Response of Lens Epithelial Cells to Injury: Role of Lumican in Epithelial–Mesenchymal Transition

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PURPOSE. Lens epithelial cells (LECs) undergo epithelial–mesenchymal transition (EMT) after injury and transform into myofibroblasts positive for α-smooth muscle actin (αSMA), an established marker of this process. Lumican is a keratan sulfate proteoglycan core protein. This study was conducted to examine whether human and mouse LECs express lumican after injury. To determine whether lumican may modulate EMT of LECs in response to injury or to exposure to transforming growth factor-β (TGFβ2), αSMA expression by the LECs was examined in lumican (Lum)-knockout mice in vivo and in organ culture.

METHODS. Human postoperative capsular specimens and healing, injured mouse lenses at various intervals were immunostained for lumican or αSMA. αSMA was also immunolocalized in healing, injured lenses of Lum-knockout mice. Finally, expression of lumican and αSMA was examined in lenses of Lum-knockout mice incubated with TGFβ2.

RESULTS. Lumican was immunolocalized in matrix in human postoperative capsular opacification. Lumican and αSMA were upregulated in mouse LECs from 8 hours and day 5 after an injury, respectively. LECs accumulated adjacent to the capsular break were of epithelial shape in Lum+/− mice and fibroblast-like in Lum−/− mice during healing; αSMA expression by LECs was significantly delayed in Lum−/− mice, indicating that lumican may modulate injury-induced EMT in LECs. TGFβ2-induced EMT appeared to be suppressed in organ-cultured lenses of Lum−/− mice compared with those of Lum+/− mice.


Lens epithelial cell (LEC) proliferation in association with extracellular matrix (ECM) production after cataract surgery can be considered a wound-healing response. Various ECM components and genes involved in ECM accumulation are upregulated in LECs after cataract surgery. However, the healing process often leads to formation of scar tissue rather than to regeneration of normal functional tissues. A scarred capsular bag containing an intraocular lens may become opaque, contract, and impair vision (postoperative capsular opacification; PCO). Wound-healing causes a recapitulation of cellular events, such as migration, proliferation, and differentiation, all of which occur in tissue morphogenesis during embryonic development. The transient expression of vimentin by migrating epithelium is a hallmark of the process of epithelial–mesenchymal transition (EMT). Although expressing α-smooth muscle actin (αSMA) and collagen I is characteristic of myofibroblasts, these molecules are also the established markers of EMT in LECs, in that LECs are positive for vimentin in the quiescent condition. Certainly, human LECs upregulate αSMA and PCO tissue is positive for collagen type I, indicating that human LECs undergo EMT in vivo after surgery. However, the mechanisms regulating the EMT process in LECs in response to injury are still not fully characterized.

Lumican belongs to the family of small leucine repeat proteoglycans (SLRPs). It is uniquely abundant as a keratan sulfate proteoglycan (KSPG) in corneal stroma. Lumican is also widely present as a glycoprotein without sulfated glycosaminoglycan (GAG) chains in ECM of various organs. In addition to serving as a component of ECM, lumican, like other SLRP members, has roles in tissue processes such as wound-healing and neoplasm, based on its ability to modulate cellular behavior, including proliferation and migration. Migrating corneal epithelium transiently expresses lumican, and lumican may facilitate healing of the corneal epithelium in mice. Both corneal and lens epithelia originate from surface ectoderm during embryonic development. We therefore speculated that lens epithelium may also upregulate lumican in response to injury, as in anterior subcapsular cataract (ASC) and PCO and that the expression of lumican may further modulate the fate of LECs in response to injury.

In the present studies, immunohistochemistry indicated that specimens of human ASC and PCO were labeled by anti-lumican antibody. We therefore hypothesized that lumican may play a role in modulating the LEC response to lens capsular injury (e.g., EMT). To investigate this possibility, we used lumican (Lum)-knockout mice developed by us. EMT of postinjury LECs in Lum-knockout mice was examined by histology and determination of the expression pattern of αSMA. TGFβ2 is abundant in aqueous humor and is known to be a
Figure 1. Immunolocalization of lumican and KS-GAG in human anterior lens epithelium obtained during surgery and healing capsular specimens obtained after cataract surgery. Uninjured human lens epithelium (case 3) did not stain for lumican (A) and KSPG (B). In a healing capsular specimen extracted 10 days after surgery (case 6), LECs are positive for lumican (E) and KSPG (F). ECM accumulation around the edge of the healing anterior capsular opacification obtained 4.6 years after cataract surgery (case 15). Matrix (⁎) deposited beneath the anterior capsule (AC) was positive for both lumican (E) and KSPG (F). Regenerated lenticular fibers of Soxemming’s ring extracted 5.4 years after cataract surgery (case 17). The lenticular fibers did not stain for lumican (G) or KSPG (F). Control staining with rabbit IgG yielded no immunoreactivity (not illustrated). The specimens were counterstained with methyl green (A, B) and hematoxylin (C-H). Bar: (A, B) 20 μm; (D-H) 50 μm.

Table 1. Summary of the Cases and the Results

<table>
<thead>
<tr>
<th>Case</th>
<th>Age*</th>
<th>Sex</th>
<th>Duration†</th>
<th>Cause of IOL Removal</th>
<th>Lumican</th>
<th>KS-GAG</th>
<th>αSMA</th>
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<tbody>
<tr>
<td>1</td>
<td>65</td>
<td>F</td>
<td>—</td>
<td>CCC</td>
<td>—</td>
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<td>—</td>
</tr>
<tr>
<td>2</td>
<td>72</td>
<td>M</td>
<td>—</td>
<td>CCC</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>58</td>
<td>M</td>
<td>—</td>
<td>CCC</td>
<td>—</td>
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<tr>
<td>4</td>
<td>72</td>
<td>F</td>
<td>—</td>
<td>Lens dislocation</td>
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<td>—</td>
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<tr>
<td>5</td>
<td>28</td>
<td>M</td>
<td>6.0d</td>
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<td>—</td>
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<td>6</td>
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<td>—</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>58</td>
<td>F</td>
<td>14.0d</td>
<td>PVR</td>
<td>+</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>8</td>
<td>68</td>
<td>F</td>
<td>0.65 y</td>
<td>PVR</td>
<td>+</td>
<td>+</td>
<td>—</td>
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<tr>
<td>9</td>
<td>75</td>
<td>F</td>
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<td>51</td>
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<td>—</td>
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<td>M</td>
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<td>—</td>
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<tr>
<td>14</td>
<td>80</td>
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<td>PVR</td>
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<td>—</td>
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<tr>
<td>15</td>
<td>76</td>
<td>M</td>
<td>4.6 y</td>
<td>IOL dislocation</td>
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<td>+</td>
<td>—</td>
</tr>
<tr>
<td>16</td>
<td>68</td>
<td>M</td>
<td>5.0 y</td>
<td>IOL dislocation</td>
<td>+</td>
<td>+</td>
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<td>5.4 y</td>
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<td>+</td>
<td>+</td>
<td>—</td>
</tr>
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<td>18</td>
<td>77</td>
<td>F</td>
<td>6.2 y</td>
<td>IOL dislocation</td>
<td>+</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>19</td>
<td>60</td>
<td>M</td>
<td>8.0 y</td>
<td>IOL dislocation</td>
<td>+</td>
<td>+</td>
<td>—</td>
</tr>
</tbody>
</table>

* Age in years at the removal of the intraocular lens (IOL). M, male; F, female.
† Duration between implantation and explantation of the IOL.
‡ CCC, continuous circular capsulorhexis; PVR, IOL removal to obtain the better observation of the fundus during vitrectomy for proliferative vitreoretinopathy; capsulotomy, obtained by anterior capsulotomy during cataract surgery.

Materials and Methods

Clinical Specimens of PCO and ASC

Human specimens were examined according to the tenets of the Declaration of Helsinki. Informed consent was obtained from each patient. Histologic analysis of extracted human specimens was approved by the IOL Implant Data System Committee (Akio Yamanaka, Chairperson, Kobe Kaisei Hospital, Hyogo, Japan) of the Japanese Society of Cataract and Refractive Surgery. (The committee is regulated by the Japanese National Institute of Health, which is affiliated with Ministry of Health, Labor, and Welfare of Japan.) All specimens examined had been removed from Japanese patients, with a mean age of 66.1 ± 12.3 years (range, 28–80; Table 1). Specimens of PCO were obtained at Wakayama Medical University Hospital or supplied by the Japanese Society of Cataract and Refractive Surgery.

Anterior capsules of nuclear cataracts in patients 1 to 3 were obtained during cataract surgery. The crystalline lens in patient 4 was extracted because of dislocation. Specimens were fixed and embedded in paraffin. ASC specimens were obtained during cataract surgery at Wakayama Medical University Hospital. Thirteen specimens from 13 patients (seven men, six women) were fixed in 10% formalin and 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. Informed consent was obtained for the usage of dislocated lenses and anterior capsular specimens was obtained from each patient.

Animal Experiments

Animal experiments were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Role of Lumican in EMT

We have shown that endogenous TGFβ2 affects in vivo LEC behavior in response to injury in mice, by showing that Smads3/4 nuclear translocation is blocked by a TGFβ2-neutralizing antibody. We therefore examined whether exogenous TGFβ2 modulates the expression of lumican and αSMA in the epithelium of organ-cultured lenses obtained from Lum−/− knockout mice.
and also with approvals of the Institutional Animal Care and Use Committees of Wakeyama Medical University and the University of Cincinnati. We used the protocol of mouse lens capsular injury approved by National Cancer Institute/National Institutes of Health (Laboratory of Cell Regulation and Carcinogenesis, Bethesda, MD). Adult male C57BL/6 mice (aged 3 weeks, n = 68) and lumican-knockout mice were usually anesthetized both generally and topically. A small incision was made in the central anterior capsule with a 26-gauge hypodermic needle through a corneal incision in one eye (right or left) after topical application of mydriatic drugs. The central anterior lens capsule was pierced one time by the blade part of the needle. The animals with an injury at the apex. The animals with an unexpectedly deeper injury in the lens were not included in the experiment. After instillation of ofloxacin ointment, the wild-type mice were allowed to heal for 0 (n = 5), 1 (n = 5), 8 (n = 4), 12 (n = 4), and 24 (n = 5) hours; 1 (n = 5), 2 (n = 5), 3 (n = 4), 5 (n = 4), and 7 (n = 5) days; and 2 (n = 5), 3 (n = 4), 4 (n = 5), 8 (n = 5), and 12 (n = 6) weeks. The lumican-knockout mice were genotyped as previously reported. Four-month-old lumican+/− and lumican−/− mice were used. The injured lens in an eye was allowed to heal up to 2 (n = 4 for lumican+/− and n = 2 for lumican−/−), 5 (n = 6 for lumican+/− and n = 8 for lumican−/−), and 10 (n = 6 for lumican+/− and n = 8 for lumican−/−) days. Uninjured eyes served as the control. The animals received ip administration of 5-bromo-2′-deoxyuridine (BrdU). Mice were killed by CO2 asphyxia and cervical dislocation. Enucleated globes were fixed and embedded in paraffin. Immunochemistry

To locate lumican protein in mouse tissue, 5-μm thick paraffin-embedded sections of mouse lenses were immunostained with rabbit anti-lumican antibody as previously reported. Each specimen was also immunostained with goat polyclonal antibodies against collagen I (100 diluted in phosphate-buffered saline [PBS]; Southern Biotechnology, Birmingham, AL), or with a mouse monoclonal anti-αSMA antibody (100 in PBS; NeoMarker, Inc., Fremont, CA). For detection of incorporated BrdU, deparaffinized sections were treated with 2 N HCl before immunohistochemistry with anti-BrdU antibody. Paraffin-embedded sections of human specimens were immunostained with another rabbit polyclonal antibody (100 in PBS; Sigma, St. Louis, MO). For detection of incorporated BrdU, deparaffinized sections were treated with 2 N HCl before immunohistochemistry with anti-BrdU antibody.

Paraffin-embedded sections of human specimens were immunostained with another rabbit polyclonal anti-human lumican antibody (200 in PBS), a mouse monoclonal anti-human αSMA antibody (200 in PBS; Sigma), or a mouse monoclonal anti-KSPG/KSGG antibody (5D4; 100 in PBS; Seikagaku-Kogyo, Tokyo, Japan).

Secondary antibody reaction and visualization of immune complex with 3,3′-diaminobenzidine were performed as previously reported. Specimens were counterstained with methyl green or hematoxylin, dehydrated, mounted in balsam, and observed under light microscopy. Control staining was performed with nonimmune IgGs derived from goats, rabbits, and mice at 10 μg/mL.

**TABLE 2.** Immunohistochemical Detection of Each Component in Lens Epithelial Cells/Matrix Accumulation in the Healing Lenses

<table>
<thead>
<tr>
<th>Time after Injury</th>
<th>Normal</th>
<th>8 h</th>
<th>12 h</th>
<th>24 h</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 5</th>
<th>1 wk</th>
<th>2 wk</th>
<th>4 wk</th>
<th>8 wk</th>
<th>12 wk</th>
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<tbody>
<tr>
<td>Lumican</td>
<td>−</td>
<td>−/−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>αSMA</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Collagen I</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
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</table>

* Positive in three of four specimens.
RESULTS

Immunolocalization of Lumican Protein and KSPG in Human Specimens of PCO and ASC

PCO tissue was characterized by the presence of fibroblast-like lens cells and accumulation of ECM, as previously reported. Table 1 summarizes the results by immunohistochemistry. LECs of uninjured lenses without ASC were negative for lumican protein and KSPG (Figs. 1A, 1B). Monolayer LECs 6 or 10 days after surgery were labeled by anti-lumican antibody (Fig. 1C), but negative by anti-keratan sulfate (KS) antibody (Fig. 1D). ECM in PCO was positive for lumican but negative for KS in a specimen 14 days after surgery (not shown). Both lumican and KS were detected in specimens from patients 0.65 year and later after the initial operation (Figs. 1E, 1F). Regenerated lens fibers of Sommerring’s ring were not reactive with antibodies against lumican protein and KS (Figs. 1G, 1H). ASC specimens were also characterized by the presence of ECM and fibroblastic lens cells beneath the anterior capsule and were positive for collagen I, as previously reported (Ref. 33 and data not shown). The ECM was also positive for lumican protein and KS in most of the specimens (data not shown). No immunoreactivity was seen in control immunostaining with nonimmune IgGs in PCO and ASC specimens (data not shown).

As previously reported, a-SMA-positive lens cells of a fibroblastic appearance were observed in the ECM of both PCO specimens, from 14 days to 8 years after surgery, and in ASC tissue. (data not shown).

Histology of an Injured Mouse Lens: Expression of Lumican Protein and a-SMA in the Epithelial Cells

We then examined the expression of a-SMA, collagen type I, and lumican in injured lenses of wild-type and Lumi-knockout mice. Histology showed a noticeable alteration of the morphology of cells accumulated at the site of injury in wild-type mice. LECs of the uninjured lens (Fig. 2A) and those at the capsular break at days 1 and 3 after injury (Figs. 2B, 2C, respectively) maintained a cuboidal epithelial-cell-like morphology. Thereafter, the cells assumed a fibroblast-like morphology, indicating that EMT was in progress at day 5 or later (Figs. 2D, 2E). At week 8, presumed regenerated lenticular structure, detected by cosinophilic staining, was also observed amid fibroblast-like cells and ECM accumulated at the site of injury (Fig. 2F).

Table 2 summarizes the results of immunohistochemical analysis of mouse lenses by time point. Lumican protein was not detected in the lens capsule and the LEC cytoplasm in an uninjured lens (Fig. 3A), nor in the LECs immediately and 4 hours after capsular injury (data not shown). Lumican protein was detected in corneal stroma, sclera, and other surrounding connective tissues. Cells near the edge of the capsular break showed lumican immunoreactivity in two of four specimens examined at 8 hours (data not shown) and then in all specimens examined at 12 hours after injury (Figs. 3B, 3C) and later. At 24 hours after injury, lens epithelium at the capsular break strongly reacted with anti-lumican antibody, but immunoreactivity diminished at the lens equator (Figs. 3D, 3E). At 48 hours, lumican protein expression was observed in the whole epithelium of the injured lens (data not shown). Lens cells became and remained multiple cell layers at the capsular break 3 days after injury and thereafter. The cells around the capsular break appeared elongated and assumed a fibroblast-like morphology. Within the multilayered lens cells and ECM were labeled by anti-lumican antibody at 1 and 2 weeks after injury (Fig. 3F). This lumican immunoreactivity in cells and matrix diminished and was almost negative after 4 weeks of injury (Fig. 5G) and thereafter (data not shown). Cortical fibers around the capsular break were negative for lumican protein at any time of healing (data not shown).

The LECs of the injured lenses were negative for a-SMA at days 1, 2 (Fig. 4A), and 3. The multilayered fibroblast-like lens cells at the capsular break from day 5 through week 2 after injury were labeled by anti-a-SMA antibody (Figs. 4B, 4C, respectively), whereas the epithelial-shaped lens cells outside the
multicell layer were negative. Four weeks (Fig. 4D) and longer after injury, the lens cells at the capsular break were no longer labeled by the anti-αSMA antibody, but they maintained a fibroblast-like morphology within the multiple cell layer. No immunoreactivity was seen with nonimmune mouse IgGs (data not shown).

**Immunolocalization of Collagen I in Healing, Injured Mouse Lenses**

Epithelial cells of injured lens at day 2 after injury were not labeled by anti-collagen I antibody (Fig. 5A), whereas weak immunoreactivity was noted in the single-layered lens epithelium around the capsular break at day 3 (Figs. 5B, 5C). Thereafter, collagen I persisted in ECM at the site of capsular break throughout the entire period examined up to 12 weeks of injury (Fig. 5D).

**Histology of LECs and Expression of αSMA in the Cells in an Injured Lens of Lum-Knockout Mice**

The findings that both lumican and αSMA were transiently expressed by LECs of wild-type mice after injury prompted us to hypothesize that lumican may modulate injury-induced EMT of LECs. To explore this possibility, we examined the expression pattern of αSMA by epithelial cells of injured lenses of Lum-knockout mice.29 At 2 days of injury, no morphologic difference was found between LECs at the capsular breaks of Lum+/− and Lum−−/− mice; the cells appeared epithelial-cell–like (not shown). At days 5 (Figs. 6A, 6B) and 10 (Figs. 6C, 6D) after injury, LECs of a Lum−/− mouse at the capsular break were multilayered and exhibited the characteristic spindle-shaped fibroblast morphology (Figs. 6A, 6C), whereas those of the Lum+/− mouse, although multilayered, maintained the cuboidal shape characteristic of epithelial cells, with relatively round nuclei (Figs. 6B, 6D). Electron microscopy confirmed the morphologic differences between the LECs of Lum−/− and Lum+/− mice. Accumulation of fibroblast-like lens cells was observed at the injury site of the lens of Lum−/− mice at days 5 (data not shown) and 10 (Fig. 6E), whereas epithelial-shaped cells were present in Lum+/− mice at the same time point (Fig. 6F).

Immunohistochemistry for αSMA also revealed prominent differences in the EMT process between Lum+/− and Lum−/− LECs. At day 2 after injury, healing LECs remained negative for αSMA in both Lum−/− and Lum+/− mice (data not shown). The LECs accumulated around the capsular break were markedly αSMA-positive in all Lum+/− mice at day 5 (Fig. 7A), whereas seven of eight Lum−/− mice were negative (statistically significant, \( P < 0.01 \) by \( \chi^2 \) test, Fig. 7B). At day 10, αSMA was expressed in lens cells accumulating beneath the anterior capsule in both Lum−/− (Fig. 7C) and Lum+/− mice (Fig. 7D). However, immunoreactivity was weaker in Lum−/− mice (Fig. 7D) than in
In the injured lens, much more elongated and were associated with ECM accumulation in Lmik/H9262 (Figs. 6A, 7A). Indirect immunostaining lines depicted anterior surface of the LECs (Figs. 6A, 7A). Multilayered LECs (Fig. 8C) were also observed at the site of capsular break in the lenses of Lmik/H11001 mice (Fig. 7C). Incidence of BrdU-positive lens cells was similar between Lmik/H9262 and Lmik/H9251 mice at each healing interval (data not shown). No specific immunoreactivity was seen in control with nonimmune IgGs (not shown).

**Discussion**

Ours is the first study to demonstrate the accumulation of lumican in specimens of human PCO and ASC, similar to other ECM proteins. LECs were cultured without TGFβ2 remained epithelial-like (Fig. 8P), and they become positive for lumican but remained negative for αSMA (Fig. 8N). αSMA was detected in Lmik/H9262 lenses without TGFβ2 (Fig. 8M) and Lmik/H9251 lenses with TGFβ2 (Fig. 8O). At day 10, wild-type LECs cultured without TGFβ2 remained epithelial-like (Fig. 8P), and they become positive for lumican but remained negative for αSMA (Figs. 8S, 8V). Wild-type LECs cultured with TGFβ2 became multicell layered, appeared elongated (Fig. 8Q), and were strongly reactive for antibodies against lumican and αSMA (Figs. 8T, 8W, respectively). In contrast, TGFβ2-treated Lmik/H9262 LECs were only slightly elongated and double layered with very weak immunoreactivity for αSMA (Figs. 8R, 8X). Lmik/H9251 lenses without TGFβ2 were also not labeled by anti-αSMA antibody (data not shown).
FIGURE 8. Exogenous TGFβ2 upregulated lumican protein expression in epithelium of an organ-cultured mouse lens and loss of lumican perturbed EMT. Expression of lumican and αSMA was determined by hematoxylin and eosin staining followed by immunohistochemistry. Uninjured epithelium of a Lum+/− mouse lens was negative for lumican (A, B) and αSMA (not shown). At day 1, control LECs were negative (C), whereas immunoreactivity for lumican protein were detected in Lum+/− epithelial cells in a TGFβ2-cultured cells (D, arrowheads). At day 2, faint, sporadic immunoreactivity for lumican was observed in a few cells of a Lum+/− lens without TGFβ2 (E, arrows) and epithelial cells in TGFβ2-cultured cells were markedly positive for lumican (F, arrowheads). At day 5 and day 10, lenses from Lum+/− and Lum−/− mice were examined. At day 5, histology showed cuboidal cells in Lum+/− LECs in control TGFβ2(−) culture (G) and Lum−/− LECs in TGFβ2(+) culture (I) and TGFβ2(−) culture (data not shown), whereas some of the Lum+/− cells in TGFβ2(+) culture were slightly elongated (H). At this time point, Lum+/− LECs in a control TGFβ2(−) culture were weakly positive for lumican protein (J, arrows), whereas those in a TGFβ2(+) culture were markedly positive (K, arrowheads). Very faint immunoreactivity for αSMA was detected in Lum+/− LECs in TGFβ2(+) cultures (N, ♦) but not in others (M, O). At day 10, Lum+/− LECs in a TGFβ2(+) culture were elongated in the cell multilayer beneath the capsule (Q, ♦), markedly expressing lumican (T, ♦) and αSMA (W, ♦), and Lum+/− LECs in TGFβ2(−) cultures were cuboidal and epithelial-like (P), positive for lumican (S, arrows), but negative for αSMA (V). TGFβ2-treated Lum−/− LECs were slightly elongated and double layered with a weak immunoreactivity for αSMA (R, X, ♦). (G–I, P–R) Hematoxylin and eosin; others, indirect immunohistochemistry, with methyl green counterstaining. Bar, 50 μm.
lumican and KS-GAG, suggesting that lumican is present in the KSPG form at these later stages of healing. Lens cells located within lumican-positive ECM of PCO, obtained 14 days or later after surgery, and cells of ASC expressed αSMA as previously reported4 (data not shown). These findings suggest that LECs express lumican during the process of EMT, although the exact time course of the expression of both αSMA and lumican is unknown.

We therefore examined the expression of lumican and its role in lens cell response (EMT) to injury in wild-type and *Lum* knock-out mice. Our results indicate that lumican was upregulated before EMT of the LECs, which is characterized by a fibroblast-like morphology and by expression of αSMA and collagen type I. To determine whether lumican modulates postinjury EMT of LECs, we compared the histology and expression pattern of αSMA in LECs after an injury in *Lum* knock-out mice. LECs in injured lenses of *Lum*−/− mice were histologically fibroblast-like, whereas in *Lum*+/− mice they were epithelial-cell-like at 5 to 10 days after injury. Injury-induced expression of αSMA protein was also significantly delayed in *Lum*−/− LECs compared with *Lum*+/− cells. All these findings are consistent with the notion that loss of lumican attenuates injury-induced EMT of LECs.

Many ECM components, such as fibronectin or vitronectin, or ECM receptors have been shown to participate in the conversion of various cell types to myofibroblasts under pathologic conditions.40–50 Although our present findings indicate that lumican expression modulates injury-induced EMT of LECs, keratocytes around the corneal incision in the present specimens were positive for αSMA in both *Lum*+/− and *Lum*−/− mice at day 5 (data not shown). This indicates that lumican is not essential for the conversion of keratocytes to myofibroblasts.51 Other ECM component(s) may account for conversion of keratocytes to myofibroblasts in *Lum*−/− mice, unlike in injured lens in which other ECM molecules may not compensate for the loss of lumican. Alternatively, these findings suggest differences in the mechanisms underlying generation of a fibroblast-like cell from an epithelial cell by EMT and those involving generation of a myofibroblast from a fibroblast. Retardation of EMT observed in *Lum*−/− mice is not due to impaired cell proliferation in the absence of lumican, because there was no difference in proliferation of LECs in injured lenses between *Lum*+/− and *Lum*−/− mice (data not shown). A cell surface receptor for lumican remains to be found, although macrophages have a receptor for low- or nonsulfated lumican.52 Osteoadherin, a novel bone-morphogenesis-related KSPG protein of SLRPs produced by osteoblasts, mediates cell attachment through α,β integrin in vitro.53 Similarly, an unknown integrin or related molecule may mediate lens cells binding to lumican protein.

Expression of lumican has been observed in various organs in pathologic conditions (e.g., healing ischemia-damaged heart,53 glomeruli in diabetic nephropathy,54 neoplastic epithelial tissues55). Lumican-modulated EMT may contribute to such pathogenic processes, as in an injured lens.56,57 TGFβ2 is the most abundant TGFβ isoform in aqueous humor and has an important role in modulating the behavior of LECs in wound healing.58,59 In the current study TGFβ2 upregulated lumican expression in LECs in association with EMT in wild-type mouse lenses in organ culture, whereas TGFβ2-induced EMT was suppressed by the absence of lumican. We have previously reported that Smad4 translocates into nuclei after 3 hours’ incubation with TGFβ2.60 In that these organ culture studies are predictive of effects of TGFβ2 in vivo, they suggest that endogenous TGFβ2 may be a candidate factor that upregulates lumican expression by healing LECs, which modulates the EMT of LECs.

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### References


