onto a diethylaminoethyl (DEAE)-Trisacryl column (Serva, Heidelberg, Germany) equilibrated in the same buffer. After stepwise washing with buffer A, and buffer A containing 0.3 *M* NaCl instead of 0.15 *M* NaCl, a bound fraction was eluted with 1 M NaCl in buffer A, dialysed against urea-free buffer A in dialysis bags pretreated with fetal calf serum, or 0.1% SDS, respectively, and dot-blotted onto nitrocellulose. At ambient temperature the nitrocellulose filter was [1] treated with buffer B (urea-free buffer A containing 3% bovine serum albumin) over night, [2] with the primary antibody (anti PG-760 diluted 1:100 in buffer B) for 90 min, [3] with a secondary peroxidase labelled antibody (Sigma goat anti rabbit IgG, diluted 1:1000 in buffer B) for 90 min, and [4] with 0.75 m*M* diaminobenzidine (Sigma) and 0.015% hydrogen peroxide (Sigma) until colour developed. After steps two to four the filter was washed five times for five min with urea-free buffer A.

Results

Immunofluorescence staining

In order to elucidate further the tissue distribution of PG-760 in vivo we studied the localization of PG-760 in skin, uterus, small intestine, and EHS-tumour using indirect immunofluorescence of cryostat sections. Three different rabbit antisera recognizing both the 300-kDa and the 460-kDa core proteins were used for all tissues investigated. Because we were not able to detect any difference in their immunospecificity (antisera 1 and 2 see Breuer et al. 1991; Faber et al. 1992; antiserum 3 see the material and methods), we use the term 'anti PG-760' as a synonym for the different antisera.

In the skin of the ear, anti PG-760 stained the epidermis and spots in the dermis (Fig. 1a, b). The epidermis of the hair follicles, and to a lesser extent the sebaceous glands were also PG-760 positive (Fig. 1a). Due to the bright fluorescence of the epithelium, staining of the basement membrane could not be unequivocally ascertained. In addition, PG-760 appeared in all smooth muscle layers investigated. In uterus the myometrium (Fig. 1d, e), and in the small intestine (Fig. 1 g, h, j) the tunica muscularis and the lamina muscularis mucosae were stained by anti PG-760 in a fibrillar pattern. As shown by comparison to laminin-specific staining of the small intestine (Fig. 1k), the PG-760 staining pattern of smooth muscles was not restricted to basement membranes. At the present level of resolution we were not able to distinguish between cellular and extracellular immunostaining of PG-760 in smooth muscle tissues. In all tissues examined the smooth muscle layers of the vessels were also positive for PG-760 (skin, Fig. 1a; uterus, Fig. 1d; striated muscle, Fig. 2a; liver, Fig. 2c). Whether the basement membrane of the vascular endothelium was PG-760 positive, could not be determined, due to the strong immunofluorescence of the media (smooth muscles). In the mucosa of the small intestine anti PG-760 stained the mucosal epithelium including its junction with the lamina propria (Fig. 1j), whereas anti laminin stained only the basement membrane appearing at this junction and not the epithelium itself (Fig. 1k).

In skeletal muscle only small muscular vessels, but not the basement membrane of the muscle fibres or of capillaries were stained by anti PG-760 (Fig. 2a). In liver, anti-PG-760 staining was only observed in the wall of blood vessels excluding the central vein (Fig. 2c). In the wall of the central vein the basement membrane as well as the myofibroblasts remained negative. By comparison, anti laminin staining of skeletal muscle and liver is shown in Figs. 2b, d; all muscular and hepatic basement membranes appeared positive for laminin. In addition, there were in liver small, discontinuous basement membrane like deposits along the space of Disse (Martinez-Hernandez 1984; Milani et al. 1989) also containing laminin.

The following experiments were undertaken as controls for the immunofluorescence staining. Firstly, every section stained with anti PG-760 was compared to immunostaining with nonspecific rabbit IgG in the same experiment (Figs. 1c, f, i, and 3c). A staining reaction was only recognized as being positive if the nonspecific fluorescence staining was much weaker than the specific one. Secondly, three different antisera possessing virtually the same specificity against both core proteins were use in parallel. We observed the same tissue distribution of PG-760 by immunofluorescence, regardless of which of the three antisera was used. Thirdly, the same immunostaining reaction was also obtained with a small amount of affinity purified antibody obtained from antiserum 3. The second and third controls were undertaken because we were not able to obtain enough PG-760 in soluble form to use as a competitive inhibitor for immunostaining of tissues.

Expression of PG-760 in EHS-tumor

Mouse EHS-tumour represents a well established source for basement membrane proteins (Timpl et al. 1979). Since PG-760 was found in or at least very close to some basement membranes we studied the immunolocalization and expression of PG-760 in this tumour. In both cells and extracellular matrix PG-760 was detected by immunofluorescence (Fig. 3a, b). No staining was detected using nonspecific rabbit IgG as a control (Fig. 3c). In order to confirm this result an extract of EHS-tumour in 4 *M* guanidine/HCl was used in a dot blot immunoassay. Prior to the dot-blotting, the extract was partially purified on a DEAE-column for proteoglycan extraction and treated with chondroitinase ABC, dithiothreitol and/or SDS, respectively. Immunoreaction of anti PG-760 was observed under all conditions (Fig. 3d). Due to the tendency of PG-760 to precipitate under various conditions, we analysed the dot blot only qualitatively. Nevertheless none of the applied treatments was required to obtain a

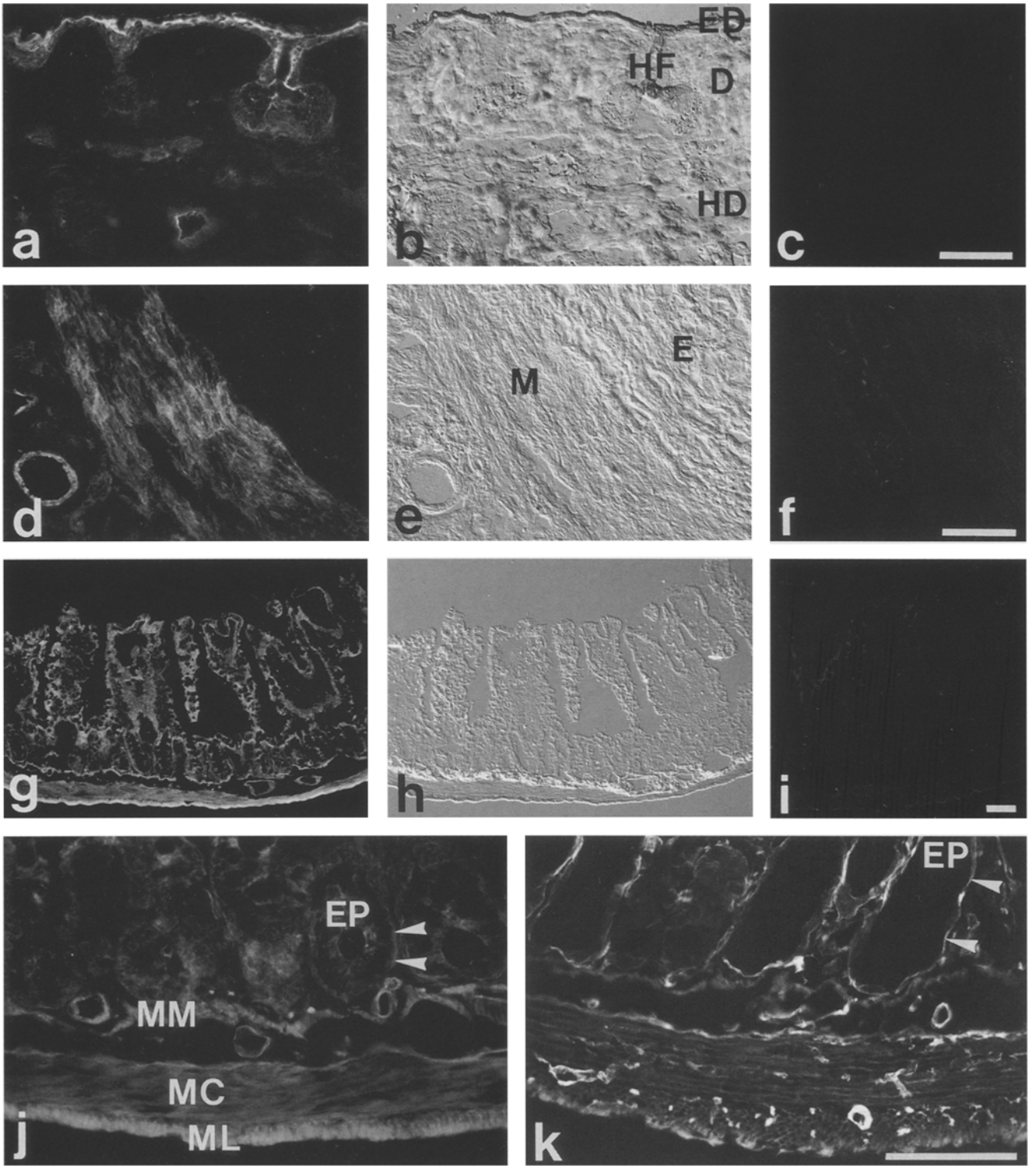

Fig. 1a–k Immunofluorescence localization of 760 kDa heterodineric dermaton sulphate proteoglycan (PG-760) in various tissues. Staining for PG-760 was observed in skin (**a, b**), in uterus (**d, e**), and in small intestine (**g, h, j**). As comparison to **j**, laminin (basement membrane) immunostaining of small intestine is shown in **k**. Negative controls were performed using unspecific rabbit serum instead of the specific antibody in skin (**c**), uterus (**f**), and small intestine (**i**). *Right* and *left* panels represent immunofluorescence images and *central* panels are interference contrast micrographs. (*D* Dermis, *E* endometrium of the uterus, *ED* epidermis, *EP* epithelium of the intestinal mucosa, *HD* hypodermis, *HF* hair follicle with sebaceous gland, *M* myometrium of the uterus, *MC* tunica muscularis – circular layer, *ML* tunica muscularis – longitudinal layer, *MM* lamina muscularis mucosae). *Arrow heads* indicate the junction of mucosal epithelium and lamina propria. *Bar*, 100 μm

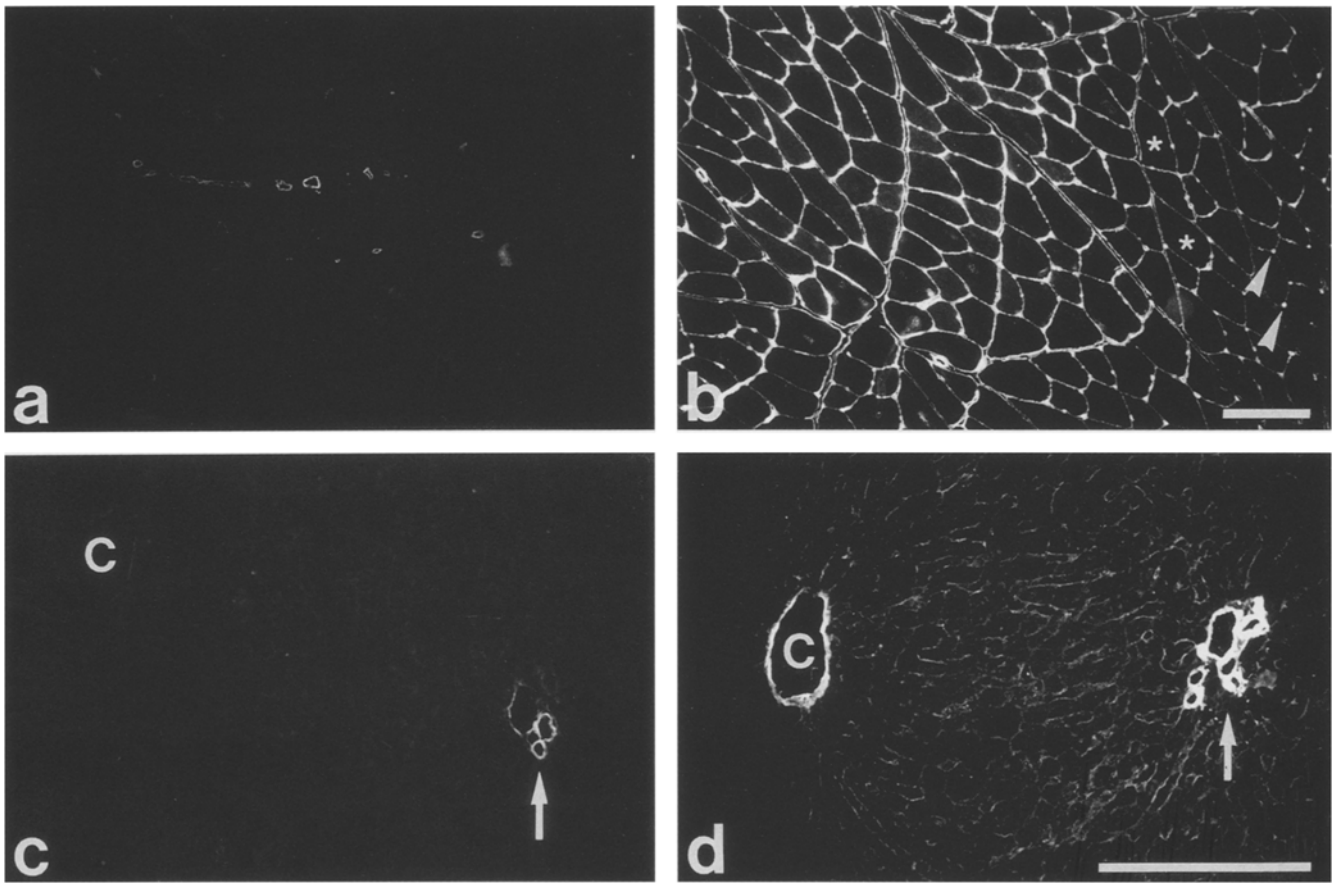

Fig. 2a–d PG-760 and laminin immunofluorescence staining of skeletal muscle and liver. Skeletal muscle (**a, b**) and liver (**c, d**) sections were stained by indirect immunofluorescence using anti PG-760 (**a, c**) and anti laminin (**b, d**). (*C* Location of central vein). The *Arrow* indicates portal canals, *arrowheads* are capillaries of skeletal muscle, and the asterisk indicates a muscle fibre. *Bar*, 200 μm

immunoreaction. Thus, we were able not only to immunostain the EHS-tumour matrix (Fig. 3a, b) and some other basement membranes (Fig. 1a, j) with anti PG-760 in situ, but also to extract PG-760 from EHS-tumour obtain an immunoreaction by dot-blotting. The latter demonstrated its polyanionic character (Fig. 3d) owing to the binding of PG-760 to the cationic matrix of the DEAE column during partial purification.

Ultrastructural resolution localization of PG-760 in rat cornea

In a previous investigation of cornea we were able to show by immunofluorescence that PG-760 appears in the epithelium, stroma, and Descemet's membrane (Faber et al. 1992). Whether or not PG-760 is a component of the epithelial basement membranes could not be determined due to the strong immunofluorescence of the epithelium. Therefore, immunocytochemistry was performed at the electron microscopic level. Freeze-substituted and Lowicryl K11M embedded mouse cornea was stained with anti PG-760 and labelled with protein A-gold. Immunogold labels for PG-760 were found over the epithelium (not shown), epithelial basement membrane (Fig. 4a), stroma (Fig. 4b), and Descemet's membrane (Fig. 4c).

As previously observed for other extracellular matrix proteins, such as laminin (Schittny 1987; Schittny 1992), the labelling intensity was not very high, but was specific compared to the negative control (nonspecific rabbit IgG; Fig. 4d, e). In Descemet's membrane, the basement membrane of the endothelium (inner corneal epithelium), the gold label predominately appeared in the layer of ~1 μm in thickness layer closest to the endothelium (Fig. 4c). This result is in good agreement with previous results of immunofluorescence staining where especially the borderline between Descemet's membrane and endothelium was high-lighted (Faber et al. 1992). In the stroma the gold grains appeared in the same location as the cross-striated collagen fibrils. Some grains were associated with the collagen fibrils, while others were observed between them situated over the ground substance (Fig. 4b). Occasionally we found some labelling in basement membrane-like structures indicated by stripes and plaques, which were recently described in the corneal stroma (not shown) (Keene et al. 1987; Schittny et al. 1988). Unfortunately, the structure of the epithelium and its basement membrane was not particularly well preserved due to ice crystal formation. Because the ice crystal damage may cause a redistribution of proteins over short distances, we

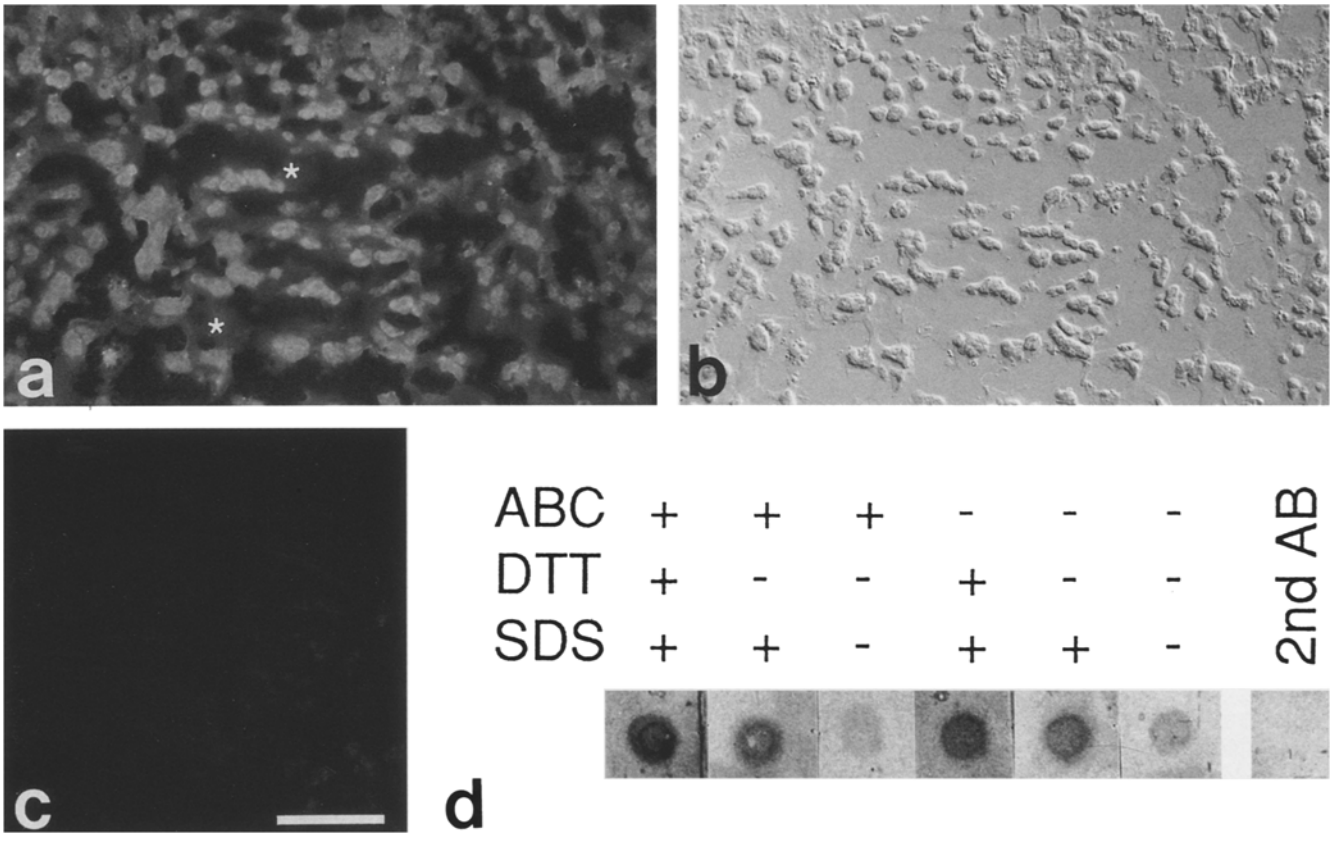

Fig. 3 Immunofluorescence and expression of PG-760 in Engelbreth-Holm-Swarm (EHS)-tumour. **a** PG-760 was detected in the matrix and the cells of EHS-tumour by indirect immunofluorescence. **b** Interference contrast image of the same area shown in **a.** **c** Negative control using nonspecific rabbit serum. The asterisk indicates EHS-tumour matrix. *Bar*, 100 µm. **d** After extraction from homogenized EHS-tumour and partial purification on a DEAE-column, PG-760 was treated with chondroitinase ABC, dithiothreitol (DTT) and/or SDS, respectively, and dotted onto nitrocellulose. Immunostaining of anti PG-760 was detected by a peroxidase-labelled secondary antibody under all conditions. (2nd AB, Negative control using only the secondary antibody)

are only able to state that the epithelium (not shown) and its basement membranes (Fig. 4a) were labelled for PG-760. Further electron microscopic investigations are necessary to determine more exactly where PG-760 appears inside the basement membrane.

Discussion

Recently, a novel heterodimeric dermatan sulphate proteoglycan, PG-760, has been described as a secretory product of an immortalized keratinocyte cell line, of normal fetal skin fibroblasts, and of endothelial cells (Breuer et al. 1991; Faber et al. 1992). Immunofluorescence studies of cultured cells yielded a fibrillar and punctuated staining pattern, and in addition, remarkable colocalization with fibronectin and the basement membrane proteins laminin, perlecan, and type IV collagen. The latter result is supported by findings obtained with rat cornea, where immunofluorescence staining for PG-760 was found in the epithelium, stroma, and Descemet's membrane (Faber et al. 1992). In the present study we investigated the distribution of PG-760 in skin (Fig. 1a–c), uterus (Fig. 1d–f,) small intestine (Fig. 1g–k), skeletal muscle (Fig. 2a, b), liver (Fig. 2c, d), and EHS-tumour (Fig. 3) at the light microscopic level, and in cornea at the electron microscopic level (Fig. 4).

In cornea, we were able to demonstrate at high resolution the appearance of PG-760 in basement membranes (Fig. 4). Due to strong immunofluorescence of the epithelial cells in skin and small intestine, we were not able to definitely localize PG-760 in respective basement membranes (Fig. 1a, j). However, because these epithelial cells produce PG-760, we may assume that the proteoglycan is secreted and could at least partly be deposited in their basement membranes. Additional evidence for the localization of PG-760 in basement membranes is provided by the investigation of EHS-tumour representing a common source of basement membrane proteins (Timpl et al. 1979). Extraction of EHS-tumour with 4 *M* guanidine and immunostaining of EHS-tumour revealed an intra- and extracellular localization of PG-760 (Fig. 3). However, anti PG-760 does not universally stain basement membranes; skeletal muscle cells, capillaries, central vein of the liver, and basement membrane-like deposits in the space of Disse (liver) are not stained (Fig.

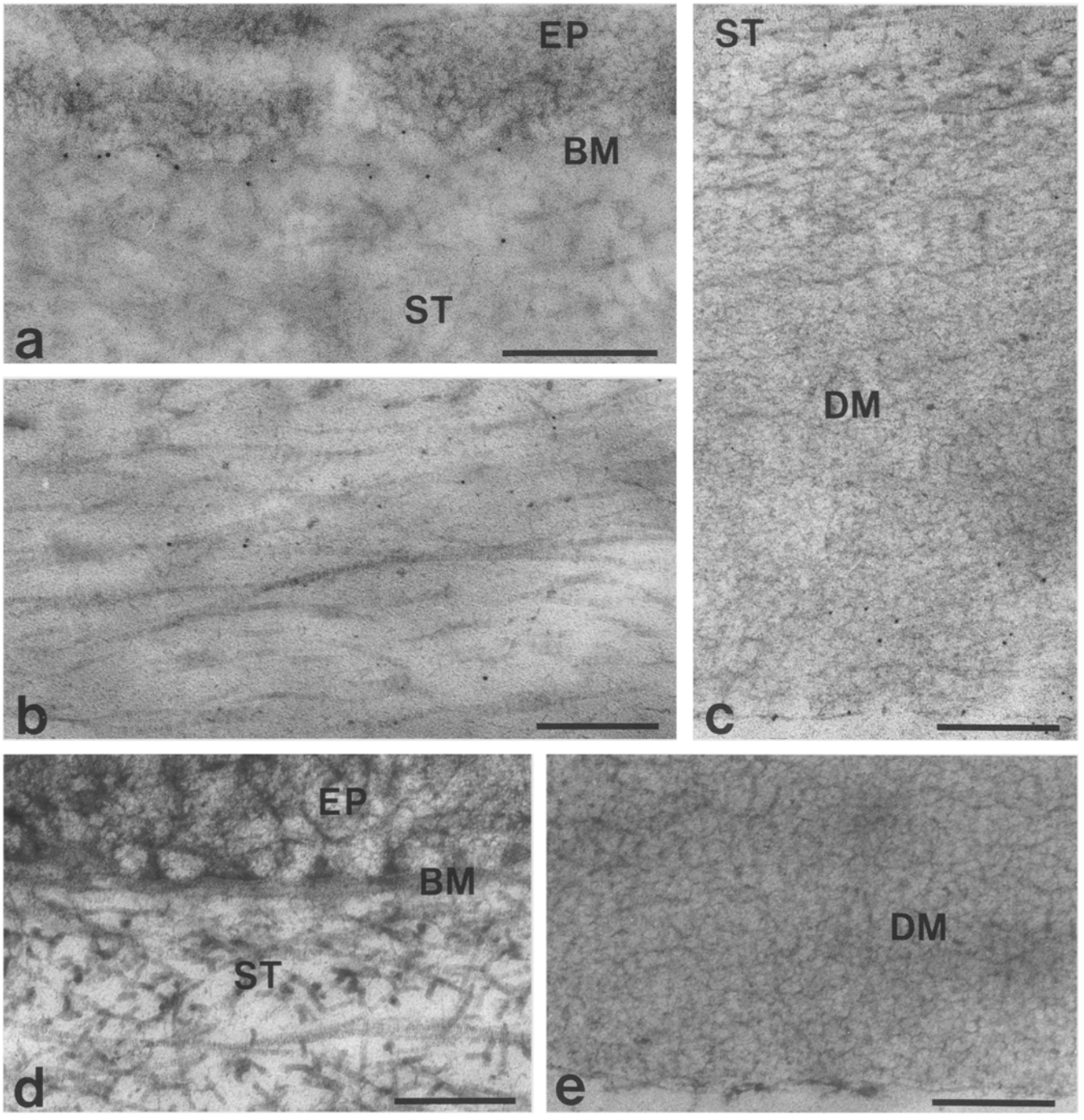

Fig. 4a–e Electron microscopic localization of PG-760 in cornea. Using the immunogold technique, anti PG-760 labelling was observed over the epithelium (not shown), epithelial basement membrane (**a**), corneal stroma (**b**), and Descemet's membrane. In the latter, gold markers were predominately found in the layer of ~1 μm thickness closest to the inner corneal epithelium (**c**). This epithelium normally underlying Descemet's membrane, was lost during sample manipulation. No markers could be detected in negative controls using nonspecific rabbit serum as shown for the epithelium, epithelial basement membrane, and stroma (**d**), and for the inner half of Descemet's membrane (**e**). (*BM* Basement membrane, *DM* Descemet's membrane, *EP* epithelium, *ST* stroma. *Bars*, 0.5 μm

2). The heterogeneity of the proteoglycan composition of basement membranes is well documented: while the heparan sulphate proteoglycan perlecan represents a component of all basement membranes (Timpl 1993), a chondroitin sulphate proteoglycan that is not identical to PG-760 has been shown to have a temporally and spatially restricted appearance (McCarthy et al. 1993). The findings are not necessarily contradictory that capillaries are PG-760 negative (Fig. 2a, b), and that cultured endothelial cells produce PG-760 (Faber et al. 1992), because the endothelial cells were obtained from large vessels (porcine aorta and human vena umbilicalis), and because

every studied vessel containing smooth muscle cells was PG-760 positive. However, due to the strong immunofluorescence of the vascular smooth muscle layers, we were not able to determine whether the endothelial basement membranes were also PG-760 positive (Figs. 1, 2).

In pulse-chase experiments of cultured fetal fibroblasts, a large pool was found of cell surface bound PG-760 representing about two thirds of the total quantity of the secreted proteoglycan (Faber et al. 1992). These results indicate that the proteoglycan may not only be anchored inside the basement membrane, but also directly attached to the cell surface via a receptor, or a potential membrane anchor of PG-760, respectively.

An unexpected finding of the present study was the occurrence of PG-760 immunostaining in smooth muscle layers: myometrium of the uterus (Fig. 1d), lamina muscularis mucosae and tunica muscularis of the small intestine (Fig. 1j), and all smooth muscle containing vessels (Fig. 1, 2); however, skeletal muscles appeared to be PG-760 negative. Interestingly, anti PG-760 did not stain myofibroblasts found in the wall of the central vein (liver). These cells contain desmin and alpha-smooth muscle actin (Seifert et al. 1994), but may be distinguished from smooth muscle cells of other hepatic vessels by the absence of a continuous basement membrane and by the lack of expression of immunoreactive glucagon (Sasaki et al. 1984). Therefore, PG-760 appears to be restricted to fully differentiated smooth muscle cells.

The extracellular distribution of PG-760 is not restricted to basement membranes. PG-760 was also found in the dermis and the stroma of the cornea. In the latter, electron microscopic investigation revealed that PG-760 is principally colocalized with cross-striated collagen fibrils (Fig. 4b), but we were not able to detect PG-760 morphologically. Therefore, it is not understood whether PG-760 forms a network that is independent of the cross-striated collagen fibrils, such as collagen VI (Keene et al. 1988), or attaches to the cross-striated collagen fibrils, such as decorin (Kresse et al. 1993), or the fibril associated collagens type XII and XIV (Keene et al. 1991).

Compared to the distribution of type XII and type XIV collagens, PG-760 possesses a different tissue distribution; generally both collagens were found to be colocalized with collagen type I (Sugrue et al. 1989). In contrast to the appearance of PG-760, collagens type XII and XIV were found in the fibrous connective tissue septa of skeletal muscles (10- and 17-day-old chicken embryos; (Wälchli et al. 1994)), but not in basement membranes (Sugrue et al. 1989). Similarly to PG-760, these collagens were found in layers of smooth muscle (gizzard of 10- and 17-day-old chicken embryos), but appeared only in the connective tissue and not in the muscle cells (Wälchli et al. 1994). Also similarly to PG-760, collagen type XII was found in the dermis (Oh et al. 1993) and in the stroma of mouse and bovine cornea (Keen et al. 1991; Oh et al. 1993)). Both decorin and biglycan maintain a tissue distribution differing from that of PG-760. While decorin is principally associated with all connective tissues containing larger amounts of collagens type I and II, biglycan is preferentially expressed in specialized cell types including skeletal myofibres, differentiating cells of the epidermis, endothelial cells, fibroblasts and chondrocytes (Kresse et al. 1993).

The results of this study indicate that PG-760 exhibits unique tissue distribution. It appears to be a constituent of some specialized basement membranes, but is also found in epithelial and interstitial tissues, and in smooth muscles. These different localizations suggest a versatile function of PG-760, although such functions remain to be elucidated in detail. There is evidence for a very strong self-association tendency of PG-760 and for the capability of forming core protein-mediated complexes with other extracellular matrix components (Faber et al. 1992). The dermatan sulphate chain could also be involved in self-association (Fransson et al. 1982). Owing to the plasma membrane-binding of PG-760 (Faber et al. 1992) participation in the modulation of cell adhesion may be envisaged. Whether or not PG-760 is able to modulate the properties of other extracellular matrix components such as laminin or type IV collagen remains to be investigated.

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