EGF, EGF Receptor, Basic FGF, TGF Beta-1, and IL-1 Alpha mRNA in Human Corneal Epithelial Cells and Stromal Fibroblasts

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We sought to determine whether human corneal epithelial cells and stromal fibroblasts synthesize messenger RNAs (mRNA) coding for epidermal growth factor (EGF), EGF receptor, basic fibroblast growth factor (basic FGF), transforming growth factor-beta 1 (TGF-beta 1), and interleukin-1 alpha (IL-1α). Total cellular RNA was extracted from cultured stromal fibroblasts and ex vivo and cultured corneal epithelial cells. Oligo dt-primed complementary DNA (cDNA) was synthesized from each RNA sample. The polymerase chain reaction (PCR) was used to amplify sequences for EGF, EGF receptor, basic FGF, TGF-beta 1, IL-1 alpha, and beta actin from cDNA samples from each cell type. Southern blots of the PCR products were probed with oligonucleotides complementary to internal sequences within each of the amplified products. The amplification products were shown to be specific. For each modulator, the amplification product of the expected size was identified with at least one specific, alternative amplification product. The alternative splicing products suggest that there may be alternative mRNA splicing for each of the modulators studied. Differences were noted in the IL-1 alpha specific amplification products in stromal fibroblasts compared to corneal epithelial cells. EGF and EGF receptor mRNA production in human corneal epithelial cells and stromal fibroblasts suggest an autocrine role for EGF in the physiology of each of these cell types. Invest Ophthalmol Vis Sci 33:1756–1765, 1992

Growth factors have been shown to have essential roles in regulating the functions of cells in the tissues of higher organisms. These functions vary depending on the cell type, but include normal homeostasis and responses to environmental stimuli such as wounding. Recent studies have demonstrated that exogenous epidermal growth factor (EGF), basic fibroblast growth factor (basic FGF), and transforming growth factor beta-1 (TGF-beta 1) can modulate corneal epithelial cell proliferation in wound healing models in vitro1–5 and in vivo.6–8 These responses are mediated through specific receptors expressed by the target cells. For example, EGF receptor has been shown to be present on the surface of corneal epithelial cells.9 We recently reported that rabbit corneal epithelial cells produce messenger ribonucleic acids (mRNAs) coding for EGF, basic FGF, and TGF-beta 1 in vitro and ex vivo.10 In the present study, the polymerase chain reaction has been used to demonstrate that human corneal epithelial cells produce EGF, EGF receptor, basic FGF, TGF-beta 1, and interleukin-1 alpha (IL-1α) mRNAs in culture and ex vivo. Primary cultures of corneal fibroblasts also were noted to produce mRNAs coding for each of these modulators.

Methods and Materials

Human corneas stored for less than 96 hr in Optisol (Chiron Ophthalmics, Irvine, CA) were obtained from eye banks. Corneas were of transplant quality, but were excluded from clinical use because of advanced donor age or other nonocular exclusion criteria. Donors varied from 7 wk to 72 yr old. Corneas were punched from the endothelial side with a 10 mm trephine, and the button was bisected with a number 22 scalpel blade. The complete Descemet's membrane-endothelial complex was stripped from the stroma of each half button using fine forceps. Human epithelial cells were scraped from the limbal zone of the corneoscleral rim and grown in tissue culture ac-

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Supported in part by an Institutional Research Grant from the University of Texas Southwestern Medical Center at Dallas, a grant-in-aid from the Fight for Sight Research Division of the National Society to Prevent Blindness, Schaumburg, Illinois (Dr. Wilson), an unrestricted grant from Research to Prevent Blindness, Inc., New York, New York, and U. S. Public Health Service Grant EY09379 from the National Eye Institute, National Institutes of Health, Bethesda, Maryland.

Submitted for publication: June 18, 1991; accepted October 22, 1991.

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cording to previously described methods with media containing EGF. Keratocytes were cultured from the central stroma. Epithelial cells and keratocytes were cultured in Costar (Cambridge, MA) 25 cm² flasks. Cells were fed twice a week with complete media and were subcultured at confluence with 1:2 split. Fetal calf serum and other reagents used for tissue culture were obtained from Hazelnut Biologics, Inc. (Lenexa, KS). Anti-cytokeratin antibody immunofluorescence studies were performed using a previously described technique to demonstrate that cultures of corneal epithelial cells (positive staining) and stromal fibroblasts (negative staining) prepared by these methods were pure cultures.

Normal human corneal epithelium was obtained for ex vivo studies by scraping the central cornea with a Paton spatula at the time of epikeratoplasty. All of the epithelium was included within an approximately 8 mm diameter central zone. Epithelium was collected after application of one drop of 0.5% proparacaine, but prior to placement of fixation sutures or other manipulations that could have resulted in contamination with blood. Epithelium was immediately transferred into 0.75 ml of guanidinium thiocyanate (GTC) solution in a 1.5 ml conical tube, ground with a micropestle, and brought to a total of 1.5 ml with additional GTC solution. This protocol was approved by the Investigational Review Board, University of Texas Southwestern Medical Center at Dallas, and informed consent was obtained prior to the procedure.

The cells in each 25 cm² flask were rinsed with Hank’s balanced salt solution, and 1.5 ml of GTC solution was transferred onto the monolayer. Total cellular RNA was otherwise isolated from the ex vivo sample, eight human corneal epithelial cultures (2, primary 50–70% confluent; 2, primary 100% confluent; 2 secondary 50–70% confluent; 2, third passage 50–70% confluent), and two human, primary stromal fibroblast cultures (100% confluent) according to a previously published method.

First strand complementary DNA (cDNA) was synthesized from total cellular RNA. Each reaction was performed in a volume of 100 µl that included all of the RNA isolated from an individual culture flask for each in vitro sample. The amount of total cellular RNA isolated from a 50–100% confluent T-25 flask of epithelial cells or stromal fibroblasts was approximately 10–15 µg. All of the RNA that was isolated from the ex vivo epithelial sample was included in a 70 µl reaction.

The PCR primers that were used to amplify the cDNA sequences for EGF, EGF receptor, basic FGF, TGF-beta 1, IL-1 alpha, and beta actin (Table 1) have been previously described. Beta actin served as an internal control for the efficiency of RNA isolation and cDNA synthesis in each sample. All primers and probes were synthesized and purified by high-pressure liquid chromatography by Midland Certified Reagent Co. (Midland, TX). EGF PCR primers and probes were designed so that TGF alpha sequences, with which EGF has considerable sequence homology, would not be detected. All six primer pairs were designed so that amplification of contaminating genomic DNA sequences would produce PCR products substantially larger than PCR products amplified from cDNA because intron sequences that were excised during RNA processing would be included in genomic DNA targets (EGF, 1811, 900 base pairs; IL-1 alpha, exact number of base pairs unknown, but a large intron sequence between exons 22 and 23 is located in the middle of the PCR product; basic FGF, 33, 900 base pairs; IL-1 alpha, 8256 base pairs; TGF-beta 1, minimum of 366 base pairs, but far longer because only 100 nucleotides of the long intron within the relevant genomic interval have been determined; beta actin, 790 base pairs). PCR amplification of each sequence was performed as previously described with 5 µl of in vitro or 10 µl of ex vivo cDNA sample in a total volume of 100 µl using 2 U of taq polymerase (Perkin Elmer Cetus Corporation, Norwalk, CT) and 1.5 mM magnesium. Negative control reactions without template were routinely included in PCR amplifications with each primer set.

Horizontal 1.5% agarose (U.S. Biochemical Corp, Cleveland, OH) gel electrophoresis was performed by a previously described technique using 18 µl of each PCR product and 2 µl of 10× loading buffer per lane with a 120 ml gel run in a wide Mini-Sub cell electrophoresis apparatus (Bio Rad, Richmond, CA). Phi X174 RF DNA/hae III fragments (Bethesda Research Laboratories, Gaithersburg, MD) were used as molecular size standards. Southern hybridization was performed according to a previously described method to confirm that the PCR amplified sequences were derived from RNA coding for EGF, EGF receptor, basic FGF, TGF-beta 1, and IL-1 alpha using 30 base oligonucleotide probes that hybridized to internal, nonprimer sequences within the amplification products. In addi-

Table 1. Expected size of PCR amplification products

<table>
<thead>
<tr>
<th>Modulator</th>
<th>Size</th>
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<tbody>
<tr>
<td>EGF</td>
<td>415</td>
</tr>
<tr>
<td>EGF receptor</td>
<td>1157</td>
</tr>
<tr>
<td>Basic FGF</td>
<td>422</td>
</tr>
<tr>
<td>TGF-beta 1</td>
<td>266</td>
</tr>
<tr>
<td>IL-1 alpha</td>
<td>729</td>
</tr>
<tr>
<td>Beta actin</td>
<td>350</td>
</tr>
</tbody>
</table>

Size indicates the expected size of the amplified sequence including the 5' clamps and restriction sites based on the previously described PCR primers.
tion, EGF PCR amplification products were probed with a second 30-mer oligonucleotide probe designed from the published cDNA sequence for the EGF precursor (bases 3140–3169).\(^\text{29}\) EGF probe 2 was used to determine whether there were specific PCR products for EGF that had lost the probing sequence identified by EGF probe 1 during RNA splicing, because the EGF amplification produced a large number of product sizes from cDNA generated from corneal epithelium and stromal fibroblasts.

Seven 5-μl portions of a single primary corneal epithelial cell EGF amplification reaction were included on a Southern blot probed with EGF probe 1 according to the method described above. However, the temperature of the final 20 min wash in 6× SSC and 0.05% sodium pyrophosphate for individual lanes on the blot was varied. Final wash temperatures were 68°, 70°, 73°, 76°, 79°, 82°, and 85°C.

**Results**

The morphology of the human stromal fibroblasts in primary culture is shown in Figure 1. Figure 2

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**Fig. 1.** Inverted phase-contrast micrograph of human stromal fibroblasts (donor age 5 years) in primary culture. Original magnification ×450.

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**Fig. 2.** PCR products amplified from the cDNA samples generated from human stromal fibroblast primary cultures 1 and 2. Lanes marked with X indicate the PhiX174/HaeIII size markers. The lengths of selected markers in base pairs are provided to the left. The primers that were used to generate the PCR products\(^\text{15}\) (Table 1) are indicated above each lane. In columns with more than one band, the small arrow indicates the product of the expected length that was detected for each growth factor and beta actin. PCR amplification products of the expected size were detected for EGF, EGF receptor, basic FGF, TGF-beta 1, and beta actin in both stromal fibroblast cultures. IL-1 alpha products were not detected in either culture by visual inspection of the PCR products on the agarose gel stained with ethidium bromide. The heavy band in the TGF-beta 1 amplification at approximately 225 base pairs (large arrow) was not identified by Southern blotting (Fig. 7). This product could represent a nonspecific amplification product or a specific product that lost the specific probing sequence during RNA splicing.
shows the PCR products that were amplified from cDNA samples prepared from the cultured stromal fibroblasts. Products of the expected length were detected for EGF, EGF receptor, basic FGF, TGF-beta 1, and beta actin. IL-1α amplification products were not detected on the ethidium bromide stained agarose gel by visual inspection of the samples prepared from primary cultures of stromal fibroblasts (Fig. 2).

The morphology of human corneal epithelial cells in the primary culture and after three passages is seen in Figure 3. The PCR products generated for EGF, EGF receptor, basic FGF, IL-1α, TGF-beta 1, and beta actin from cDNA produced with primary and third passage corneal epithelium are shown in Figure 4. Figure 5 is a composite that shows the PCR products for EGF, EGF receptor, basic FGF, IL-1α, TGF-beta 1, and beta actin generated from nonconfluent and confluent primary, nonconfluent first passage, and nonconfluent third passage human corneal epithelial cells. Figure 6 shows the PCR products generated for EGF, EGF receptor, basic FGF, IL-1α, TGF-beta 1, and beta actin from cDNA produced with cor-

*Fig. 3. Inverted phase-contrast micrograph of human corneal epithelial cells in primary (A) and third-passage (B) culture. The donor age was 2 years old for both cultures. Original magnifications ×450.*
Fig. 4. PCR products amplified from the cDNA samples generated from primary and third-passage corneal epithelial cells. Lanes marked with φX indicate the PhiX174/HAIR III size markers. The lengths of selected markers in base pairs are provided to the left. The primers that were used to generate the PCR products15 (Table 1) are indicated above each lane. In primary epithelial cell columns for EGF, EGF receptor, basic FGF, IL-1 alpha, and beta actin the arrowhead indicates the product of the expected length. For TGF-beta 1, however, the expected size product cannot be detected by visual inspection, and the arrow indicates that location where the expected TGF-beta 1 product would have migrated. PCR amplification products of the expected size were detected for EGF, EGF receptor, basic FGF, IL-1 alpha, and beta actin in both cultures. Note the large number of products generated with the EGF primers, and the prominent bands that were smaller than the expected product for TGF-beta 1.

Fig. 5. Composite of the PCR products amplified from the cDNA samples generated from nonconfluent (NC) and confluent (C) primary, nonconfluent first-passage (1), and nonconfluent third-passage (3) cultured human corneal epithelial cells. The primers that were used to generate the PCR products15 (Table 1) are indicated for each row. In amplifications with more than one band the arrowhead indicates the product of the expected length. PCR amplification products of the expected size were present for EGF, basic FGF, IL-1 alpha, and beta actin in each culture. The product of the expected size for TGF-beta 1, however, is barely detectable by visual inspection in the first NC primary culture, and cannot be clearly distinguished in the remaining samples.

Hybridization of Southern blots of the PCR products generated with each primer pair with internal oligonucleotide probes complementary to 30 nucleotide intervals within the amplified EGF, EGF receptor, basic FGF, and TGF-beta 1 sequences detected amplification products of the expected sizes for cultured and ex vivo corneal epithelial cells and cultured stromal fibroblasts (Fig. 7). PCR products of the expected size for IL-1 alpha were detected in cultured and ex vivo epithelial cells but not in cultured stromal fibroblasts (Fig. 7). These Southern blots demonstrated that the PCR amplified sequences were specific for EGF, EGF receptor, basic FGF, IL-1 alpha, and TGF-beta 1. In addition to the expected amplification products, specific

neal epithelium removed at the time of epithelial ablation for epikeratophakia (ex vivo). Amplification products of the expected length were detected for each of the growth factors, IL-1 alpha, and EGF receptor in each in vitro and ex vivo epithelial sample, except for TGF-beta 1, which could not be detected by visual inspection of the PCR products from cultured epithelial cells resolved on an ethidium bromide stained agarose gel. However, it could be clearly detected in the ex vivo epithelial sample (Fig. 6). In addition, PCR products that varied from the expected size also were present for EGF, basic FGF, TGF-beta 1, and beta actin in the ex vivo cDNA sample (Fig. 6) and each of the cDNA samples generated from the cultured epithelial cells (Figs. 4 and 5). The pattern of the PCR amplification products on the agarose gels (Figs. 4-6) was similar for each of the growth factors and EGF receptor in all of the epithelial samples.
products of other sizes also were detected with the probes for EGF (approximately 750, 495, 450, and 270 base pairs with EGF probe 1 and 2; EGF receptor, approximately 1050 base pairs; basic FGF, approximately 910 and 525 base pairs; IL-1α, approximately 970 base pairs; and TGF-beta 1, approximately 330 base pairs. PCR products of the expected size for IL-1α (729 base pairs) were detected in stromal fibroblasts (Fig. 7). The approximate 970 base pair amplification product for IL-1α, however, was detected by Southern blotting the cDNA samples prepared from stromal fibroblasts (Fig. 7).

The bands on the seven lanes of the Southern blot of epithelial cell EGF amplification products probed with EGF probe 1 and 2 and washed at increasing final temperatures up to 85°C did not differ from those on the epithelial EGF blot in Figure 7 that had a final wash temperature of 68°C (data not shown). Thus, none of the alternative products identified by hybridization with EGF probe 1 were eliminated by more stringent washing conditions.

Discussion

The results of this study demonstrate that messenger RNAs coding for EGF, EGF receptor, basic FGF, IL-1α, and TGF-beta 1 are produced in cultured (Figs. 4, 5, and 7) and ex vivo (Figs. 6 and 7) human corneal epithelial cells. These results confirmed our previous study that demonstrates EGF, basic FGF, and TGF-beta 1 messenger RNA production in ex vivo and in vitro rabbit corneal epithelial cells. Messenger RNA-cDNA PCR amplification products for the beta actin control and each modulator were identical in primary nonconfluent, primary confluent, first passage nonconfluent, and third passage nonconfluent human corneal epithelial cells. The exception was that TGF-beta 1 mRNA levels appeared to be markedly decreased in third passage epithelial cells that had a cell morphology (Fig. 3) noted in nearly senescent epithelial cells. The differentiated state of the epithelial cells regarding qualitative EGF, EGF receptor, basic FGF, and IL-1α mRNA production remained stable for at least one passage in culture. TGF beta-1 mRNA expression appeared to be more variable in the cells in culture, with only a small amount detected in one of the primary epithelial cell cultures and none detectable in either third passage epithelial culture (Fig. 7B). Therefore, further study is needed with methods such as quantitative PCR to detect more subtle changes that may occur in the levels of these messenger RNAs in corneal epithelial cells during wound healing and senescence. Additional study also is needed to determine whether corneal epithelial cells in culture could provide an appropriate model for studying the in vivo expression of these growth factor, growth factor receptor, and cytokine mRNAs following corneal wounding.

Our results also demonstrate that stromal fibroblasts in primary culture produce mRNAs of the expected sizes for EGF, EGF receptor, basic FGF, and TGF-beta 1 (Fig. 2) based on the published cDNA sequences. The IL-1α sequence of the expected size was not detected in stromal fibroblasts by ethidium bromide staining of PCR products (Fig. 7). Southern blotting demonstrated, however, that the alternative IL-1α mRNA that yielded a PCR product that was larger than expected (970 base pairs) was produced in the stromal fibroblasts. The functional significance of this finding is unknown.

Previous studies have demonstrated that EGF receptor protein is produced by corneal epithelial cells. Demonstration of the protein in a tissue, however, does not conclusively demonstrate synthesis. For example, a recent study used immunodetection methods to demonstrate that retinol-binding protein (RBP) was present in the corneal endothelium. In the same study, however, in situ hybridization detected RBP messenger RNA only in the retinal pigmented epithelial cells, suggesting that the endothelial
Human Corneal Epithelium and Stromal Fibroblasts

Fig. 7. Southern blotting of PCR products from ex vivo, primary, and third-passage epithelial cell samples and primary stromal fibroblasts for (A) EGF (probe 1) and EGF receptor and (B) basic FGF, IL-1 alpha, and TGF-beta 1 with specific 30-mer oligonucleotide probes. For EGF, a is the expected size PCR product of approximately 415 base pairs. Other specific PCR products for EGF that are approximately 270 (b), 750 (c), 495 (d), and 450 (e) base pairs were detected with probe 1. The pattern, however, is complex, and there may be products of other sizes that are difficult to identify with this method. For EGF receptor, a is the expected PCR product of approximately 1157 base pairs in size. Another specific PCR product (b) approximately 1050 base pairs in size was also detected. Basic FGF product a is the amplification product of the expected size of approximately 422 base pairs. Other specific products approximately 910 (b) and 525 (c) base pairs in length were also detected. Note that the 910 base pair product was not detected in the ex vivo or primary cultured epithelial samples. IL-1 alpha PCR product a is the amplification of the expected size of approximately 729 base pairs. Another IL-1 alpha product approximately 970 base pairs in size (b) was also detected. Note that the IL-1 alpha PCR product of the expected size could not be detected in the primary stromal fibroblasts, although the 970 base pair product was present. A TGF-beta 1 PCR product of the expected size of approximately 266 base pairs (a) was detected in ex vivo and primary cultured corneal epithelial and one primary stromal fibroblast sample. The a band is very faint in one of the primary epithelial cell samples. A product that was approximately 330 base pairs (b) was also detected in these samples. Note that neither product was detected in the third-passage cultured epithelial cells or one of the stromal fibroblast cultures.

RBP was synthesized elsewhere and subsequently localized to the corneal endothelium. Studies should be performed, however, to demonstrate that EGF, basic FGF, IL-1alpha, and TGF-beta 1 proteins are present in corneal epithelial cells. One previous study did not detect EGF protein in human corneal epithelium or in tears with a radioimmunoassay. The epithelium should be reevaluated, however, because EGF subsequently has been identified in the tears of the human and the mouse. Similarly, EGF, EGF receptor, basic FGF, IL-1alpha, and TGF-beta 1 protein production should be confirmed in stromal fibroblasts.

Southern blotting of PCR products and hybridization with specific oligonucleotide probes demonstrated that the PCR products of the expected lengths were specific (Fig. 7). In addition, hybridization with the specific oligonucleotide probes also identified at least one alternative PCR product that was of a different length for EGF, EGF receptor, basic FGF, IL-1alpha, and TGF-beta 1. For example, in addition to the expected 415 base pair PCR product, other PCR products approximately 750, 495, 450, and 270 base pairs in length were amplified with the EGF PCR primers and identified by the EGF-specific oligonucleotide probes 1 and 2. None of the alternative EGF bands were eliminated by increasing the stringency of the final wash of a Southern blot probed with EGF probe 1 up to 85°C. This suggests that the complementary sequence in the alternative products was highly homologous, if not identical, to that in the EGF PCR product of the expected size.

Southern blots performed with PCR products characteristically have higher background and bands that are not as discrete as Southern blots performed with restriction enzyme digested DNA. This is attributable
adds additional bases terminal to the primers (usually adenosine that is not specified by the complementary strand). Therefore, there is usually a limited amount of size heterogeneity among the amplified DNA molecules that make up a specific band. In addition, aborted amplifications commonly occur during a PCR reaction. The products of aborted PCR amplifications migrate according to their size and will be recognized on a Southern blot if they contain the sequence complementary to the probe.

Depending on the size and number of these random aborted products, an increase in background may be noted on the Southern blot. Therefore, it was difficult to distinguish all of the alternative PCR products of the EGF amplification (Fig. 7), as an example. The alternative products were too small to represent genomic DNA amplifications for any of the modulators being studied, and are likely derived from alternative messenger RNA splicing products. It also is possible that some of these products represent specific PCR mispriming events. Several recent studies, however, have demonstrated that alternative splicing products for growth factors and growth factor receptors may be identified with PCR. The latter study demonstrated that liver cells have the capacity to produce 12 different messenger RNA splicing products for basic FGF receptor from a single genomic DNA sequence. Liver cells were found to contain a minimum of 6 of these 12 alternative RNA products. Interestingly, we did not detect the PCR product of the expected size for IL-1α in primary stromal fibroblasts, although the larger 970 base pair product was present (Fig. 7). The PCR product patterns in human cells for each of the growth factors and for EGF receptor were otherwise identical between the ex vivo and primary epithelial cells and the primary stromal fibroblasts, except for the previously mentioned variation in TGF beta-1 products. Alternative PCR products for each of these modulators are being cloned so the relationships between these products can be conclusively determined by nucleic acid sequencing.

A portion of the mRNAs coding for EGF, EGF receptor, basic FGF, IL-1α, and TGF-beta 1 could have been derived from cell types other than the epithelium in the ex vivo sample obtained at the time of epikeratophakia. For example, the corneal epithelium contains Langerhans cells. However, because the results obtained with cultured epithelial cells directly paralleled those noted in the ex vivo sample, it is likely that the detected mRNA molecules were produced in the corneal epithelium. Also, Langerhans cells are concentrated at the limbus and decrease to nearly zero in the central corneal epithelium. In the rabbit, we detected the EGF, basic FGF, and TGF-beta 1 mRNAs in the central and peripheral epithelium with similar efficiency. Therefore, Langerhans cells are likely not the only source of these modulators. In situ hybridization will be needed to demonstrate unequivocally that the mRNAs for these growth factors are produced ex vivo by corneal epithelial cells.

Several studies have suggested that EGF, basic FGF, IL-1α, and TGF-beta 1 can regulate specific functions in corneal epithelial cells and stromal fibroblasts. EGF1,2,3,5-8 and basic FGF1,5,6 have been shown to stimulate proliferation of corneal epithelial cells in culture or to increase the rate of healing of epithelial wounds. TGF beta2,4,5 has been shown to have an inhibitory effect on corneal epithelial cell proliferation. Another study29 recently demonstrated that exogenous IL-1α and TGF-beta can modulate the expression of metalloproteinases in corneal stromal cells. Further work will be needed to determine the functions of the endogenous growth factors and the effects of exogenous growth factors added to experimental systems on the expression of the endogenous modulators and their receptors.

EGF receptor protein has been detected in the plasma membrane of corneal epithelial cells.9 FGF receptors and TGF receptors have not been identified on corneal epithelial cells. However, it is probable they are present because exogenous basic FGF1,5,6 and TGF beta2,4,5 have been shown to have specific regulatory effects on the proliferation of these cells. As was previously noted, IL-1α and TGF-beta have been shown to have specific effects on the production of metalloproteinases in stromal fibroblasts.35 Thus, it is likely that EGF, basic FGF, IL-1α, and TGF beta-1 produced by the corneal epithelial cells and stromal fibroblasts have autocrine or paracrine effects on the cells producing the modulators, surrounding cells of the same type, or adjacent cells of a different type. Although messenger RNAs coding for EGF, EGF receptor, basic FGF, and TGF-beta 1 are produced by corneal epithelial cells, stromal fibroblasts, and corneal endothelial cells,15 each growth factor and cytokine may have differing functions in the three primary cell types of the cornea.

Corneal epithelial cells, stromal fibroblasts, and endothelial cells have very different embryologic origins, structures, and functions. Epithelial cells are nonkeratinized, stratified squamous cells derived from surface ectoderm.40 Basal epithelial cells proliferate throughout life, and progeny cells migrate from the basal layer to the surface where they are eventually shed into the tear film. Corneal epithelial cells provide a protective layer on the surface of the cornea, establish a smooth anterior corneal optical surface, and perform a barrier function in the regulation of corneal...
hydration.41 In response to wounding, surface defects are healed by proliferation and migration of the epithelial cells.41 Stromal fibroblasts and corneal endothelial cells are derived from neural crest cells.42-44 Stromal fibroblasts (keratocytes) produce collagen, glycosaminoglycans, and other components of the corneal stroma.45 Following penetrating corneal injury, they become activated, proliferate, migrate into the stromal wound, and participate in the wound healing response.44 Corneal endothelial cells play a dominant role in the regulation of corneal hydration and produce Descemet’s membrane.45 Human corneal endothelial cells have limited capacity to proliferate following wounding, and endothelial defects are closed by a combination of cell hypertrophy and migration.45 Variations in individual growth factors,35 growth factor receptors,36 and the associated signal transduction systems46 may produce disparate functions in different cell types such as corneal epithelial cells, stromal fibroblasts, and corneal endothelial cells that have qualitative similarities in the expression of specific growth factors and their receptors. The tools are now available to begin revealing these differences at the molecular level.

Key words: corneal epithelium, stromal fibroblasts, epidermal growth factor, epidermal growth factor receptor, basic fibroblast growth factor, transforming growth factor beta-1, interleukin-1 alpha, polymerase chain reaction

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