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Cell Adhesion Glycoproteins in the Human Lamina Cribrosa

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Purpose: The distribution of the cell adhesion glycoproteins, laminin, fibronectin, tenascin, vitronectin, thrombospondin, and entactin/nidogen, was examined in the human lamina cribrosa.

Methods: Frozen sections of the optic nerve head from 7 normal human elderly donors were stained by immunohistochemistry.

Results: All six glycoproteins were detected in this tissue. While laminin and entactin/nidogen were observed linearly, reflecting the localization of basement membranes, fibronectin was identified diffusely. Marked tenascin immunoreactivity was apparent in the lamina cribrosa, but little or no tenascin staining was detected in the sclera. Vitronectin showed a fine fibrillar staining pattern in the lamina cribrosa, and, to a lesser extent, in the sclera and pial septa. Thrombospondin staining was apparent only in the sclera and the lamina cribrosa, which traversed the optic nerve.

Conclusions: These results indicate that extracellular matrix components in the lamina cribrosa differ from those in the sclera or pial septa. This study is the first report that the human lamina cribrosa includes vitronectin and thrombospondin. **Jpn J Ophthalmol 2001;45:363–367**

Key Words: Cell adhesion glycoprotein, extracellular matrix, human, lamina cribrosa, optic nerve head.

Introduction

The extracellular matrix occupies a large portion of the extracellular space and has important functions in maintaining the architecture and mechanical stability of tissue.[1,2] In addition, the extracellular matrix interacts with cells and influences critical cellular functions, including migration, differentiation, establishment and maintenance of polarity, and tissue-specific gene expression. The lamina cribrosa[3,4] in the optic nerve head has been postulated to provide mechanical and functional support to optic nerve fiber bundles. This tissue contains types I, III, IV, V, VI,[5–10] and VIII[11] collagen; glycoproteins, such as laminin, fibronectin, tenascin[12], α-elastin, and fibrillin,[8] and proteoglycans.[13–15] Abnormalities or alterations of the extracellular matrix have been thought to affect the strength and elasticity of the laminar beams, as a result of glaucomatous changes in the optic nerve head.[12,16–23]

The cooperation or interaction between cells and matrix has attracted more attention recently. Various glycoproteins have been identified in plasma and extracellular matrix and also their molecular and gene structures have been revealed. In an attempt to increase our understanding of the extracellular matrix of the optic nerve head and its roles in glaucoma and other types of optic neuropathy, we have now examined the localization of the cell adhesive glycoproteins, tenascin,[12,24–26] vitronectin,[27] thrombospondin,[28–30] and entactin/nidogen,[31] as well as that of laminin and fibronectin, in the normal elderly human lamina cribrosa.

Materials and Methods

Seven eyes from 7 elderly human donors were obtained within 36 hours of death from the Illinois Eye Bank (Chicago, IL, USA). The donors were 5 men, aged 62, 64, 72, 78 and 92 years; and 2 women, aged 72 and 84 years. None of the donors had any known

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ocular diseases, and the eyes did not show any abnormalities under the dissection microscope. The optic nerve head tissues were dissected and snap-frozen in dry-ice isopentane (Fischer Scientific, Itasca, IL, USA). Six-micrometer-thick frozen sections were cut and placed on glass slides that were coated with poly-L-lysin. They were kept in the freezer at −20°C until the experiment was ready to begin.

Immunohistochemical staining was performed as described previously[10,22] with a biotin-streptavidin method. Sections were fixed in cold acetone, followed by washing in 0.01 M phosphate-buffered saline (PBS, pH 7.2). They were treated with 3% hydrogen peroxide for 15 minutes to block endogenous peroxidase. After incubation for 30 minutes at room temperature in 10% normal goat (for laminin and fibronectin experiments) or rabbit (for tenascin, vitronectin, thrombospondin, and entactin/nidogen experiments) serum, slides were set aside to react overnight at 4°C with one of the following antibodies: rabbit anti-mouse laminin (1:500; Collaborative Research, Bedford, CA, USA), rabbit anti-human fibronectin (1:500; Collaborative Research), mouse anti-human tenascin (1:100; GIBCO BRL, Gaithersburg, MD, USA), mouse anti-human vitronectin type 1 (1:100; Calbiochem, San Diego, CA, USA), mouse anti-human thrombospondin (1:50; Chemicon, Temecula, CA, USA), and rat anti-mouse entactin/nidogen (1:100; Chemicon). As negative control, some sections were incubated with normal rabbit IgG or with mouse anti-digoxigenin (Boehringer Mannheim, Indianapolis, IN, USA) with the same dilution as each primary antibody. The specificity of each primary antibody was confirmed by Western blotting using cultured trabecular meshwork cells or scleral fibroblasts.[32]

Sections were overlaid with biotinylated secondary antibodies (Vector, Burlingame, CA, USA) diluted 1:500, and were exposed to streptavidin-peroxidase (BioGenex, San Ramon, CA, USA) and mixed 1:100 for 45 minutes for each section. They were developed in 3-3-diaminobenzidine with 0.02% hydrogen peroxide in 20 mM Tris-HCl buffer (pH 7.2) washed with PBS and distilled water. Some sections were counterstained with hematoxylin. They were dehydrated, mounted with mounting media (Premount, Fischer Scientific) and then examined under a light microscope.

Results

The results of this study are summarized in Table 1. No differences in staining patterns were apparent among the 7 eyes studied. In addition, sections incubated with nonimmuno rabbit immunoglobulin or with antibodies to digoxigenin did not show any significant staining.

Table 1. Summary of Results

Glycoprotein	Location			Staining Pattern
	Lamina	Pial Septa	Sclera	
Laminin	+	+	(+)	Linear, basement membrane
Fibronectin	+	+	+	Diffuse
Tenascin	+	+	−	Diffuse and enhanced linear
Vitronectin	+	+	+	Short, fine-fibrillar
Thrombospondin	+	−	+	Diffuse
Entactin/nidgen	+	+	(+)	Linear, basement membrane

All six glycoproteins were detected in the normal human lamina cribrosa (Figures 1 and 2). Laminin immunostaining showed a linear-like pattern surrounding the laminar beams and a ring-like pattern around the vascular system, (Figures 1A, 2A–C). Laminin immunoreactivity was also detected on the surface of the pial septa (Figure 2B). Marked staining for fibronectin was apparent in the laminar beams, whereas the sclera and pial septa showed a diffuse pattern of fibronectin immunoreactivity (Figures 1B, 2D–F). Staining for tenascin was intense in the lamina cribrosa, faint in the pial septa and none or a little in the sclera (Figures 1C, 2G–I). The staining in the insertion region between the lamina cribrosa and the sclera or choroid was similar to that in the lamina cribrosa; staining appeared generally but was enhanced in a linear pattern along the surface of the laminar beams (Figure 2G). Vitronectin was present in short fine fibrillar structures within the lamina cribrosa, pial septa, and sclera (Figures 1D, 2J–L). These structures traversed the optic nerve in the lamina cribrosa (Figure 2J), and, in the posterior optic nerve, they were oriented almost parallel to the surface of the pial septa. Diffuse thrombospondin immunostaining was apparent along the sclera and in the lamina cribrosa (Figures 1E, 2M–O). Intense deposit-like staining also was detected in the beams of the lamina cribrosa. Little or no thrombospondin staining was observed in the pial septa, with the exception of faint ring-like immunoreactivity associated with small capillaries (Figure 2N). The insertion region and the junction between the sclera and dura mater showed little reactivity with antibodies to thrombospondin (Figure 2O). Entactin/nidogen staining showed a pattern virtually identical to that of laminin (data not shown).

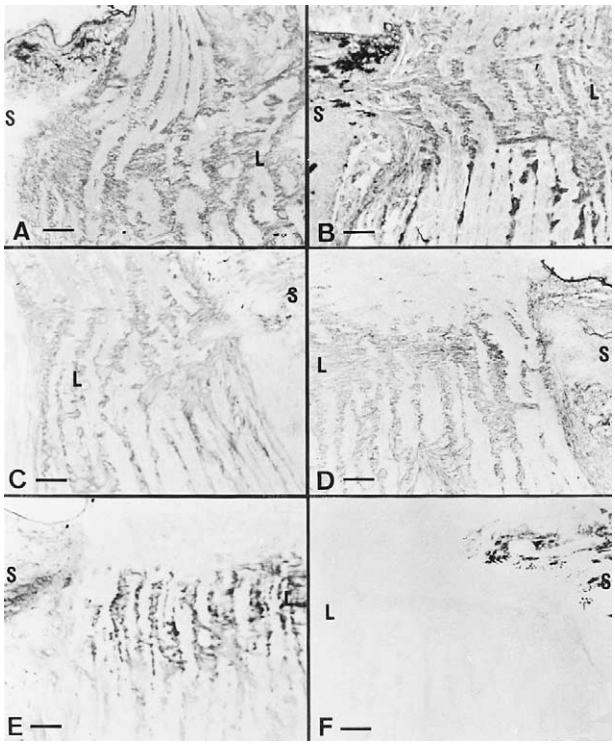

Figure 1. Localization of laminin (**A**), fibronectin (**B**), tenascin (**C**), vitronectin (**D**), and thrombospondin (**E**) in normal human optic nerve head. (**A**) 72-year-old man: Immunostaining for laminin showed linear pattern surrounding beams of lamina cribrosa and surface of pial septa. (**B**) 64-year-old man: Fibronectin showed diffuse pattern of staining in lamina cribrosa, pial septa, and sclera. (**C**) 78-year-old man: Reactivity to tenascin antibodies was marked in lamina cribrosa, faint in pial septa, and not detected in sclera. (**D**) 62-year-old man: Vitronectin immunoreactivity was present in fine fibrillar structures in lamina cribrosa, as well as in pial septa and sclera. (**E**) 72-year-old woman: Thrombospondin staining was intense in lamina cribrosa and less so in sclera. (**F**) 62-year-old man: No significant staining was apparent with mouse anti-digoxigenin antibody as negative control. L: lamina cribrosa, S: sclera. Bar = 100 μm.

Discussion

We have demonstrated the presence of six glycoproteins in the normal elderly human lamina cribrosa. This study is the first report that the human lamina cribrosa includes vitronectin and thrombospondin.

Tenascin[12,24–26] is a large (240 kDa) polymorphic glycoprotein, which is expressed in many developing organs and reappears during wound healing and in tumor cues. It is a highly specialized product of mesenchymal and glial cells and its expression correlates with cell proliferation and migration as well as growth and remodeling of the extracellular matrix. The presence of tenascin in the normal human lamina cribrosa is somewhat surprising given that the expression of this molecule is highly restricted in adult tissues. It is possible that tissue remodeling occurs in the lamina cribrosa even under normal conditions, especially in aged human eyes, because the optic nerve head is constantly exposed to intraocular pressure and fluctuation therein. In addition, the number of optic nerve fibers decreases with age, and this change may be associated with tenascin expression. In this study, we used tissues from elderly donors only; further studies with younger donor tissues and biochemical analysis will be needed. More recently, Pena et al[12] reported tenascin expression in human optic nerve heads from primary open angle glaucoma (POAG) patients. Tenascin was slight but detectable even in normal eyes by immunostaining and in situ hybridization. In addition, it was curious to find that this molecule was markedly enhanced in the eyes of POAG patients. The present study indicates that tenascin may play an important maintenance role in the optic nerve head, both in normal and pathological conditions. Tervo et al[26] detected tenascin-like immunoreactivity at the corneoscleral margin even in normal human tissue.

Vitronectin is a cell adhesion and spreading factor present in plasma and the extracellular matrix.[27] Immunohistochemical studies suggest that vitronectin is deposited in fibrillar forms in a number of connective tissues, including skin and renal tissue. This molecule is also associated with elastin and may serve as a link between elastin and the surrounding collagen or proteoglycan scaffold. In our study, the fine fibrillar staining pattern for vitronectin was similar to that of elastin. The intensity of immunostaining for vitronectin as well as elastin was much greater in the lamina cribrosa than that in the sclera and pial septa. Elastin is thought to be important in the pathogenesis of glaucomatous optic nerve damage.[33,34] Interaction between elastin and vitronectin also may be related to glaucoma-induced optic nerve changes. Thrombospondin[28–30] is secreted rapidly at sites of injury and thrombosis as a major component of activated platelet. This molecule is also synthesized and secreted by many other cell types, including fibroblasts and smooth muscle cells. Although thrombospondin is also present in the extracellular space, its functional roles here are unclear. Previous studies suggest that inhibition of angiogenesis may be an important function of this molecule.[30] Entactin/nidogen is an integral component of basement membranes.[31] It associates specifically with both laminin and type IV collagen and is thought to play an important role is linking these two molecules. Finally, we have showed the staining for laminin and fibronectin again in this study. As many previous reports have in-

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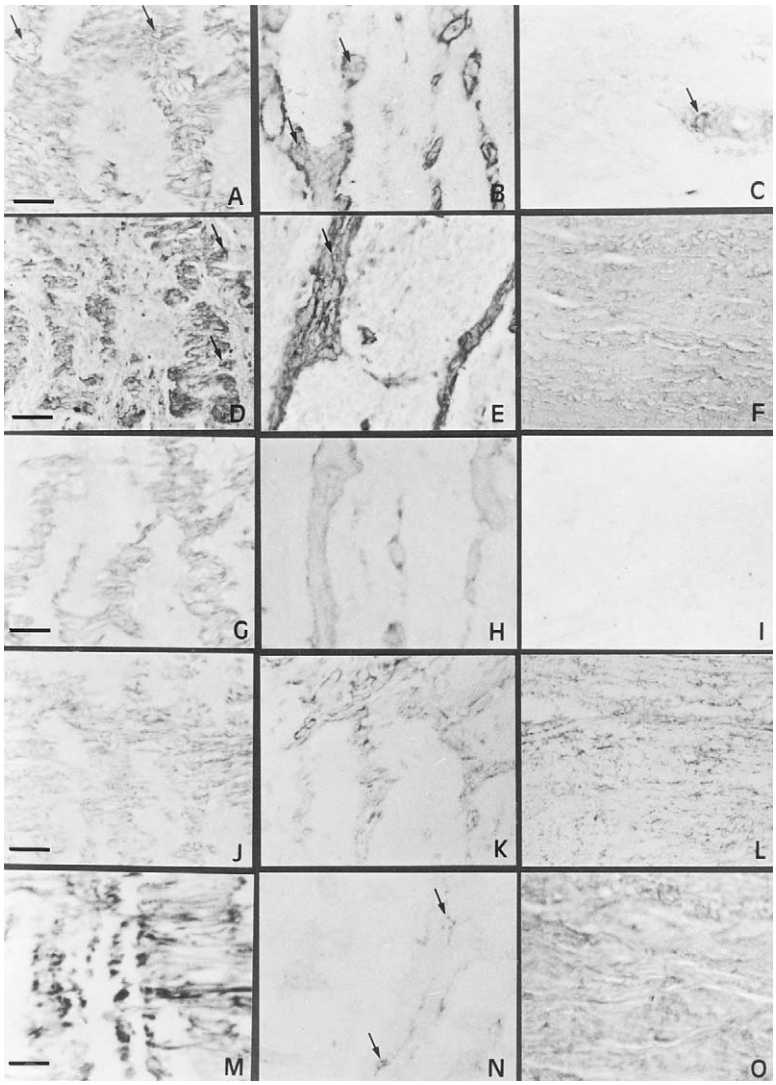

Figure 2. Immunostaining for laminin (**A**, **B**, **C**), fibronectin (**D**, **E**, **F**), tenascin (**G**, **H**, **I**), vitronectin (**J**, **K**, **L**), and thrombospondin (**M**, **N**, **O**) in lamina cribrosa (**A**, **D**, **G**, **J**, **M**), pial septa (**B**, **E**, **H**, **K**, **N**), and sclera (**C**, **F**, **I**, **L**, **O**). (**A**) Laminin was distributed on surface of laminar beams in linear pattern. (**B**) In optic nerve, laminin staining was localized on structure between pial septa and optic nerve fibers. (**C**) Only vasculature was stained for laminin in sclera. (**D**) Staining for fibronectin appeared diffuse in lamina cribrosa, although capillaries inside beams showed marked staining. (**E**) Pial septa also stained diffusely with antibodies to fibronectin. (**F**) Fibronectin was also detected in sclera. (**G**) Immunoreactivity for tenascin was generally diffuse but was enhanced in a linear pattern in lamina cribrosa. (**H**) Surface part of pial septa was stained with antibodies to tenascin. (**I**) Little or no staining for tenascin was apparent in peripapillary sclera. (**J**) In lamina cribrosa, vitronectin was localized to fine fibrillar structures that traversed optic nerve. (**K**) Vitronectin was also detected in pial septa, but density of fine fibrils was markedly less than that in lamina cribrosa. (**L**) Vitronectin was also present in sclera. (**M**) Thrombospondin was distributed diffusely in lamina but also showed intense deposits. (**N**) Thrombospondin was present only in capillaries, in pial septa. (**O**) Sclera showed diffuse staining with antibodies to thrombospondin. Arrows indicate capillaries. Bar = 50 μm.

dicated,[5–10] laminin shows linear staining by immunohistochemical study, which confirms the distribution on the basement membranes. Diffuse staining for fibronectin can be identified in the lamina cribrosa as well as in the pial septa and sclera. We again demonstrated in this study how easy it is to understand and to compare the distribution of four more glycoproteins because of the specific distribution of laminin and fibronectin in the optic nerve head.

The lamina cribrosa, sclera, and pial septa show both similarities and differences with regard to the expression of extracellular matrix components. Fibronectin and vitronectin were distributed in the lamina cribrosa, sclera, and pial septa. Because tenascin was identified in the lamina cribrosa and pial septa but not in sclera, it was along the optic nerve. In contrast, the staining of thrombospondin was localized in the sclera and lamina cribrosa across the optic nerve. The lamina cribrosa might be a part of the optic nerve as well as a part of connective tissue like sclera. These differences in the extracellular matrix, which probably reflect the different developmental histories of these regions of the eye, may have functional implications that are important with regard to glaucomatous optic nerve damage, considering that the lamina cribrosa and sclera are pressure-bearing structures, even in normal eyes, which are constantly exposed to intraocular pressure.

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