

# TGF $\beta$ -Smad signalling in postoperative human lens epithelial cells

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*Br J Ophthalmol* 2002;**86**:1428–1433

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Accepted for publication  
17 July 2002

**Aims:** To localise Smads3/4 proteins in lens epithelial cells (LECs) of fresh and postoperative human specimens. Smads3/4 are involved in signal transduction between transforming growth factor  $\beta$  (TGF $\beta$ ) cell surface receptors and gene promoters. Nuclear localisation of Smads indicates achievement of endogenous TGF $\beta$  signalling in cells.

**Methods:** Three circular sections of the anterior capsule, one lens, and 17 capsules undergoing postoperative healing were studied. Immunohistochemistry was performed for Smads3/4 in paraffin sections of the specimens. The effect of exogenous TGF $\beta$ 2 on Smad3 subcellular localisation was examined in explant cultures of extracted human anterior lens epithelium.

**Results:** The cytoplasm, but not the nuclei, of LECs of uninjured lenses was immunoreactive for Smads3/4. In contrast, nuclear immunoreactivity for Smads3/4 was detected in LECs during capsular healing. Nuclei positive for Smads3/4 were observed in monolayered LECs adjacent to the regenerated lens fibres of Sommerring's ring. Interestingly, the nuclei of LECs that were somewhat elongated, and appeared to be differentiating into fibre-like cells, were negative for Smads3/4. Fibroblast-like, spindle-shaped lens cells with nuclear immunoreactivity for nuclear Smads3/4 were occasionally observed in the extracellular matrix accumulated in capsular opacification. Exogenous TGF $\beta$  induced nuclear translocation of Smad3 in LECs of anterior capsule specimens in explant culture.

**Conclusions:** This is consistent with TGF $\beta$  induced Smad signalling being involved in regulating the behaviour of LECs during wound healing after cataract surgery.

Cytokines are thought to orchestrate the behaviour of lens epithelial cells during healing after cataract extraction. In many cases this results in aberrant growth of residual lens epithelial cells and the formation of fibrotic scar tissue (posterior capsular opacification, also referred to as secondary cataract or after-cataract).<sup>1–6</sup> Transforming growth factor  $\beta$  (TGF $\beta$ ), mainly TGF $\beta$ 2, is abundant in the aqueous humour.<sup>7–9</sup> TGF $\beta$  is pivotal in regulating proliferation, differentiation, and extracellular matrix (ECM) expression by cells in a positive or negative manner.<sup>10–11</sup> Lens cells express TGF $\beta$  isoforms and TGF $\beta$  receptors.<sup>12–15</sup> Moreover, we have reported that human lens epithelial cells and macrophages adhering to implanted intraocular lenses (IOLs) express TGF $\beta$  family members.<sup>15–17</sup>

Smads are proteins involved in mediating intracellular signal transduction between TGF $\beta$ /bone morphogenic protein (BMP) receptors and gene promoters,<sup>18–19</sup> although other signalling pathways have also been reported.<sup>20</sup> On ligand binding to the TGF $\beta$  receptor, phosphorylated Smad2 or Smad3 translocates to the nucleus in a complex with Smad4, and binds to a site in a promoter region. We observed that Smads3/4 translocate to the nuclei of lens epithelial cells in the healing murine lens following an anterior capsular injury. This is also the case in lens epithelial cells from murine lenses organ cultured in the presence of TGF $\beta$ 2. Taken together this indicates that healing murine lens cells are regulated by endogenous TGF $\beta$ 2 through the Smad system.<sup>14</sup> We hypothesised that human lens epithelial cells are also regulated by endogenous TGF $\beta$  during healing following cataract extraction and implantation of an IOL. To explore this hypothesis, in the present study we examined Smads3/4 localisation in lens cells in postoperative lens capsule specimens from humans. We also examined phenotypic alterations in lens cells by immunodetection of  $\beta$ -crystallin and  $\alpha$ -smooth muscle actin ( $\alpha$ SMA), these being key markers for lens fibres and myofibroblastic cells respectively.<sup>21–23</sup>

## MATERIALS AND METHODS

### Specimens

All specimens examined had been removed from Japanese patients, with a mean age of 64 years (range 28–80; Table 1). Specimens were obtained at the Wakayama Medical College Hospital, Wakayama, Japan, or were supplied by the IOL Implant Data System Committee of the Japanese Society of Cataract and Refractive Surgery. The specimens were fixed immediately after the removal. They were processed for histological examination as follows after informed consent was obtained. Circular sections of the anterior capsule of cases 1–3 were obtained during cataract surgery. The crystalline lens of case 4 was extracted because of its dislocation and was removed from an enucleated globe. Anterior capsular specimens with anterior subcapsular cataract were excluded in this study. Cases 5–21 were capsules in various stages of postoperative healing obtained during a subsequent operation to treat proliferative vitreoretinopathy, dislocation of the capsular bag with an IOL, or malignant glaucoma. Cases 6 and 8 were postoperative capsules without IOL implantation. Cases 7 and 16 had undergone implantation of an Acrysof IOL (Alcon, Fort Worth, TX, USA) and a silicone IOL, respectively. Other postoperative specimens had a polymethylmethacrylate IOL. Specimens were fixed in 10% formalin and embedded in paraffin as previously reported.<sup>24</sup>

### Immunohistochemistry

Deparaffinised sections cut at 5  $\mu$ m thickness were immunostained with rabbit polyclonal anti-Smad3 antibody (diluted  $\times$  100 in phosphate buffered saline (PBS); Zymed, South San Francisco, CA, USA), goat polyclonal antibody against Smad4 (diluted  $\times$  200 in PBS; Santa Cruz Biotechnology, Santa Cruz, CA, USA), monoclonal anti- $\alpha$ SMA antibody ( $\times$ 100, Sigma, Saint Louis, MI, USA) or rabbit polyclonal anti- $\beta$ -crystallin antibody (McAvoy, 1978, 5  $\mu$ g/ml in PBS). After washing in

**Table 1** Summary of the cases and the results

Case No	Age*	Sex	Duration	Cause†	Presence/absence of nuclear Smads3/4 positive cells			
					Epithelial-type cells adjacent to regenerated lens fibres in Sommerring's ring		Cells amid fibrous matrix accumulation	
					Smad3	Smad4	Smad3	Smad4
1	65	F	–	CCC‡	–	–	–	–
2	72	M	–	CCC	–	–	–	–
3	58	M	–	CCC	–	–	–	–
4	72	F	–	Lens dislocation	–	–	–	–
5	28	M	6 days	IOL dislocation	+	+	NE	NE
6	64	M	10 days	PVR	+	+	NE	NE
7	58	F	14 days	PVR	+	+	NE	NE
8	68	F	0.65 y	PVR	+	+	+	+
9	75	F	0.75 y	PVR	+	+	+	+
10	51	M	1 y	Malignant glaucoma	+	+	+	+
11	53	M	3 y	PVR	+	+	NE	NE
12	75	M	2.7 y	IOL dislocation	+	+	+	+
13	78	M	4 y	PVR	+	+	+	+
14	80	M	4 y	PVR	+	+	+	+
15	76	M	4.6 y	IOL dislocation	+	+	+	+
16	68	M	5 y	IOL dislocation	+	+	+	+
17	77	F	5.4 y	PVR	+	+	+	+
18	77	F	6.2 y	IOL dislocation	–	–	+	+
19	60	M	8 y	IOL dislocation	–	–	NE	NE
20	68	M	9 y	IOL dislocation	+	+	NE	NE
21	31	M	10 y	IOL dislocation	–	–	–	–

\*Age at the removal of the intraocular lens (IOL); † duration between implantation and explantation of the IOL; y = year(s); ‡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; – = negative; + = positive (regardless the incidence of positive cells); NE = not examined.

PBS, specimens were allowed to react with peroxidase conjugated polyclonal secondary antibodies ( $\times 200$  in PBS; Cappel, Organon-Teknika, West Chester, PA, USA). Reactivity/specificity of the antibodies used here was referred to the previous publication by Flanders *et al.*<sup>25</sup> After another wash, the reaction product was visualised with 3,3'-diaminobenzidine and sections were counterstained with methyl green and mounted in balsam. Negative control staining was performed with rabbit, goat or mouse non-immune IgG as primary antibody, as appropriate.

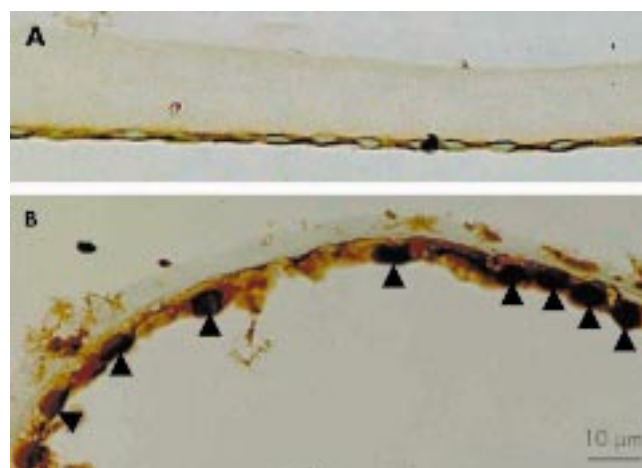
#### Explant culture experiment

In order to define whether nuclear translocation of Smad3 is induced by TGF $\beta$ 2, we cultured the anterior capsular sections excised during cataract surgery in the presence and absence of TGF $\beta$ 2. An anterior capsule was put into the Eagle's medium (Gibco BRL, Life Technologies Inc, Gaithersburg, MD, USA) in the presence or absence of the active form of porcine TGF $\beta$ 2 (1.0 ng/ml, R & D system, Minneapolis, MN, USA) immediately after extraction. We have confirmed that this TGF $\beta$ 2 is effective to human cells *in vitro* by determining collagen I upregulation in human subconjunctival fibroblast culture. Altogether 15 specimens were studied, the ages ranged from 58 to 80 years of age and the mean age was 67.7 (SD 6.31) years; the ratio of male to female specimens was 8 to 7. Capsular specimens with anterior subcapsular cataract were excluded from this study. After incubation at 37°C in 5.0% carbon dioxide/95.0% air for 0 (n=2), 30 minutes (n=3), 60 minutes (n=3), 3 (n=2), 4 (n=3), and 24 hours (n=2), the specimens were fixed in 2.0% paraformaldehyde in 0.1M phosphate buffer for 24 hours. Then the specimens were routinely embedded in paraffin. Deparaffinised sections 5.0  $\mu$ m thick were immunostained for Smad3 with a polyclonal antibody (Zymed) as described above. After counterstaining in methyl green, the sections were mounted in balsam after dehydration.

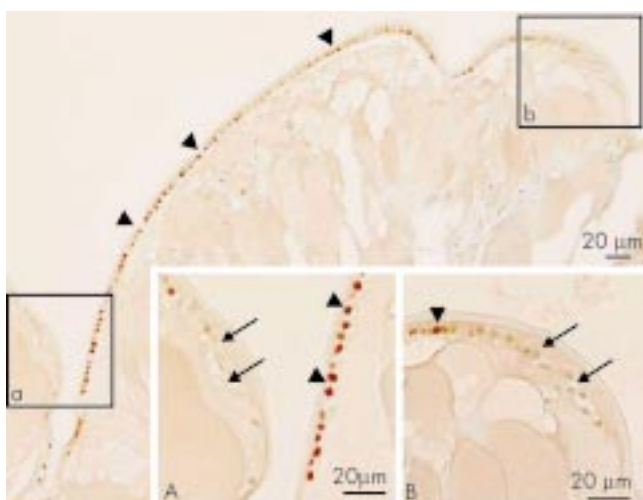
#### RESULTS

As shown in Table 1 we obtained specimens for analysis from patients at the time of cataract surgery (cases 1–4) and at

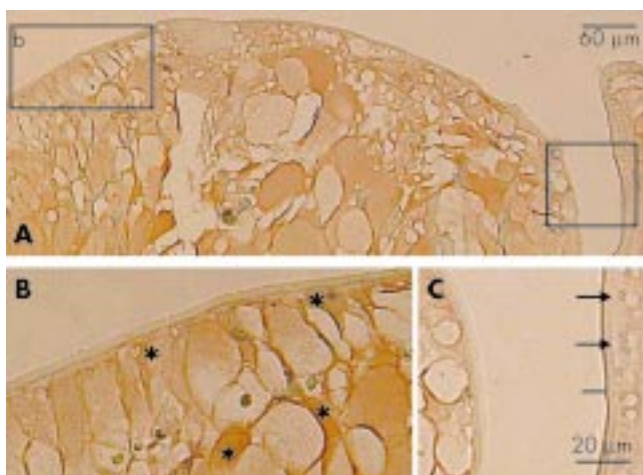
various times after cataract surgery (cases 5–21). This allowed us to analyse the distribution of TGF $\beta$  signalling molecules, the Smads, in relation to postoperative cellular changes. In particular, we examined Smads 3/4 localisation patterns in cells of the epithelium, Sommerring's ring and the ECM accumulated next to the implanted lens optic. In general, we noted that, beginning at 10 days after surgery, indications of lenticular fibre regeneration (Sommerring's ring) could be observed in the peripheral capsular bag. Fibroblast-like spindle-shaped lens cells were commonly observed in areas of fibrous opacification of the capsule in the specimens obtained at or beyond 0.65 year after initial surgery. Histology was similar to that previously reported.<sup>13</sup>



**Figure 1** Intracellular immunolocalisation of Smad3 in human lens epithelial cells on the capsular specimen obtained during cataract surgery (A, case 2) or extracted 10 days after cataract surgery (B; case 6). Lens epithelial cells on the capsular specimen, obtained during first cataract surgery are immunoreactive for Smad3 in the cytoplasm, but not in the nuclei. On the other hand, lens cells 10 days postoperatively show nuclear immunoreactivity for Smad3 with a weak reaction in the cytoplasm. Indirect immunostaining. Bar, 10  $\mu$ m.



**Figure 2** Immunolocalisation of Smad3 in lens cells in Sommering's ring 2.7 years postoperatively (case 12). Lens epithelial cells beneath the capsule (arrowheads) are positive for nuclear Smad3. (A) and (B) Higher magnification pictures of boxed areas a and b, respectively, show the progressive disappearance of nuclear Smad3 in elongating lens cells (arrows) as they differentiate into the fibre-like cells. Indirect immunostaining. Bar, 20 µm.

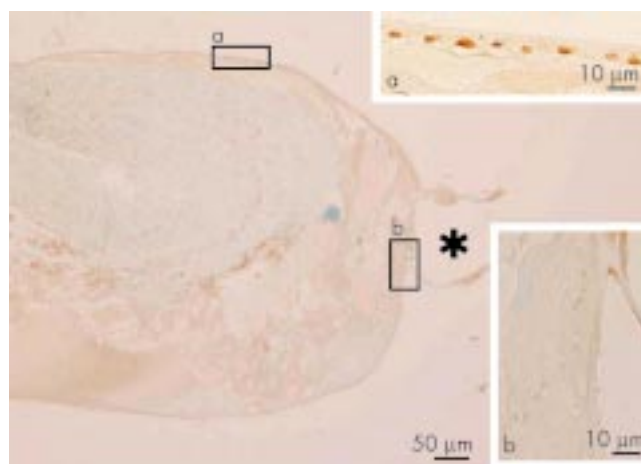


**Figure 3** Immunolocalisation of  $\beta$ -crystallin in Sommering's ring 2.7 years postoperatively (case 12). (A) Enlarged/elongated lens cells forming the Sommering's ring structure are immunostained for  $\beta$ -crystallin, whereas monolayered epithelial-shaped lens cells are unstained; (B) and (C) are the high magnification pictures of boxed areas b and c, respectively. Asterisks in (B) show immunostained enlarged/elongated cells reactive for fibre specific  $\beta$ -crystallin. Arrows in (C) show a monolayer of unstained lens cells. Indirect immunostaining. Bar, 60 µm (A); 20 µm (B and C).

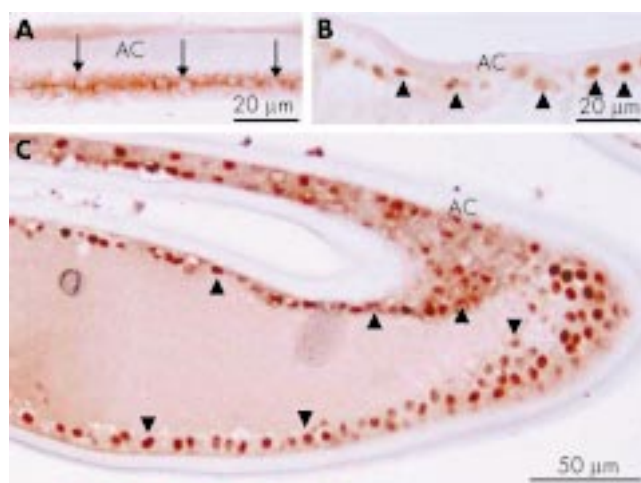
### Epithelial cells and cells adjacent to Sommering's ring

To determine if there was any evidence of TGF $\beta$  signalling through the Smad pathway as a result of cataract surgery, we compared the intracellular localisation of Smads in epithelial cells excised at the time of cataract surgery with epithelial cells in specimens collected postoperatively. Smad3, a TGF $\beta$  signalling Smad, was detected in the nuclei of epithelial-shaped lens cells as early as 10 days postoperatively, whereas it was negative in the nuclei of freshly isolated lens epithelia (Fig 1).

At 2.7 years postoperatively, in the area of the peripheral capsular bag, cuboidal, epithelial-like lens cells were positive for nuclear Smad3. Adjacent cells that were somewhat elongated, lacked immunoreactivity for Smad3 in their nuclei (Fig 2). The cuboidal cells between the regenerated fibre-like cells and residual capsule appeared morphologically similar to lens epithelial cells by light microscopy, whereas the elongated cells looked like epithelial cells undergoing the transition into



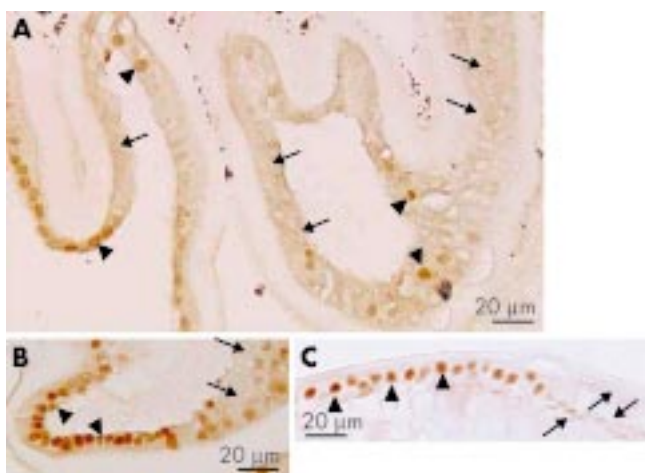
**Figure 4** Immunolocalisation of Smad3 in lens cells in Sommering's ring 5 years postoperatively (case 16). Lens epithelial cells beneath the anterior capsule (boxed area a) are positive for nuclear Smad3, but somewhat elongated lens cells in the equator (boxed area b) do not show nuclear immunoreactivity. The asterisk indicates the position of the haptic loop of the intraocular lens. Indirect immunostaining. Bar, 50 µm; inserts bar, 10 µm.



**Figure 5** Intracellular immunolocalisation of Smad4 in lens epithelial cells following cataract surgery. (A) (Case 1), epithelial cells (arrows) in an uninjured lens show Smad4 immunoreactivity in the cytoplasm. In (B) a specimen obtained 6 days after cataract surgery (case 5), some of lens epithelial cells show Smad4 in the nuclei (arrowheads), while others do not. (C) Nuclei of the lens epithelial cells (arrowheads) in the closed capsular bag 1 year postoperatively exhibit nuclear Smad4 immunoreactivity (case 10). AC = anterior capsule. Indirect immunostaining. Bar, 20 µm (A) and (B), and 50 µm (C).

fibres. These epithelial and fibre-like phenotypes were consistent with the distribution of fibre specific  $\beta$ -crystallin; cuboidal cells were negative for  $\beta$ -crystallin, whereas both denucleated lenticular fibres and elongated cells containing nuclei were immunoreactive for  $\beta$ -crystallin (Fig 3). Five years postoperatively, the cuboidal, epithelial-like lens cells maintained their nuclear Smad3 immunoreactivity and a similar loss of nuclearly localised Smad3, as described above, was evident in the differentiating fibre-like cells (Fig 4).

Smad4 is the member of Smad family of proteins which works in both TGF $\beta$  and BMP signal transduction pathways. Similar to smad3, Smad4 was detected in the cytoplasm, but not in the nuclei, of lens epithelial cells lining the inner surface of the uninjured anterior lens capsule (Table 1, Fig 5A). Postoperatively, Smad4 was located in the nuclei of cuboidal lens epithelial cells between the anterior and posterior capsules in the peripheral capsular bag and in lens epithelial cells adjacent to the regenerated lenticular structure



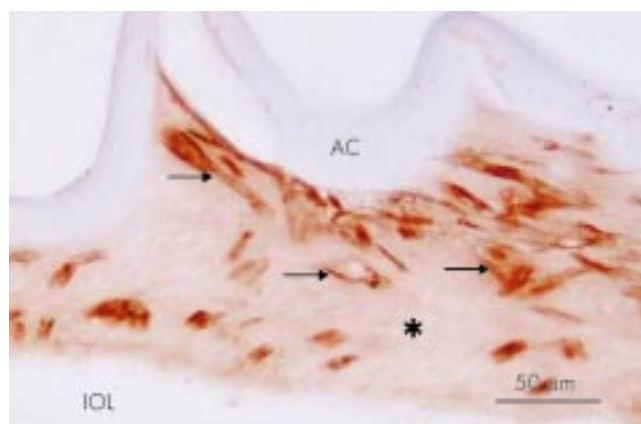
**Figure 6** Reduction of nuclear immunoreactivity of Smad4 in elongating lens cells. In (A) (case 9), some of the lens cells are positive for nuclear Smad4 (arrowheads), whereas others are negative for nuclear Smad4 (arrows). In (B) (case 7) and (C) (case 20), the cells with strong nuclear immunoreactivity tend to be cuboidal (arrowheads) whereas in regions where the cells appear to be elongating, Smad4 immunoreactivity tends to be absent or reduced (arrows). Indirect immunostaining. Bar, 20  $\mu$ m.

(Fig 5B, C). Smad4 was also detected in most cell nuclei of cuboidal lens cells in the capsular bag, but not in the nuclei of the somewhat elongated lens cells that appear to be undergoing differentiation into lenticular fibre-like cells (Fig 6).

Lens epithelial cells in specimens obtained 6.2, 8, and 10 years postoperatively or later did not show positive immunoreactivity for nuclear Smads (Table 1).

**Fibroblast-like lens cells amid extracellular matrix (ECM) accumulation**

Fibroblastic, spindle-shaped lens cells with nuclear immunoreactivity for Smads3/4 were observed in regions of ECM

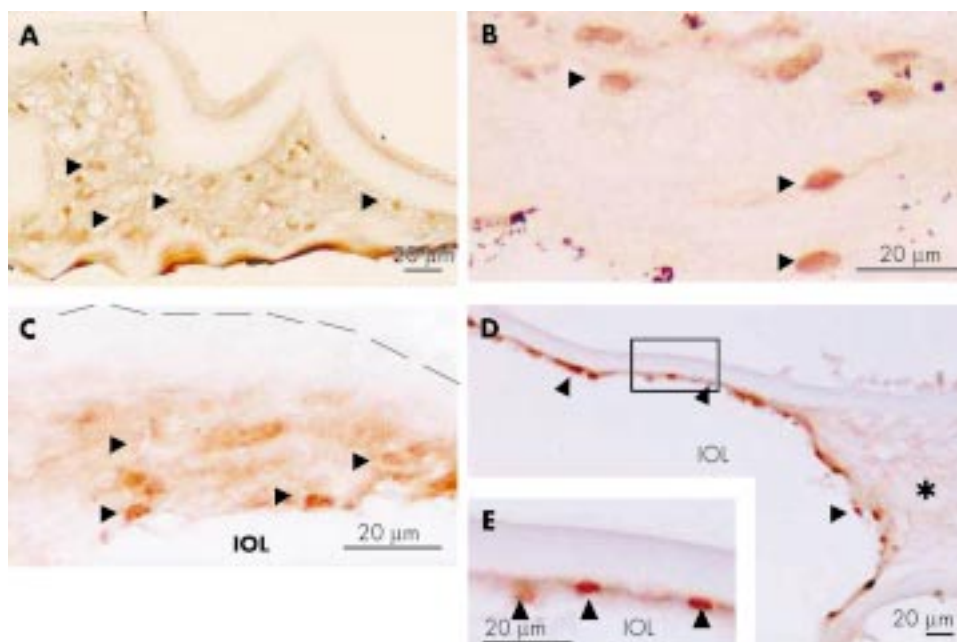


**Figure 8** Expression of  $\alpha$ -smooth muscle actin in elongated, (myo)fibroblast-like lens cells (case 15). Almost all the cells with such a configuration (arrows) are positive for  $\alpha$ -smooth muscle actin in a filamentous pattern in the cytoplasm. Indirect immunostaining. Asterisk, extracellular matrix accumulation; Bar, 50  $\mu$ m.

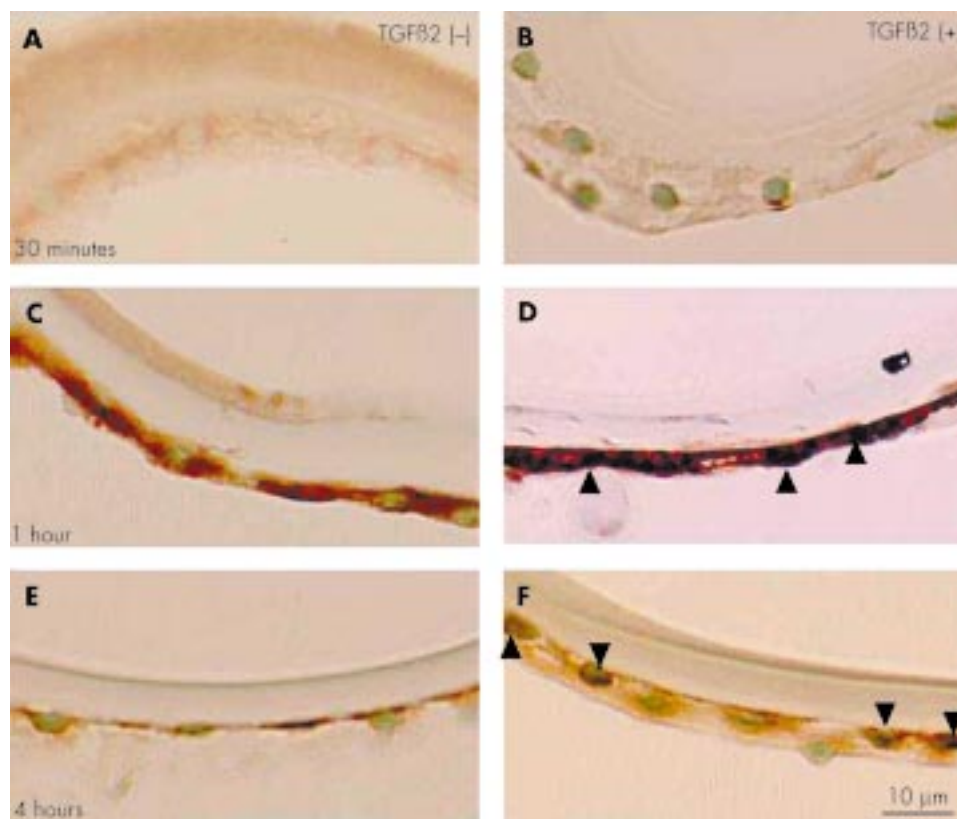
accumulation (Fig 7A–C). Although cells with unstained nuclei were also present amid matrix, the majority of the fibroblast-like lens cells there were positive for  $\alpha$ SMA as previously reported (Fig 8).<sup>13</sup> In specimens with an IOL, which was associated with ECM accumulation, however, cells with nuclear Smad3 (not illustrated) and Smad4 (Fig 7D, E) were prominent in the fibrous tissue adjacent to the optic portion of the IOL, while most elongated lens cells within the ECM accumulation did not stain for nuclear Smads3/4. Fibroblastic lens cells in specimens obtained 8 and 10 years postoperatively or later did not show positive immunoreactivity for nuclear Smads (Table 1).

**Smad3 translocation in lens epithelial cells cultured with exogenous TGF $\beta$ 2**

To determine if TGF $\beta$  induces nuclear translocation of Smads in vitro, we cultured anterior capsule specimens in the



**Figure 7** Intracellular immunolocalisation of Smads3/4 in lens cells amid ECM on the healing capsules. (A) Lens cells embedded in ECM exhibit nuclear immunoreactivity for Smad3 (arrowheads) in specimens from case 9 (0.75 years postoperatively). (B) Lens cells with nuclei positive for Smad4 (arrowheads) amid extracellular matrix accumulated inside the capsule of the specimen (case 9). (C) Some lens cells within the ECM show Smad4 immunoreactivity in nuclei (arrowheads), while others exhibit only faint cytoplasmic immunoreactivity. Lens cells attached to the intraocular lens (IOL) optic tend to exhibit the strongest nuclear Smad4 staining (case 13). (D) Nuclei of lens cells in the ECM that has accumulated around the optic portion of an IOL still stain for Smad4 2.7 years after surgery (arrowheads, case 12). No nuclear Smad4 positive cells are seen in the ECM (asterisk) that is not attached to the lens optic. (E) Shows increased magnification of the lens cells in the boxed area in (D). Broken line in (C) indicates the anterior surface of the anterior capsule. Indirect immunostaining. Bar, 50  $\mu$ m for (A) and (B) and 20  $\mu$ m for (C) to (E).



**Figure 9** Nuclear translocation of Smad3 in the epithelium of human lens anterior capsule specimens cultured in the presence of exogenous TGF $\beta$ 2. In controls, cultured in the absence of TGF $\beta$ s, Smad3 immunoreactivity was weak, or absent, from the cytoplasm of lens epithelial cells after 30 minutes' incubation (A). Cytoplasmic immunoreactivity was very strong after 1 hour (C) and weak after 4 hour (E) incubation periods. The cells in the presence of TGF $\beta$ 2 at 30 minutes showed faint nuclear Smad3 (B), whereas those at 1 hour exhibited marked Smad3 nuclear immunoreactivity (arrowheads, D). After 4 hours, weak nuclear immunoreactivity was detected in some cells (arrowheads), but not in others (F). Indirect immunostaining. Bar, 10  $\mu$ m.

presence or absence of this growth factor. In the absence of TGF $\beta$ , Smad3 protein was not detected in the nuclei of lens epithelial cells after 30 minutes, 1 hour, or 4 hours culture (Fig 9A, 9C, 9E). Cytoplasmic immunoreactivity was weak, or absent, after 30 minutes but was particularly strong after 1 hour culture (Fig 9C). In specimens cultured with TGF $\beta$  nuclear localisation of Smad3 was evident. Little reactivity was detected after 30 minutes (Fig 9B); however, after 1 hour with TGF $\beta$  there was strong nuclear reactivity (Fig 9D). After 4 hours with TGF $\beta$ , Smad3 was absent from many nuclei and only weakly detected in others (Fig 9F). Nuclear immunoreactivity was also absent after 3 hours in TGF $\beta$  treated specimens (not shown).

#### Controls

No specific immunoreactivity was observed in each negative control when non-immune serum was substituted for the primary antibody (not illustrated).

#### DISCUSSION

Results from the present study indicate that human lens epithelial cells are regulated by endogenous TGF $\beta$  during postoperative healing. Nuclear localisation of Smads3/4 was demonstrated in postoperative lenses, while these Smads were not detected in the nuclei of lens epithelial cells of uninjured lenses or in the cells of anterior capsule freshly obtained during ocular surgery. It has long been hypothesised that TGF $\beta$ s might influence postoperative lens cell behaviour. This is because it was shown that aqueous humour collected at the time of surgery contains abundant TGF $\beta$ 2,<sup>7-9</sup> although the ratio of active/total TGF $\beta$  in aqueous humour is reportedly altered during the relatively earlier phase of healing interval following cataract surgery.<sup>26</sup> Moreover, we have shown that

TGF $\beta$ 2, but not  $\beta$ 1 and  $\beta$ 3, is involved in ocular morphogenesis in mice.<sup>27</sup> A similar phenomenon was observed by us in injured mouse lenses; *in vivo* neutralisation of TGF $\beta$ 2, but not TGF $\beta$ 1 and TGF $\beta$ 3, by exogenous antibodies inhibits nuclear translocation of Smad4 during lens wound healing in mice, indicating that endogenous TGF $\beta$ 2 utilises the Smad signalling pathway during healing of the mouse lens epithelium.<sup>14</sup>

In Sommerring's ring of the peripheral capsular bag, lens epithelial cells located between regenerated lenticular fibre-like cells and lens capsule were found to be positive for nuclear Smads3/4. This finding indicates that TGF $\beta$  signals are modulating these postoperative lens cells. Although these cells were attached to fibre-like cells and were epithelial-like in morphology similar to the epithelial cells in uninjured lenses, the presence of nuclear Smads3/4 positive lens cells indicates that they may be physiologically and transcriptionally different. Interestingly, the nuclei of elongated,  $\beta$ -crystallin positive, lens fibre-like cells, lacked nuclear immunoreactivity for Smads3/4. This finding might indicate that the TGF $\beta$ -Smad signalling is not essential to the differentiation of these fibre-like cells during wound healing. Although there is evidence that TGF $\beta$  signalling is required for events in normal fibre differentiation in mice<sup>28</sup>, as yet there are no indications of whether this process is mediated by Smads. TGF $\beta$  is known to activate other signalling pathways; for example, the JNK pathway, rather than the Smad proteins, is involved in the response of muscle cells to TGF $\beta$ .<sup>29</sup>

TGF $\beta$  upregulates expression of ECM components in various cell types including lens cells.<sup>30,31</sup> We detected accumulation of collagen types, laminin, and fibronectin in human opacified lens capsules with an IOL in the previous study<sup>13</sup> and also in injured mouse lenses in a late phase of repair (Saika *et al*, data submitted). These ECM components are likely to be accumulated by the fibroblast-like lens cells

induced by TGF $\beta$ . In the present series of human specimens, some fibroblast-like lens cells in the accumulated ECM were positive for nuclear Smad3/4, although many were negative. There was some variation among specimens, in particular, nuclear Smads3/4 positive cells were more common in one specimen extracted at a relatively early phase of healing (0.75 year postoperatively; case 9, Fig 7A). Interestingly, lens cells attached to the IOL were more likely to stain for nuclear Smad4, while many of those within the ECM were unstained (Fig 7C, D). This suggests that attachment to a foreign body, such as an IOL, could influence susceptibility to TGF $\beta$  and fibrotic changes. On the other hand,  $\alpha$ SMA positive myofibroblastic cells were found to be distributed amid the matrix, indicating that the cells with nuclear Smad and cytoplasmic  $\alpha$ SMA did not overlap. One of the explanations may include that nuclear Smads can be degraded by ubiquitine system.<sup>32</sup> In this context, further studies on how different materials influence Smad mediated TGF $\beta$  signalling in lens cells may prove useful in the construction of more biocompatible IOLs.

The present study also shows that exogenous TGF $\beta$ 2 induces Smad3 translocation to cell nuclei of organ cultured human lens epithelia, further indicating that TGF $\beta$  is capable of translocating Smad3 to human lens cell nuclei. TGF $\beta$ 2 was added at the concentration of 1.0 ng/ml to the organ culture medium and this corresponds with estimates of physiological levels.<sup>9</sup> This is consistent with our conclusion that the presence of Smads in the nuclei of lens cells during postoperative healing indicates that TGF $\beta$  is a mediator of this process. However, in the in vitro study, the Smads nuclear translocation was transient and Smads were no longer detected after 3 hours' incubation. The ubiquitin system reportedly degrades the Smads after transcription has been switched on,<sup>32</sup> so this might account for the rapid loss of nuclear reactivity in these cultures. In contrast, the analysis of the in vivo material showed that the cuboidal lens epithelial cells, which lined the regenerated fibre mass, still have Smads3/4 in their nuclei even up to 5.4 years postoperatively. Detection of Smads 3/4 in the nuclei of lens cells for such a long period after surgery is an important finding. It indicates that the lens cells continue to be influenced by TGF $\beta$  over an extended period. On the other hand, it is also possible that ongoing ocular diseases—that is, diabetic retinopathy or proliferative vitreoretinopathy, might affect the TGF $\beta$ -Smad signalling in lens cells. Moreover, cytoplasmic Smad3 immunoreactivity seemed markedly increased at 1 hour's culture compared with that in 30 minutes' culture both in TGF $\beta$ 2(+) and (-) cultures. Although immunohistochemistry is not a quantitative method, we consider it possible that exogenous TGF $\beta$ 2 upregulates de novo biosynthesis of Smad3 protein by some stimulation in association with removal of the epithelium from lens cortical fibres. In the absence of exogenous TGF $\beta$ 2, however, upregulated Smad3 protein might not be phosphorylated and translocated. This should be borne in mind when strategies to protect lens cells from the damaging effects of wound healing, are being considered.

## ACKNOWLEDGEMENT

The authors thank the members of IOL Implant Data System Committee of the Japanese Society of Cataract and Refractive Surgery for providing specimens.

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