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Immunohistochemical Identification of Proteoglycan Types in Fibrotic Human Capsules With Intraocular Lens Implants

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Abstract: Lens capsules become fibrotic after cataract extraction. A variety of extracellular matrix (ECM) components accumulate on these capsules in association with the proliferation of lens epithelial cells. To provide a better understanding of the process of capsular fibrosis, we assessed the types of proteoglycans (PG) in human lens capsules with intraocular lenses (IOL). Lens capsules containing IOLs were removed from 1 patient with proliferative vitreoretinopathy and 1 patient with proliferative diabetic retinopathy. After treatment with chondroitinase ABC, tissue sections were processed for immunohistochemical detection of the proteoglycans including chondroitin, large PG, chondroitin 4-sulfate PG, chondroitin 6-sulfate PG, dermatan sulfate PG, and keratan sulfate PG. Extracellular matrix was found on the inner surface of the capsular bag. In association with what appeared to be proliferating lens epithelial cells, each of the six types of PG was present in the ECM on the capsules. All six types of PG might be involved in the fibrosis and opacification of lens capsules after extraction of the cataract and implantation of the IOL. **Jpn J Ophthalmol 1998;42:368–372**

Key Words: Cataract surgery, human eye, immunohistochemistry, posterior capsule opacification, proteoglycan.

Introduction

Lens epithelial cells, which originally line the inner surface of the anterior capsule, have been reported to proliferate on the posterior capsule after cataract extraction[1–4] and also on the surface of the intraocular lenses (IOL).[5–8] These cells are thought to be responsible for the fibrotic change observed in the capsules. In culture, lens epithelial cells of animals are transformed into fibroblast-like cells and produce collagens, including types I and III,[9,10] the major components of opacified lens capsules. They also produce proteoglycan types in vitro.[11–14]

Collagen types I and III have been detected in opacified human posterior capsules by immunoelectron microscopy.[15] We have detected the deposition of collagen types I, III, IV, V, and VI, as well as laminin and cellular fibronectin, in the extracellular matrix (ECM) that accumulates on the capsule of patients after the extraction of the cataract and the implantation of the IOL.[16] Lens epithelial cells of humans or animals express prolyl 4-hydroxylase, an enzyme involved in procollagen hydroxylation, and produce collagens on the capsules.[16–18] Similar components have been observed on the surface of IOLs explanted from humans and rabbits.[19–24]

Proteoglycans also deposit in capsular fibrosis. Ishibashi and his coworkers[25] detected a cuprolinic blue-stained material in the opaque posterior capsules of patients with IOL implants. The exact distribution of the various proteoglycans was not eluci-

Received November 27, 1997
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Published by Elsevier Science Inc.

0021-5155/98/$19.00
PII S0021-5155(98)00033-1

dated by these histochemical methods, although these investigators concluded that the opacified capsules contained chondroitin sulfate and dermatan sulfate proteoglycans. Chondroitin sulfate isomers are present in connective tissues with chondroitin 4-sulfate, chondroitin 6-sulfate and dermatan sulfate proteoglycans being the most abundant components. In addition, the proteoglycans, dermatan sulfate and keratan sulfate, are associated with the formation of collagen fibers.

More recently, immunohistochemical methods have been developed to distinguish among the types of proteoglycans.[26–31]

To provide a better understanding of the fibrotic process that occurs in lens capsules after IOL implantation, we utilized immunohistochemical methods to identify the types of proteoglycans present in the lens capsules of patients with IOL implants.

Materials and Methods

Specimens

Lens capsules containing polymethylmethacrylate (PMMA) IOLs were removed during vitreous surgery in 2 patients, a 62-year-old woman with proliferative vitroeretinopathy, and a 55-year-old man with proliferative diabetic retinopathy. In these patients, the length of time between the implantation of the IOL and its removal was 6 and 9 months, respectively. The IOL was removed through a limbal incision prior to the intravitreal procedure, and the capsule obtained was embedded in OCT-Compound (Miles Laboratories, Elkhart, IN, USA) for immunohistochemical study. Informed consent for the present study was obtained from each patient.

Immunohistochemistry for Proteoglycan Types [26–31]

Each specimen was cryosectioned in 8.0 μm sections that were fixed with cold acetone for 5.0 minutes. After being immersed in 0.1 mmol/L Tris-HCl buffer, pH 8.0, for 60 minutes at 37°C, the specimens were incubated with chondroitinase ABC (5 U/mL in 0.1 mmol/L Tris-HCl buffer, pH 8.0) for 60 minutes at 37°C to unmask the epitopes. Endogenous peroxidase was inactivated by treatment with methanol that contained 0.3% hydrogen peroxide for 30 minutes at 4°C. The specimens were subsequently treated with avidin, biotin, and normal rabbit serum for 30 minutes each at room temperature.

They were then allowed to react with a panel of primary mouse monoclonal antibodies directed against each type of proteoglycan (Seikagaku Kogyo, Tokyo) for 120 minutes at 37°C. The antibodies used were directed against unsulfated chondroitin (1-B-5), core protein of large proteoglycan molecule (2-B-1), chondroitin 4-sulfate proteoglycan (2-B-6), chondroitin 6-sulfate proteoglycan (3-B-3), dermatan sulfate proteoglycan (6-B-6), and keratan sulfate proteoglycan (5-D-4). 2-B-6 recognizes a stub of the unit of 2-acetamide-2-deoxyl-3-O-(β-D-gluco-4-enepyranosyluronic acid)-4-O-sulfo-D-galactose bound to the core protein via a linkage tetrasaccharide; 3-B-3 recognizes the unit of 2-acetamide-2-deoxyl-3-O-(β-D-gluco-4-enepyranosyluronic acid)-6-O-sulfo-D-galactose, also bound to the core protein via a linkage tetrasaccharide. Each of these units had been obtained from chondroitin sulfate proteoglycan by treatment with chondroitinase ABC. 2-B-1 reacts with the intact proteoglycan core molecule and with the chondroitinase-ABC treated core protein. After being washed in PBS, the specimens were incubated with a biotinylated secondary mouse Ig antibody for 60 minutes at RT. The samples were again washed in PBS and the specimens were treated with peroxidase-conjugated streptavidin for 15 minutes at RT. The color of the antibody complex was developed with 0.05% 3,3′-diaminobenzidine in Tris-HCl buffer (pH 9.6) containing 0.06% hydrogen peroxide. The specimens were mounted in Canada balsam, and observed under light microscopy.

Negative control staining was performed by omission of the primary antibodies.

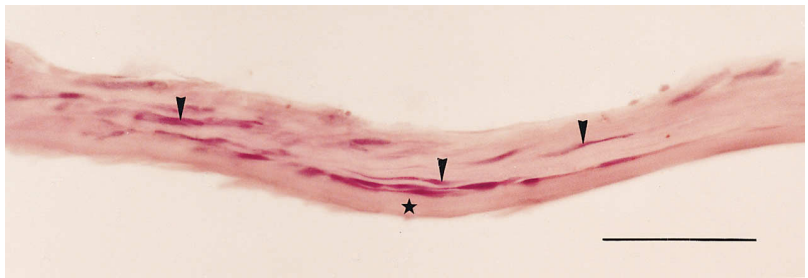

Figure 1. Light microscopy of fibrous posterior capsule with intraocular lens implant obtained from 55-year-old man with proliferative diabetic retinopathy. Cells were stained by hematoxylin and eosin. Slender cells are denoted by arrowheads. ★Posterior capsule (Bar: 25 μm).

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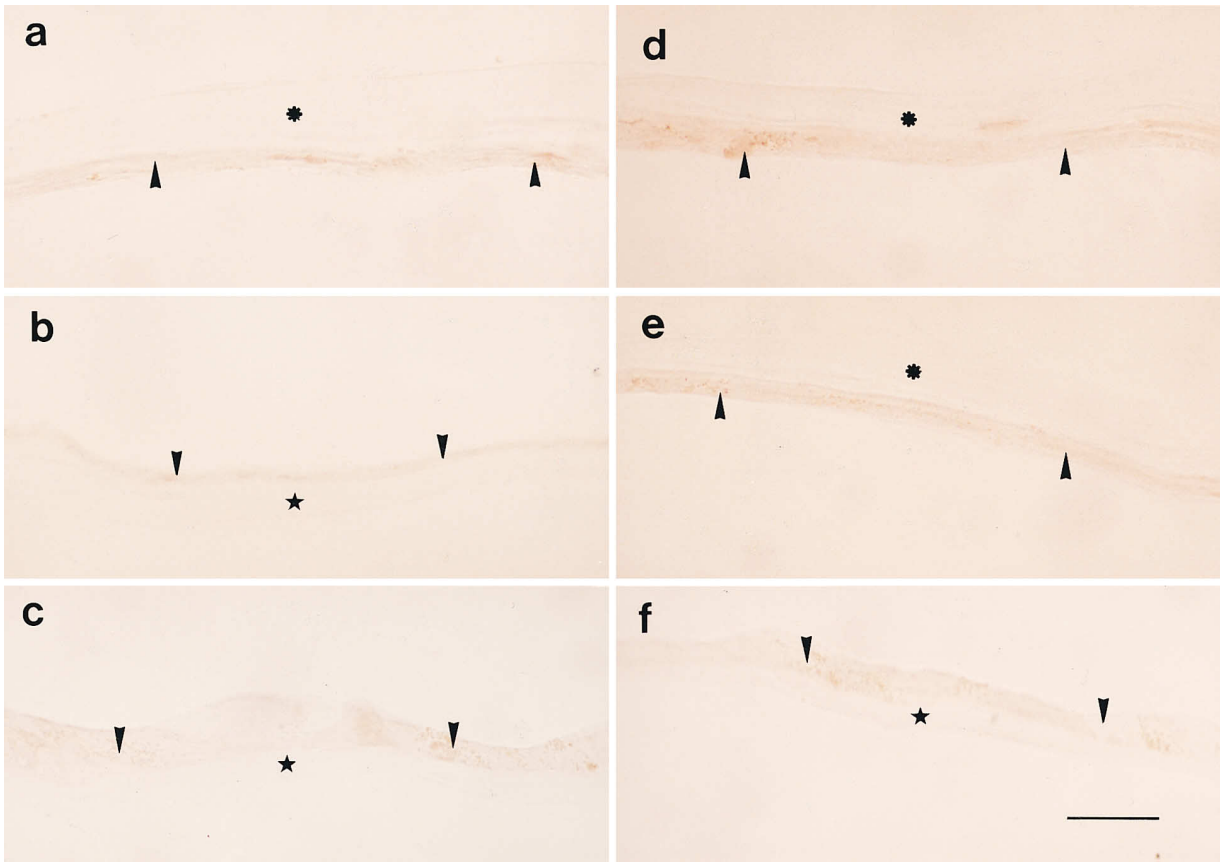

Figure 2. Immunolocalization of types or proteoglycans (PGs) in extracellular matrix on capsule removed from 55-year-old man with proliferative diabetic retinopathy. Tissue sections were reacted with antibodies directed against chondroitin (a); large proteoglycan (b); chondroitin 4-sulfate PG (c); chondroitin 6-sulfate PG (d); dermatan sulfate PG (e); keratan sulfate PG (f). Immunoreactivity against each PG is denoted by arrowheads. * anterior capsule, ★ posterior capsule (Immunostaining, Bar: 25 μm).

Results

Accumulation of ECM was observed on the inner surface of the capsular bag derived from each patient. Slender cells, which were presumed to be lens epithelial cells, proliferated in this accumulated matrix (Figure 1). In both patients, the ECM exhibited staining for each of the antibodies either diffusely or in a fine granular pattern (Figure 2). Immunoreactivity for each antibody in the matrix was similar in the anterior and posterior capsules. No specific immunoreactivity was detected in negative control staining (Figure 3).

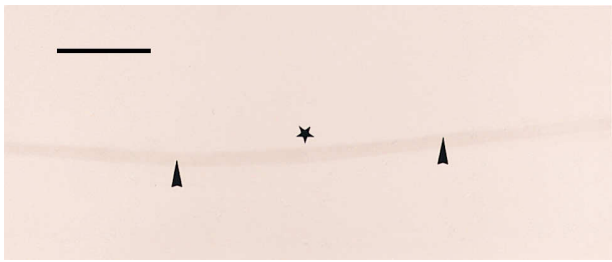

Figure 3. Negative control specimen obtained from same 55-year-old man as in Figure 2 exhibits no specific immunoreactivity. (arrows: matrix accumulation, ★ posterior capsule). (Immunostaining, Bar: 25 μm).

Discussion

The present study showed that the ECM that accumulated on the inner surfaces of the capsular bag contained proteoglycans, including chondroitin, chondroitin 4-sulfate, chondroitin 6-sulfate, dermatan sulfate, large proteoglycan, and keratan sulfate proteoglycans, although quantitative analysis had not been performed. These proteoglycans are considered to be present among the collagenous fibers accumulated in the fibrosis on the capsules.

Cultured lens epithelial cells produce collagen

types[9,10] as well as proteoglycan types.[11–14] Utilizing immunohistochemical methods, we have previously shown that several types of collagen are deposited on fibrotic lens capsules[16] and on IOLs that were explanted from patients during vitreous surgery.[19–22] Experimentally, similar deposition of collagenous matrix was detected by scanning electron microscopy and immunohistochemical methods on lens capsules and on PMMA and silicone IOLs of rabbit eyes under conditions free of postoperative complications.[23,24] Collagenous deposits on the surface of IOLs in humans and animals are believed to be produced by prolyl 4-hydroxylase-positive lens epithelial cells that proliferate on the surface of an IOL or on the posterior capsule.[16–18] Our finding of the deposition of these types of proteoglycan in the ECM suggest that the lens epithelial cells, which proliferate on the capsules after cataract extraction, also synthesize these proteoglycans and secrete them into the ECM, similar to that noted for collagen deposition. Together with our previous finding of the presence of latent transforming growth factor (TGF)-β1 binding protein in human capsular fibrosis,[16] the results reported here suggest that TGF-β1 may influence the production of collagen and proteoglycan by lens epithelial cells on the capsules. Indeed, addition of TGF-β1 into culture medium induces an accumulation of ECM by lens epithelial cells in organ-cultured rat lenses.[32] Addition of TGF-β2 into culture medium reportedly enhances the biosynthesis of collagen in cultured human lens epithelial cells,[33] and also induces the accumulation of ECM components, including collagen and heparan sulfate proteoglycan, in organ-cultured rat lenses.[34] Moreover, studies using other types of cultured cells revealed that TGF-βs increase the synthesis of various types of proteoglycans.[35,36] Various cytokines and growth factors secreted by lens epithelial cells may also influence the biosynthesis of ECM in the epithelial cells. Moreover, the growth factor components found in the intraocular fluid were elevated under the condition of proliferative vitreoretinopathy or proliferative diabetic retinopathy.[37] Growth factors contained in the aqueous humor may influence the biosynthesis of the components of extracellular matrix, including proteoglycan types in lens epithelial cells.

These proteoglycans may, in turn, influence the behavior of lens epithelial cells on the capsules.[38] Proteoglycans can be a reservoir for growth factors.[39] TGF-βs both locally secreted by the lens epithelial cells and derived in the aqueous humor can be trapped in the ECM via interaction with proteoglycans. Further detailed study is needed to clarify the roles of proteoglycans in the fibrotic change of the lens capsules after cataract surgery.

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