Osteopontin: A Component of Matrix in Capsular Opacification and Subcapsular Cataract

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PURPOSE. To examine whether tissues of human capsular opacification and subcapsular cataract contain osteopontin, an adhesive matrix protein, and whether mouse lens epithelium expresses osteopontin after injury.

METHODS. An immunohistochemical examination was conducted to determine whether matrices in human postoperative capsular specimens and anterior subcapsular cataract contain osteopontin. The spatial and temporal protein expression patterns of osteopontin were then determined in epithelium of a healing mouse lens after a capsular incision.

RESULTS. Human lens epithelial cells in the specimens extracted at the time of vitrectomy 10 days after cataract surgery and also after longer healing intervals were labeled with an anti-osteopontin antibody, whereas uninjured lens epithelium was not. In the later healing phase, matrix of capsular opacification was positive for osteopontin. Lens cells amid anterior subcapsular cataract tissue were also positive. Osteopontin was detected in the cell surface and membrane and the cytoplasm of lens cells, as well as in the matrix. Unlike normal uninjured specimens, anterior lens capsule of some of the healing postoperative specimens and anterior subcapsular cataract specimens also faintly or weakly stained for osteopontin. Mouse lens epithelium started to express osteopontin protein at 8 hours after injury, before the cells changed their shape from epithelial cell type to fibroblast type. Expression of osteopontin lasted during the healing interval, even after the cells transformed into fibroblast-like cells.


L ens epithelial cells (LEC s) are responsible for postoperative capsular opacification (PCO), the most common complication of implantation of an intraocular lens (IOL), which is caused by cell proliferation and deposition of extracellular matrix (ECM) on the capsule. LECs upregulate various molecules, such as prolyl 4-hydroxylase, fibrous collagens types, fibril-associated collagens with interrupted triple helices (FACITs) collagen types, fibronectin, laminin, fibrillin-1, and metalloproteinases and their inhibitors after cataract surgery. These proteins are considered to modulate cell adhesion and migration of LECs. LECs respond similarly in the injured animal crystalline lens. In the process of the formation of PCO, LECs undergo epithelial–mesenchymal transition and behave similarly to myofibroblasts, expressing α-smooth muscle actin. Although proliferation of LECs in association with production of ECM is therefore considered part of the wound-healing process in the postoperative crystalline lens, such a process is not desirable for restoration of vision by cataract surgery.

Osteopontin is a glycosylated protein in body fluid and ECM of various tissues, and it has characteristics of ECM components. Molecular mass deduced from SDS-polyacrylamide gel electrophoresis mobility ranges from 55 to 80 kDa. In ECM, it modulates collagen fibrillogenesis. Osteopontin is also expressed in macrophages themselves and modulates their locomotion. Moreover, it has been reported that many cell types upregulate osteopontin under pathologic conditions (e.g., during wound healing or in neoplastic tissues). All these pathologic conditions are characterized by abnormal cell proliferation with or without epithelial–mesenchymal transition and ECM deposition. Because PCO is caused by a wound response reaction by LECs as just described, the tissue may contain osteopontin.

In the present study, to explore our hypothesis that LECs upregulate osteopontin during healing after surgery, we first examined immunolocalization of osteopontin in human PCO or anterior subcapsular cataract (ASC) tissues. We included ASC specimens in this study, because it has been reported that lens cell behavior in this form of cataract is similar to that seen in healing lens after surgery or injury. LECs transform to fibroblast-like cells in association with expression of α-smooth muscle actin in both conditions. Lens cells in ASC undergo epithelial–mesenchymal transition and secrete various wound-healing–related matrix macromolecules. We then examined the protein expression pattern of osteopontin in a mouse lens epithelium after healing of a capsular incision, to determine the spatial and temporal expression pattern of osteopontin in LECs activated by injury.

MATERIALS AND METHODS

Animal experiments were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and with approvals of the Institutional Animal Care and Use Committee of Wakayama Medical University. We applied the mouse lens injury protocol approved by the National Cancer Institute (NIH, Bethesda, MD). Human specimens were examined in compliance with the tenets of the Declaration of Helsinki.

Clinical Specimens of PCO and ASC

All specimens examined had been removed from Japanese patients, with a mean age of 66.1 ± 12.3 years (range, 28–80; Table 1). Circular sections of the anterior capsules of nuclear cataracts of cases 1 to 3

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were obtained during cataract surgery. The crystalline lens in case 4 was extracted because of dislocation. These four specimens served as control, uninjured, anterior capsular specimens. Specimens were fixed in 10% formalin and embedded in paraffin, as previously reported. ASC specimens were obtained during cataract surgery. Thirteen specimens from 13 patients (male-female: 7:6) were fixed in 10% formalin and routinely embedded in paraffin. Mean age of the patients was 55.2 ± 18.4 years (range, 25–81). Two patients had atopic dermatitis, three had diabetes mellitus, and one woman had retinitis pigmentosa.

**Animal Experiments**

Male C57BL/6 mice (3 weeks old, n = 68) were anesthetized with an intraperitoneal injection of pentobarbital sodium, as well as with topical anesthesia by 0.4% oxybuprocaine. To mimic the anterior capsulotomy in human cataract surgery, we made an incision in the central anterior lens capsule with a 26-gauge hypodermic needle through the incision in one eye (right or left) after topical application of mydriatic drugs, as previously reported. After instillation of ofloxacin ointment, the animals were allowed to heal for 0, 4, 8, or 12 hours; 1, 2, 3, 5, or 7 days; or 2, 3, 4, 8, or 12 weeks. Three to five eyes were used at each interval except for the 12-week time point when six were used. The mice were killed by CO2 asphyxiation and cervical dislocation. These four specimens served as control, uninjured, anterior capsular specimens. Specimens were obtained during cataract surgery. The crystalline lens in case 4 was extracted because of dislocation. These four specimens served as control, uninjured, anterior capsular specimens. Specimens were fixed in 10% formalin and embedded in paraffin, as previously reported.

**Antibody Specificity**

We used a goat polyclonal anti-osteopontin antibody (X150 in phosphate-buffered saline [PBS]; Santa Cruz Biotechnology, Santa Cruz, CA). Specificity of the antibody was evaluated by Western blot analysis with recombinant osteopontin protein containing a carboxyl-terminal His-Tag (catalog no., CC1074; Chemicon, Temecula, CA). Protein (0.5 μg) was subjected to SDS-polyacrylamide gel electrophoresis (5%–20%) and electrotransferred to a PVDF membrane. The membrane was washed in PBS supplemented with 5% skim milk and 0.05% Triton X and then immunostained with the antibody (1:500 in PBS containing 5% skim milk) for 12 hours at 4°C or was stained with Coomassie brilliant blue (CBB). The membrane was again washed and allowed to react with a peroxidase-conjugated anti-goat IgG in PBS with 5% skim milk (×500; ICN-Cappel, Aurora, OH) for 1 hour at room temperature. Nonimmune goat IgG at 5 μg/mL served as the negative control stain. After another wash in PBS, the antibody complex was visualized with 3,3′-diaminobenzidine (DAB).  

**Table 1. Summary of the Cases**

<table>
<thead>
<tr>
<th>Case</th>
<th>Age*</th>
<th>Sex</th>
<th>Duration†</th>
<th>Cause of Surgery or IOL Removal</th>
<th>Osteopontin in Cell/Matrix</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>65</td>
<td>F</td>
<td>—</td>
<td>CCC***</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>72</td>
<td>M</td>
<td>—</td>
<td>CCC</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>58</td>
<td>M</td>
<td>—</td>
<td>CCC</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>72</td>
<td>F</td>
<td>—</td>
<td>Lens dislocation</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>28</td>
<td>M</td>
<td>6 days</td>
<td>IOL dislocation</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>64</td>
<td>M</td>
<td>10 days</td>
<td>PVR</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>58</td>
<td>F</td>
<td>14 days</td>
<td>PVR</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>68</td>
<td>F</td>
<td>0.65 y</td>
<td>PVR</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>75</td>
<td>F</td>
<td>0.75 y</td>
<td>PVR</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>51</td>
<td>M</td>
<td>1 y</td>
<td>Malignant glaucoma</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>53</td>
<td>M</td>
<td>3 y</td>
<td>PVR</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>75</td>
<td>M</td>
<td>2.7 y</td>
<td>IOL dislocation</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>78</td>
<td>M</td>
<td>4 y</td>
<td>PVR</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>80</td>
<td>M</td>
<td>4 y</td>
<td>PVR</td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td>76</td>
<td>M</td>
<td>4.6 y</td>
<td>IOL dislocation</td>
<td>+</td>
</tr>
<tr>
<td>16</td>
<td>68</td>
<td>M</td>
<td>5 y</td>
<td>IOL dislocation</td>
<td>+</td>
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<tr>
<td>17</td>
<td>77</td>
<td>F</td>
<td>5.4 y</td>
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<td>18</td>
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<td>F</td>
<td>6.2 y</td>
<td>IOL dislocation</td>
<td>+</td>
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<tr>
<td>19</td>
<td>60</td>
<td>M</td>
<td>8 y</td>
<td>IOL dislocation</td>
<td>+</td>
</tr>
</tbody>
</table>

CCC, continuous circular capsulorhexis; PVR, IOL removal to obtain the better observation of the fundus during vitrectomy for proliferative vitreoretinopathy.

* Age at the cataract surgery (cases 1–4) or at the removal of the IOL (cases 5–19).
† Duration between implantation and explantation of the IOL.

**Figure 1.** Western blot analysis of recombinant human osteopontin with a goat polyclonal anti-osteopontin antibody. Recombinant osteopontin protein containing a carboxyl-terminal His-Tag (0.5 μg) was subjected to SDS-polyacrylamide gel electrophoresis (5%–20%) and electrotransferred to a PVDF membrane. The PVDF membrane was stained with CBB or washed and immunostained with the goat polyclonal anti-osteopontin antibody or nonimmune goat IgG. After another wash in PBS, the antibody complex was visualized with 3,3′-diaminobenzidine (DAB).
Immunohistochemistry
To locate osteopontin protein in tissue, we immunostained the deparaffinized human specimens by an indirect method. Paraffin sections were cut at 5 μm, as previously described. In brief, the sections were cut through the anterior capsular break made with a needle. Deparaffinized sections were treated with H2O2-methanol to inactivate endogenous peroxidase, rinsed in PBS, and allowed to react with the primary antibody overnight at 4 °C after blocking by PBS supplemented with both 5% bovine serum and 5% skim milk. After a wash in PBS, the sections were treated with a peroxidase-conjugated secondary antibody (1:200; ICN-Cappel). The antibody complex was visualized with DAB. Specimens were counterstained with methyl green, dehydrated through an ethanol series, mounted, and observed under light microscopy. Control staining was performed with goat nonimmune IgG derived at 10 μg/mL.

RESULTS

Western Blot Analysis with the Anti-osteopontin Antibody
A single protein band was detected on the CBB-stained PVDF membrane (Fig. 1, lane 1). The anti-osteopontin antibody reacted with this band of protein (Fig. 1, lane 2), and no reaction was seen with nonimmune goat IgG (Fig. 1, lane 3).

Immunolocalization of Osteopontin in Human Specimens of PCO and ASC
Anterior lens capsule and LECs on the capsule in uninjured lenses without ASC were negative for osteopontin (Fig. 2A). Monolayered LECs, 6 (data not shown) or 10 days (Fig. 2B) after surgery, were positive for osteopontin. Human specimens of PCO were characterized by the presence of fibroblast-like lens cells and accumulation of ECM, as previously reported. ECM in postoperative capsular specimens was positive for osteopontin in the specimen 14 days after surgery or later (Figs. 2C, 2D). Regenerated lenticular fibers of Soemmerring’s ring were unstained for osteopontin, whereas cuboidal epithelial cells adjacent to regenerated lenticular fibers were stained for osteopontin (Figs. 2E, 2F). Lens capsule (AC) adjacent to osteopontin-positive epithelial cells also stained weakly for osteopontin (case 16, 5 years after surgery). A higher magnification shows obvious osteopontin labeling in epithelial cells and a weak immunoreactivity in the anterior lens capsule (AC). Negative control staining yielded no immunoreaction in the specimen shown in (F).
cataractous tissue was also very faintly stained with anti-osteopontin antibody. Osteopontin was detected in cell surface and membrane and in the cytoplasm, especially in the Golgi area of lens cells, as well as in the ECM (Fig. 3B). No immunoreactivity was seen with the control stain (Fig. 3C).

**Expression of Osteopontin in Healing Epithelial Cells of Mouse Lenses after a Capsular Incision**

Human PCO and ASC tissues were positive for osteopontin. With this finding, we were inclined to hypothesize that LECs are responsible for the accumulation of osteopontin in human cataractous conditions. To examine the spatial and temporal expression patterns of osteopontin by injury-stimulated LECs, we conducted immunohistochemistry for osteopontin in healing mouse lenses after an anterior capsular incision. Histologic findings were similar to that previously reported by us. Cuboidal lens epithelium was observed beneath the broken anterior capsule. At day 3, lens cells formed a multicell layer. At and after day 5, cells in the multicell layer were elongated and fibroblast-like in morphology.

Osteopontin protein was not detected in the epithelial cells of the uninjured lens (Fig. 4A) and in the epithelial cells in the injured lens immediately and 4 hours after the injury (data not shown). Cells near the edge of the capsular break showed osteopontin immunoreactivity in two of four specimens examined at 8 hours (data not shown) and in all three specimens examined at 12 hours after surgery (Fig. 4B) or later. At 24 hours after injury, the lens epithelium was markedly positive for osteopontin, with a gradual fade toward the lens equator (Figs. 4C, 4D). At 48 hours after injury, osteopontin protein expression was observed throughout the epithelium of the injured lens (data not shown). From day 3, lens cells were multilayered around the capsular break. At day 3, some of the multilayered lens cells were positive for osteopontin, although the cells were not well elongated, but were relatively epithelial-like (Fig. 4E). In the multilayers, lens cells were positive for osteopontin at 1 to 8 weeks after injury (Figs. 4F–H). The cells around the capsular break were found to be elongated and fibroblast-like and expressed osteopontin protein in the cytoplasm (Fig. 4G). ECM accumulation was also positive for osteopontin (Figs. 4F, 4G). Moreover, the cells in a monolayer outside the multilayer were also labeled by anti-osteopontin antibody (Fig. 4H). Findings at week 12 were similar to those at week 8 (data not shown). No specific immunoreactivity was seen in negative control staining with nonimmune IgGs (Fig. 5).

**DISCUSSION**

PCO is thought to be caused by the response of LECs to injury. Moreover, ASC exhibits histology similar to that of PCO. This notion prompted us to hypothesize that specimens of human
PCO and ASC may contain osteopontin because of its localization in pathologic tissues (i.e., tissues undergoing fibrosis or neoplastic tissues). Osteopontin is one of the key ECM molecules in modulating the behavior of local cells and also that of inflammatory cells. Our results first showed an ectopic accumulation of osteopontin protein in specimens of human PCO and ASC, in addition to other ECM components (e.g., collagens I, III, IV, V, VI, XII, and XIV; laminin; hyaluronan; fibronectin; and fibrillin-1, as previously reported). The anterior lens capsule was also sometimes weakly or faintly stained for osteopontin in healing postoperative specimens and in ASCs. It is possible that osteopontin expressed by activated or fibroblast-like LECs in such specimens accumulate in both ECM and lens capsule. Osteopontin was detected both in the cytoplasm and in the cell surface and membrane, as well as in the ECM surrounding the cells in ASC specimens. This interesting finding is suggestive of an adhesive function for osteopontin amid the ECM. A similar cell surface and cytoplasm localization of osteopontin was observed in porcine cultured lens epithelial cells in culture (Saika S, unpublished data, 2002).

Osteopontin is through to modulate cell behavior, possibly through cell surface integrin receptors. CD44 and αvβ3 integrin reportedly serve the specific cell surface receptors to osteopontin. We have reported the expression of CD44 in lens cells in PCO. Accumulation of osteopontin in the Golgi apparatus (Fig. 3B) may illustrate the processing and export of this protein to the ECM through the membrane by normal cellular transport pathways. In the present study cuboidal epithelial-type lens cells located between the peripheral capsule and regenerated lenticular fibers of Soemmerring’s ring were positive for osteopontin, whereas the epithelial cells of freshly isolated anterior capsule were not labeled by anti-osteopontin antibody. These findings indicate a difference of cellular activation status—that is, signal-transduction kinetics between these two epithelial-type lens cells—although morphology does not differentiate these two types of cells. We
have reported that Smad-3 and -4, the protein family members that convey TGFβ signaling from cell surface receptors to nuclei, are located in the cytoplasm of epithelial cells of freshly isolated capsules and that they are localized to the nuclei of epithelial-type cells in Soemmerring’s ring.26 Further study is needed, however, to clarify whether TGFβ-Smad signaling is responsible for the upregulation of osteopontin in lens cells.

To explore further the hypothesis that osteopontin is upregulated in lens epithelium during wound response and to know the spatial and temporal expression patterns of this molecule in injury-stimulated LECs, we examined the expression of osteopontin in a mouse lens after a capsular incision. The results from the animal experiment showed that epithelial cells of injured mouse lenses ectopically and transiently expressed osteopontin as early as 8 hours after a capsular incision injury, an event before morphologic changes that are characterized by transformation of single-cell-layered lens epithelium to a cell mass of multilayered fibroblastic cells (epithelial-mesenchymal transition). We have revealed that collagen type I and -smooth muscle actin, both markers for epithelial-mesenchymal transition, are upregulated in healing mouse lens epithelial cells at and after 3 and 5 days after injury, respectively (Saika S, unpublished data, 2002). Therefore, upregulation of osteopontin occurs well before overt epithelial-mesenchymal transition in the cells. Moreover, osteopontin was found to accumulate in ECM tissue formed beneath the broken capsule at the later postinjury periods and may modulate behavior of fibroblastic lens cells.

Such ectopic expression of osteopontin has also been observed in various organs in pathologic conditions: recovering heart tissue after ischemia, glomeruli in diabetic nephropathy, and neoplastic epithelial tissue.12–16 All these pathologic processes are characterized by the proliferation of fibroblasts and/or fibroblastic epithelial cells undergoing epithelial-mesenchymal transition and accumulation of ECM. Such ectopic expression of osteopontin may serve as an ECM component and also may be a chemoattractant to draw macrophages toward wounded tissues. Further in vitro study is needed to examine the effect of osteopontin on modulation of cell phenotype, migration, and proliferation in lens cells during wound healing.

Acknowledgments

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