Growth Factors Influence Contractility and α-Smooth Muscle Actin Expression in Bovine Lens Epithelial Cells

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Purpose. To determine whether basic fibroblast growth factor (bFGF) and transforming growth factor-β (TGF-β2) influence the contractile activity and the expression of α-smooth muscle actin (α-SMA) in bovine lens epithelial cells (LECs). To examine whether modulation of contractile activity by these growth factors depends on changes of α-SMA expression.

Methods. Bovine LECs were cultured in collagen gel in MED 5 medium (F-12 nutrient mixture supplemented with 5% fetal bovine serum) with or without bFGF (1 to 100 ng/ml) or TGF-β2 (0.01 to 10 ng/ml). To evaluate collagen gel contraction, the longest and shortest diameters of the gels were measured daily for 7 days, and the area was determined. Detection of α-SMA in the gels was performed immunohistochemically using a mouse monoclonal antibody against α-SMA. The percentage of α-SMA-positive cells to the total number of cells was determined.

Results. Control gels cultured with MED 5 medium alone contracted to 15.8% ± 3.4% of their original area after 7 days. TGF-β2 significantly increased this contraction in a dose-dependent manner, whereas bFGF significantly decreased it. Approximately 30% of cells in the control gels were α-SMA positive. TGF-β2 significantly increased the α-SMA positivity dose dependently, whereas bFGF significantly decreased it. The percent positivity for α-SMA and the gel area showed a significant negative correlation.

Conclusions. TGF-β2 increased collagen gel contraction and α-SMA expression in bovine LECs, whereas bFGF decreased these parameters. Because collagen gel contraction was correlated with α-SMA expression, the modulation of LEC contractile activity by growth factors may be related to an effect on α-SMA. Invest Ophthalmol Vis Sci. 1995;36:1701-1708.

Because lens epithelial cells (LECs) possess contractile activity, they produce contraction of the lens capsule after cataract surgery1-5 and in patients with anterior subcapsular cataract.6 This capsular contraction directly causes visual disturbance1,8 and brings about postoperative complications such as decentration of the intraocular lens,3 narrowing of the anterior capsular opening,3-5 retinal detachment,5,7 and postoperative hypotony.8 Collagen gel culture has been used to evaluate the contractile activity of various cells,9-24 including LECs.25,26 Transforming growth factor-β (TGF-β) and basic fibroblast growth factor (bFGF) are present in the aqueous humor,27-29 and these growth factors influence collagen gel contraction induced by various cells.13,17-20 It was reported recently that TGF-β can alter the morphology of rat lens epithelial explants, including the induction of capsular wrinkling.30,31 These observations suggest that TGF-β may play a role in lens capsular contraction mediated by LECs, but it is unclear whether growth factors can actually influence collagen gel contraction mediated by these cells.

In addition, the mechanisms by which growth factors modulate collagen gel contraction mediated by other cells are not fully understood. Alpha-smooth muscle actin (α-SMA) is an isoform of actin32 that has an important role in collagen gel contraction mediated by lipocytes25 and fibroblasts.9 Collagen gel contraction mediated by fibroblasts depends on α-SMA expression and is inhibited by electrinojection of an α-SMA antibody into these cells.9 Recently, it was re-
ported that TGF-β and bFGF influence the expression of α-SMA in various cells, but it is unknown whether this effect of these growth factors is related to their influence on cellular contractile activity.

To determine whether bFGF and TGF-β2 influence the contractile activity of LECs, we cultured bovine LECs in collagen gel with or without these growth factors. In addition, to determine the effect of these growth factors on α-SMA expression in LECs, we performed an immunohistochemical study. Furthermore, to examine whether the ability of these growth factors to influence LEC contractile activity depends on α-SMA, we examined the relationship between collagen gel contraction and α-SMA expression.

METHODS

Bovine LECs were obtained using previously described methods. Cells were cultured in a humidified atmosphere of 5% CO₂ and 95% air at 37°C in IMD 10 medium, which consisted of F-12 nutrient mixture (Gibco, Grand Island, NY), 10% fetal bovine serum (Bioserum, Victoria, Australia), 0.15% sodium bicarbonate solution (Gibco), 50 U/ml penicillin (Gibco), and 50 μg/ml streptomycin (Gibco). Cells from the third passage were used for experiments. All procedures adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

To evaluate the effects of TGF-β2 and bFGF on collagen gel contraction, we performed an experiment. Collagen gels were prepared as previously described by other investigators with the following modifications. Type I collagen (3 mg/ml) derived from porcine tendon (cell matrix type I-A) was obtained from Nitta Gelatin (Osaka, Japan). To make a collagen solution, the porcine collagen, 10-fold concentrated F-12 medium, and 50 mM NaHCO₃ containing 260 mM NaHCO₃ and 200 mM HEPES were mixed at a proportion of 8:1:1 (by volume) at 4°C. Bovine LECs
added to each well of a 24-well plate (Falcon, Lincoln Park, NJ) and incubated for 30 minutes at 37°C to polymerize the collagen. We also prepared MED 5 medium, which was similar to MED 10 medium except that it contained 5% (instead of 10%) fetal bovine serum. After polymerization of the collagen, 0.5 ml of MED 5 medium alone (control) or MED 5 medium supplemented with bFGF (100, 10, or 1 ng/ml) or TGF-β2 (10, 1, 0.1, 0.01 ng/ml) was added each well. The collagen gel was positioned with a 25-gauge needle so that it floated on the medium. Then the longest and shortest diameters of each collagen gel were measured daily for 7 days to determine the gel area. The medium was changed on day 3. TGF-β2 was derived from porcine platelets and obtained from R&D Systems (Minneapolis, MN), and bFGF was derived from bovine brain and obtained from R&D Systems.

To evaluate the effects of TGF-β2 and bFGF on collagen gel contraction in the absence of LECs, the next experiment was performed using procedures similar to those described above. The differences were as follows: Collagen solution was mixed with MED 10 medium instead of bovine LEC solution, and collagen gels were cultured with MED 5 medium alone or MED 5 medium supplemented with 10 ng/ml bFGF or 1 ng/ml TGF-β2.

To determine the effect of TGF-β2 and bFGF on α-SMA expression, an immunohistochemical study was performed using the labeled-streptavidin-biotin method and a HISTOSTEIN-SP kit (Zymed, San Francisco, CA) for the detection of α-SMA. After collagen gels had been cultured for 7 days, they were immersed in 10% neutral buffered formalin for fix-
Collagen gels containing LECs that were floated on the medium immediately after detachment from the 24-well culture plate maintained their original area. However, the control gels containing LECs that were cultured with MED 5 medium alone significantly contracted with time during the 7-day culture period \((P < 0.0001; \text{repeated measures ANOVA})\) and to 15.8% ± 3.4% of their original area after 7 days (Figs. 1A, 2A). In contrast, collagen gels without LECs did not contract spontaneously and maintained their original area during the 7-day culture period when cultured with MED 5 medium alone (data not shown).

TGF-β2 significantly increased collagen gel contraction mediated by LECs in a dose-dependent manner (Fig. 1B), whereas bFGF significantly decreased it (Fig. 1B). In contrast, gels without bovine LECs that were cultured in MED 5 medium containing 10 ng/ml bFGF or 1 ng/ml TGF-β2 did not contract spontaneously (data not shown).

Approximately 30% of the cells in the control gels cultured with MED 5 medium alone were α-SMA positive (Figs. 1C, 2C). TGF-β2 significantly increased the P/T ratio in a dose-dependent manner (Figs. 1C, 3), whereas bFGF significantly decreased it (Fig. 2C). There was a significant negative correlation between the collagen gel area and the P/T ratio (Fig. 4).

**DISCUSSION**

The current study showed that TGF-β2 significantly promoted collagen gel contraction mediated by bovine LECs in a dose-dependent manner, whereas bFGF significantly inhibited it. TGF-β2 significantly increased the P/T ratio, an indicator of α-SMA expression, whereas bFGF significantly decreased this ratio. Because there was a significant negative correlation between the collagen gel area and the P/T ratio, the ability of these growth factors to influence contractile activity of bovine LECs may depend in part on changes in the expression of α-SMA.

Collagen gel contraction basically depends on three factors: cell-matrix interactions, matrix remodeling, and the number of cells in the gel. With respect to the influence of TGF-β and bFGF on cell-matrix interactions, these points can be considered. Cellular fibronectin, but not serum fibronectin, is required for collagen gel contraction by fibroblasts. Collagen gel contraction by retinal pigment epithelial cells and fibroblasts is mediated by the integrins, which also mediate cell adhesion to the extracellular matrix and to other
FIGURE 3. (top) Immunohistochemical staining of lens epithelial cells with a monoclonal antibody against α-SMA in collagen gels cultured with MED 5 medium containing transforming growth factor-β2 (1 ng/ml). (bottom) Negative control. The sections were stained with 3-amino-9-ethyl carbazole and counterstained with hematoxylin. Original magnification, ×200.

cells. TGF-β increases fibronectin expression in various cells, including LECs, and upregulates the expression of integrins for collagen and fibronectin in fibroblasts. In contrast, bFGF reduces the synthesis of fibronectin by LECs and upregulates expression of integrins for collagen and fibronectin in endothelial cells. Thus, the modulation of fibronectin and integrins by these growth factors may influence collagen gel contraction.

With regard to the influence of the growth factors on matrix remodeling, TGF-β upregulates plasminogen activator inhibitor and the tissue inhibitor of metalloprotease, both of which inhibit extracellular matrix-degrading enzymes. TGF-β also upregulates the synthesis of type I collagen. These activities of TGF-β may influence collagen gel contraction, but gel contraction mediated by retinal pigment epithelial cells has been found to be independent of metalloproteinase secretion by these cells.

The number of cells in the gel also influences collagen gel contraction by various cells, including LECs. DNA synthesis by fibroblasts is suppressed in floating collagen gels, but not in attached gels, and this inhibition is independent of the extent of gel contraction. TGF-β and bFGF have no noticeable effect on DNA synthesis by fibroblasts in contracted floating collagen gels, whereas TGF-β decreases and bFGF increases fibroblast DNA synthesis in monolayer cultures. The growth of mammary epithelial cells is inhibited by TGF-β in monolayer cultures but is unaffected in floating collagen gels. These observations suggest that modulation of collagen gel contraction by growth factors is independent of a mitogenic effect, although TGF-β inhibits the proliferation of LECs in monolayer culture, whereas proliferation is increased by bFGF.

Although LECs in normal lens do not express α-SMA in vivo, the LECs involved in lens capsular contraction after cataract surgery and in patients with anterior subcapsular cataract are surrounded by collagen fibrils and express α-SMA in vivo. The similarity of these observations with our culture system suggests that our in vitro contraction assay was suitable for evaluating the contractile activity of these LECs.

We demonstrated that TGF-β increased the percentage of α-SMA-positive cells in cultured LECs and that bFGF decreased it. TGF-β has biologic proper-

FIGURE 4. Relationship between the P/T ratio and the gel area. The P/T ratio is the ratio of α-SMA-positive cells to the total number of cells. A significant negative correlation was detected between P/T ratio and gel area (r = −0.969; P < 0.0001) using Pearson’s correlation coefficients and simple regression analysis: Gel area = −2.37 × P/T ratio + 183.7.
ties similar to those of TGF-β2 and has been reported to increase the percentage of α-SMA-positive cultured fibroblasts,endothelial cells,brain pericytes, and corneal keratocytes.TGF-β1 induces the transcription and translation of α-SMA mRNA by fibroblasts.In contrast, bFGF reduces the percentage of α-SMA-positive cultured fibroblasts, brain pericytes, and corneal keratocytes.TGF-β2 and bFGF are reported to be present in aqueous humor and in the vitreous. Jampel et al detected TGF-β in human aqueous humor at levels ranging from 2.3 to 8.1 ng/ml (mean ± SD; 4.5 ± 1.7 ng/ml), with 61% present in the active form and TGF-β2 as the main isoform. Cousins et al also reported the presence of active TGF-β in human aqueous humor at 0.45 ng/ml and stated that most of the biologic activity was caused by TGF-β2. Tripathi et al reported that human aqueous humor contains bFGF at 1.074 ± 0.158 ng/ml. It is unclear whether the concentrations of TGF-β2 and bFGF in aqueous humor are altered by cataract surgery. Although the TGF-β concentration does not change after breakdown of the blood–aqueous barrier after anterior chamber paracentesis in the cynomolgus monkey, these observations generally suggest that TGF-β2 and bFGF may influence LEC behavior after cataract surgery.

In conclusion, this study showed that TGF-β2 increased the contractile activity and α-SMA expression in bovine LECs, whereas bFGF decreased them. Because contractile activity was correlated with α-SMA expression, the influence of these growth factors on collagen gel contraction by bovine LECs may partly depend on the modulation of α-SMA expression. Thus, TGF-β2 and bFGF may have an important role in lens capsular contraction observed after cataract surgery and in patients with anterior subcapsular cataract.

Key Words
alpha-smooth muscle actin, basic fibroblast growth factor, collagen gel, lens epithelial cell, transforming growth factor-beta

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References


