Purpose. To study the expression of the neurotrophic pigment epithelium-derived factor (PEDF), a protein with neurotrophic and neuronal-survival activities, by the human ocular ciliary epithelium.

Methods. Total RNA extracted from human and bovine ocular tissues were screened by Northern blot analysis with cDNA probes for PEDF. Antibodies to PEDF were used to monitor its synthesis and secretion by metabolically labeling ciliary processes in vitro with 35S-methionine, followed by immunoprecipitation. Pigment epithelium-derived factor antibodies also were used to visualize the cellular distribution of PEDF along the human and bovine ciliary epithelium. Polymerase chain reaction (PCR) and reverse transcription (RT)–PCR was used to screen cDNA libraries of tissue and cell lines derived from the ciliary epithelium to demonstrate PEDF expression.

Results. From a subtractive library of the human ocular ciliary body, the authors identified a cDNA clone exhibiting nucleotide homology with the PEDF. Northern blot analysis indicated that PEDF transcripts are present in all the ocular tissues in the human eye; in the bovine eye, it is expressed preferentially in the retinal pigment epithelium. RT–PCR and PCR demonstrated that the PEDF gene is still transcriptionally active in cultured cell lines derived from the bilayer of the ciliary epithelium. Immunoprecipitation and Western blot (immunoblot) analyses with antisera to the PEDF protein demonstrated that a predominant PEDF form of 46 kDa is synthesized in the ciliary body and is secreted as a glycoprotein of 50 kDa. By indirect immunofluorescence and immunocytochemistry, PEDF antibodies decorated both cell types that comprise the ciliary epithelium (nonpigmented and pigmented) and, more distinctively, the plasma-membrane domain of nonpigmented cells in the pars plicata region.

Conclusions. These results reveal a new site of synthesis (ciliary epithelium) and accumulation (aqueous humor) of PEDF, and they emphasize its potential importance as a trophic factor in the neuro-differentiated functions of the human ciliary epithelium. Investigative Ophthalmology & Visual Science. 1996;37:2759–2767.
tors,\(^1\) and neuropeptides,\(^2\) indicates that this tissue displays multifunctional activities.

Recently, we have identified a known cDNA clone, CBS-211, from among a large number of clones isolated from a subtracted ciliary body library that shared 100% homology with the pigment epithelium-derived factor (PEDF),\(^4\) a member of the serine protease inhibitor (serpin) gene superfamily.\(^8,20\) Recombinant PEDF induces in vitro neuronal differentiation in the human retinoblastoma tumor cell line Y-79\(^10\) and promotes neuronal survival of cerebellar granule cells in culture.\(^11\) Pigment epithelium-derived factor was discovered as a 50-kDa secretory protein in conditioned medium of retinal pigment epithelial cells in culture.\(^12\)

In this article, we report the expression, synthesis, and secretion of PEDF by ciliary epithelial cells. We show that the neurotrophic factor PEDF is expressed in several tissues of the human and bovine eye. In cell lines derived from the ciliary epithelium, Western blot analysis and radioimmunoprecipitation experiments with metabolically radiolabeled proteins in explants of ciliary processes demonstrate that the 50-kDa PEDF protein is the predominant secretory form, whereas a 46-kDa protein is the predominant form found intracellularly. These results, together with the specific cellular localization of PEDF along the ciliary epithelium, support the idea that this tissue contributes in part to the naturally occurring PEDF glycoprotein in aqueous humor, where it may exert its neurotrophic–survival functions.

**MATERIALS AND METHODS**

**Source of Eye Tissues**

Human eyes were obtained from cadavers within 24 hours of enucleation through the National Disease Research Interchange (Philadelphia, PA). Under a dissecting microscope, ciliary body, cornea, iris, retinal pigment epithelium, and retina were microdissected and stored in liquid nitrogen until further analysis. Aqueous humor fluid was obtained from donor patients through the Yale Eye Center (New Haven, CT).

Bovine eyes from 2-week-old calves were obtained through a local slaughterhouse. Ocular tissues (ciliary body, cornea, lens, retinal pigment epithelium, and retina) were microdissected within 2 hours of enucleation. Investigations with the human subjects, which involved only residual material from autopsy, was approved by the Human Subjects Committee of Yale University and, as far as it applies, followed the tenets of the Declaration of Helsinki. Research using experimental animals adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

cDNA Libraries

The cDNA libraries used in this work were constructed from human and bovine ciliary bodies and from human and bovine ciliary epithelial cell lines in the Uni-ZAP XR lambda vector (Stratagene, La Jolla, CA) and agtI vector (Promega, Madison, WI) as previously described.\(^1,7,13\)

**Polymerase Chain Reaction Assays for Human Pigment Epithelium-Derived Factor and Nucleotide Sequencing**

Polymerase chain reaction conditions were determined according to the PrimerSelect program (DNASTAR, Madison WI). The PCR method of Saiki et al\(^14\) was used to anneal a pair of primers corresponding to the human nucleotide sequence of the PEDF cDNA (GenBank accession number M76979)—a forward primer, 5’-CAT GAT GGG CCC CAC GAC CAA C-3’ and a reverse primer, 5’-TGC GCA ACA CCG AGG AGA AT-3’—to DNA from human and bovine cDNA libraries of ciliary bodies\(^1,3\) and to DNA from human and bovine cDNA libraries of ciliary epithelial cell lines.\(^7\) The forward primer is located at position 326 to 347, and the reverse primer is located at position 751 to 729 of the human nucleotide sequence of the PEDF cDNA (GenBank accession number M76979).\(^8\) Reaction mixtures prepared in 100 µl in MicroAmp tubes (Perkin Elmer, Emeryville, CA) contained 10 mM Tris–HCl (pH 9), 50 mM KCl, 0.1% Triton X-100, and 1.5 mM MgCl₂ as buffering components, and DNA from 10⁶ pfu of λUni-ZAP, 200 µM each deoxynucleoside triphosphates, each oligonucleotide primer at 1 µM, and 5U of Taq polymerase (Promega) as reaction components. Polymerase chain reaction was completed in a Perkin Elmer DNA Thermal Cycler 480 (Perkin Elmer Cetus, Norwalk, CT). Samples were inserted into the machine during an initial incubation of the lambda phage particles at 94°C for 1 minute. Each cycle consisted of a denaturation step at 94°C for 1 minute, 1 minute of annealing at the optimal temperature of 61°C, and 1 minute of polymerization at 72°C. This was repeated for 35 cycles; the final polymerization step was extended an additional 5 minutes. Five per cent of the resultant PCR product was size fractionated by electrophoresis on 1% agarose-SeaPlaque gels (FMC BioProducts, Rockland, ME).

An expected 462-nt PCR product for PEDF was obtained and sequenced directly without subcloning using a Sequenase PCR sequencing kit (United States Biochemical, Cleveland, OH). The DNA sequence was compiled and aligned using the DNASTAR program. The sequence obtained from the PCR DNA product shared complete homology with published nucleotide sequence for human PEDF.
Reverse Transcription–Polymerase Chain Reaction Analysis

Total RNA was prepared from human ciliary processes microdissected from donors eyes or from the human NPE cell line, and cDNA was synthesized from 5 μg of total RNA as template according to the instructions of the manufacturer of the RT–PCR kit (Stratagene). Polymerase chain reaction amplification of the human PEDF transcripts was performed as described above.

Northern Blot Analysis

RNA was extracted according to the method of Chomczynski and Sacchi15 using guanidinium thiocyanate. When specified, poly A+ mRNA from ciliary processes was selected using cellulose oligo dT. Total RNA samples (20 μg/lane) or poly A+ mRNA (2 μg/lane) were fractionated by electrophoresis on a 1% agarose gel containing formaldehyde and were transferred by capillary action onto a Nytran filter (Schleicher & Schuell, Keene, NH) as previously described.1 Blots were hybridized with a 32P-labeled cDNA probe for PEDF. A human PEDF probe was generated after the 0.8-kb cDNA insert in clone CBS-211 [pBluescript SK(−)] was excised with EcoRI and XhoI.1 The 0.8-kb DNA fragment was purified and labeled with 32P-dCTP (Amersham) by random priming. Hybridization and subsequent washing of the filters was carried out under standard conditions.1 Filters were autoradiographed at −70°C with intensifying screens.

Western Blots and Immunoprecipitations

Whole-cell lysates were prepared from ciliary processes, and proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred onto Nytran filter by semidy electroblotting (Schleicher & Schuell) as previously described.7 Blots were probed with the rabbit anti-PEDF (dilution 1:100).18 Secondary antibodies were peroxidase-conjugated goat anti-rabbit immunoglobulin G (Amersham, Arlington Heights, IL) by random priming. Hybridization and subsequent washing of the filters was carried out under standard conditions.1 Filters were autoradiographed at −70°C with intensifying screens.

Indirect Immunofluorescence and Immunocytochemistry

For immunofluorescence studies, semithin cryostat sections (0.5 μm) from bovine ciliary processes were used as previously described.8 Sections were incubated at 37°C with the primary antisera anti-PEDF at a dilution of 1:100 for 90 minutes. The sections were rinsed in PBS and were incubated further with the secondary antisera (100-fold-diluted rhodamine-conjugated goat anti-rabbit immunoglobulin G) for 60 minutes. After washing in PBS and mounting in a solution of glycerol mounting medium, the specimens were analyzed with a Zeiss Axioskop fluorescent microscope (Carl Zeiss, Göttingen, Germany). Photographs were taken using TMAX-400 film (Eastman Kodak, Rochester, NY). As a control, sections were stained with secondary antibodies alone or with the primary antibody preadsorbed with recombinant PEDF protein. In both cases, no signal was obtained.

For immunocytochemistry, ciliary processes were microdissected from the donor eye of a 50-year-old cadaver, fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) embedded in paraffin and serially sectioned at 4 to 5 μm. Deparaffinized sections were stained with PEF antibodies (1:300 dilution), and immunoreactivity was detected after a biotin–streptavidin-amplified system (BioGenex, San Ramon, CA). Sections were counterstained with 1% of Alcian blue, viewed on a Zeiss Axioskop and photographed using Ektachrome 64T color film (Kodak).

RESULTS

Expression of Pigment Epithelium-Derived Factor in the Human Ciliary Body

Partial nucleotide sequencing of a cDNA clone (CBS-211), recently isolated from a subtractive human ciliary body library1 (GenBank accession number R36800), showed extensive nucleotide homology with the nucleotide sequence reported for the human PEDF cDNA, which was isolated from a fetal human eye library6 (accession number M78979). We extended the nucleotide sequence of the 0.8-kb cDNA insert in CBS-211 and verified that it shares 100% identity with PEDF (data not shown). To determine the pattern of expression of PEDF mRNA in an adult human eye, we probed a Northern blot containing RNA from distinct ocular tissues dissected from a pair of human donor eyes with the 0.8-kb cDNA insert of clone CBS-211. Figure 1 shows the result of this hybridization. An expected, 1.5-kb mRNA (the size previously reported for human PEDF) hybridized to the

0.5 ml of 200 mM glycine–HCl, 0.25% bovine serum albumin (pH 3.3) and neutralized by the addition of 2 ml of 2M Tris–HCl (pH 9.1) to the elution medium.16
FIGURE 1. Pigment epithelium-derived factor (PEDF) mRNA expression in ocular tissues of a human eye donor. Ciliary body (C.B.), cornea, iris, retinal pigment epithelium (RPE), and retina were dissected from a pair of donor eyes, and total RNA from each tissue was separated in a 1% agarose gel, blotted, and hybridized with the 0.8-kb cDNA insert of clone CBS-21I.1 (A, B, arrows) Position of the 1.5-kb specific PEDF transcript size. B shows a longer exposure (4 days) than A (18 hours) to reveal PEDF transcript signal in cornea. (C) 28S ribosomal RNA from each tissue, stained with ethidium bromide. (A, B, left) Migration positions of standard RNA molecular weight markers are indicated.

0.8-kb DNA probe in all the ocular tissues tested, including ciliary body, iris, RPE, and retina (Figs. 1A, 1B). Thus, despite the unexamined human lens and weak detection in the cornea, PEDF transcripts are expressed widely in human eye tissues. Northern blot analysis of RNA from cell lines established from the ocular ciliary epithelium and from nonpigmented and pigmented ciliary epithelial cells failed to detect PEDF transcripts (not shown). However, a more sensitive assay, such as PCR or RT-PCR, demonstrated that transcripts for PEDF are expressed in these cells in culture (see below).

In contrast, Northern blot analysis with bovine ocular tissues indicated that the 1.5-kb mRNA for PEDF was expressed preferentially in RPE (Fig. 2A); PEDF mRNA was not detected in the rest of the ocular tissues. The detection of PEDF transcripts in the ciliary body required the isolation of poly A+ mRNA (Fig. 2B). Under these conditions, the 1.5-kb mRNA specific for PEDF was detected clearly in two areas of the pars plicata region of the ciliary epithelium (regions A and B) (Fig. 2B). This region comprises approximately 90% of the surface area of the entire ciliary epithelium in the bovine eye, and it corresponds to the anterior and posterior regions of this epithelium.3

These results suggest possible species differences in the expression of PEDF mRNA between human and bovine eye tissue. Although in the human eye PEDF appears to be ubiquitous, in bovine eyes, PEDF appears to be predominantly expressed in the RPE. When the Northern blot shown in Figure 2A was hybridized with a bovine DNA probe for PEDF obtained by PCR amplification (see below), the hybridization pattern was similar to that seen with the human PEDF probe (data not shown). This result confirms that the human and bovine eye shows clear, distinct patterns of expression for the PEDF gene.

Polymerase Chain Reaction Amplification of the Human and Bovine DNA Homologous of Pigment Epithelium-Derived Factor in Cultured Ciliary Epithelial Cell Lines

We used PCR and RT-PCR to determine whether PEDF mRNA is expressed in cultured pigment epithelial (PE) and nonpigment epithelial (NPE) cell lines established from the ciliary epithelium. Using a set of oligonucleotide primers based on the human PEDF cDNA nucleotide sequence (GenBank accession number M76979), we amplified by PCR the DNA inserts from human and bovine cDNA libraries constructed
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PEDF cDNA reported earlier8 (data not shown). These were sequenced using a Sequenase PCR sequencing kit NPE (lane 4). We also detected the 426-nt product in human cDNA libraries of the ciliary body and established cell lines of the ciliary epithelium. Equivalent lambda particle form-

PEDF was 100% (unpublished results, 1996). Align-

cDNA PCR product obtained from the human NPE cell line cDNA library (lane 4) was selected for direct nucleotide se-
ting units (pfu) particles from libraries constructed in lambda gt11 Sfi-Not or in Uni-ZAP XR vectors were amplified in parallel by PCR using oligonucleotide primers complementary to the human PEDF gene, as described in Material and Methods. The predicted 426-nt fragment amplified from all the libraries was resolved on a 1% agarose gel with ethidium bromide stain, and its migration position was marked with an arrow at right. The DNA PCR product obtained from the human NPE cell line cDNA library (lane 4) was selected for direct nucleotide sequencing and verified to exhibit 100% homology with the reported human PEDF nucleotide sequence.8 Lane M = 1-kb ladder DNA marker; lane 1 = bovine ciliary body library; lane 2 = bovine PE cDNA library; lane 3 = bovine NPE cDNA library; lane 4 = human NPE cDNA library; lane 5 = human ciliary body cDNA library.

from cell lines established from the human and bovine ciliary epithelium. As expected, a 426-bp product (Fig. 3) was obtained in all the libraries, including a bovine PE (lane 2), a bovine NPE (lane 3), and a human NPE (lane 4). We also detected the 426-nt product in the ciliary epithelial cell lines by RT–PCR (data not shown). The DNA PCR product obtained with the human NPE cell line cDNA library (Fig. 3, lane 4) was sequenced using a Sequenase PCR sequencing kit (United States Biochemical). Furthermore, the homology between the human and bovine nucleotide sequences along the 426-bp DNA PCR products for PEDF was 100% (unpublished results, 1996). Alignment and comparison of our nucleotide sequence indicated that it shares 100% identity with the human PEDF cDNA reported earlier8 (data not shown). These results demonstrate that PEDF mRNA is expressed in the intact ciliary body and in cultured cell lines derived from both layers of the ciliary epithelium.

FIGURE 3. Polymerase chain reaction (PCR) amplification of pigment epithelium-derived factor (PEDF) from bovine and human cDNA libraries of the ciliary body and established cell lines of the ciliary epithelium. Equivalent lambda particle forming units (pfu) particles from libraries constructed in lambda gt11 Sfi-Not or in Uni-ZAP XR vectors were amplified in parallel by PCR using oligonucleotide primers complementary to the human PEDF gene, as described in Material and Methods. The predicted 426-nt fragment amplified from all the libraries was resolved on a 1% agarose gel with ethidium bromide stain, and its migration position was marked with an arrow at right. The DNA PCR product obtained from the human NPE cell line cDNA library (lane 4) was selected for direct nucleotide sequencing and verified to exhibit 100% homology with the reported human PEDF nucleotide sequence.8 Lane M = 1-kb ladder DNA marker; lane 1 = bovine ciliary body library; lane 2 = bovine PE cDNA library; lane 3 = bovine NPE cDNA library; lane 4 = human NPE cDNA library; lane 5 = human ciliary body cDNA library.

FIGURE 4. Pigment epithelium-derived factor (PEDF) protein in ciliary body, vitreous, and aqueous humor. (A) Autoradiogram of gel after SDS-PAGE of radio-immunoprecipitation results, the additional bands (36-, 40-, 53- and 60-

metabolically labeled in vitro with 35S-methionine. Radiolabeled PEDF proteins were selected by immunoprecipitation with antisera to recombinant PEDF (Ab-
rPEDF).18 Figure 4 shows that in total cellular extracts, the main PEDF-immunoreactive protein migrated as a 46-kDa polypeptide (lane 1). Several additional proteins of higher (53 and 60 kDa) and lower (36 and 40 kDa) molecular weight also were detected. In contrast, the main radioimmunoprecipitated protein from the culture medium migrated as a 50-kDa protein (lane 2). To determine the size of the PEDF protein that accumulates intracellularly within the ciliary body, a Western blot analysis was performed (Figs. 4B, 4C). In ciliary process extracts from bovine and human eyes, a band migrating as a 46-kDa protein was immunodetected (lanes 3 and 6), whereas, in aqueous humor from bovine and human eyes, a protein of 50-

kDa protein was the main band detected (lanes 4 and 9). Western transfer of human vitreal proteins also revealed the presence of a 50-kDa PEDF protein (lane 8). The levels of the 50-kDa protein were greater in vitreous than in aqueous humor, as is the case in bovine eyes.18 In contrast to our radioimmunoprecipitation results, the additional bands (36-, 40-, 53- and 60-

kDa proteins) in lane 1 were not detected by Western blot analysis of total protein extracts of ciliary processes. Transfers of NPE cell extracts were not sensitive to immunodetection of proteins of either 46- or 50-

d; however, an immunoreactive molecule was re-

FIGURE 4. Pigment epithelium-derived factor (PEDF) protein in ciliary body, vitreous, and aqueous humor. (A) Autoradiogram of gel after SDS-PAGE of radio-immunoprecipitation assays conducted with antisera to rPEDF. Bovine ciliary explants were labeled metabolically with 35S-methionine for 14 hours. Lane 1 = immunoprecipitates from total labeled protein ciliary explant extracts; lane 2 = immunoprecipitate from labeled proteins from culture medium. (B) Western blot: (lane 5), purified recombinant human rPEDF protein (25 ng); (lane 6), total extracts of human ciliary processes (60 μg); (lane 7), total extracts of human NPE cells (60 μg); (lane 8), an aliquot of human vitreous (10 μg); and (lane 9) and aliquot of human aqueous humor (10 μg). Arrows at right (A to C) indicate the migration position of the 50-kDa (lanes 2, 4, 8, and 9) and the 46-kDa PEDF (lanes 1, 3, and 6) proteins.
tained at the top of the gel, as observed for aqueous humor (lanes 4 and 9). Note that the purified antiserum specifically recognized the expected 43-kDa rPEDF protein (lane 5).

These results demonstrate that PEDF is synthesized constitutively in ciliary body and that it is produced in two forms, the intracellular 46-kDa polypeptide molecule and the extracellular 50-kDa protein. The apparent molecular weight of the extracellular protein is consistent with the glycosylated form found in bovine interphotoreceptor matrix. The apparent molecular weight of the intracellular form is consistent with the size of the coding sequence (418 amino acids) from PEDF cDNAs.

Cellular Distribution of Pigment Epithelium-Derived Factor in the Ciliary Epithelium

To detect the cellular distribution of PEDF in the ciliary epithelium, we used semithin frozen sections (0.5 μm) of bovine ciliary processes and paraffin-embedded sections of human ciliary processes. In Figure 5, the pattern of staining with Ab-rPEDF along two regions of the bovine ciliary epithelium is shown. Both cells of the bilayer—pigmented and nonpigmented—were uniformly labeled intracellularly along the pars plicata (Figs. 5A, 5B) and pars plana (Figs. 5C, 5D). Furthermore, the basal plasma membrane of NPE cells at the pars plicata region was also labeled distinctly. At the pars plana region, however, the plasma membranes in both NPE and PE cells were labeled as well. These observations are consistent with the evidence that PEDF is a secretory protein targeted to the extracellular domain of the NPE that faces the aqueous humor. When Ab-rPEDF was immunoadsorbed with rPEDF protein or the first antibody was replaced by preimmune serum, no signal was detected along the ciliary epithelium (Figs. 5E, 5F).

When antiserum against rPEDF was applied to paraffin-embedded sections of human ciliary processes, it preferentially stained NPE cells intracellularly all along the ciliary epithelium. Labeling appears also in a region that may represent the basal plasma membrane of NPE and PE cells (Figs. 6A to 6D). Pigment epithelium-derived factor antibodies preadsorbed with rPEDF protein failed to stain the bilayer of NPE and PE cells (Figs. 5E, 5F, 6E, 6F).

DISCUSSION

So far, cDNA clones coding for the PEDF protein have been isolated from a fetal human eye cDNA library, WI-38 lung fibroblasts, a bovine RPE library (Perez et al, manuscript in preparation) and, most recently, a subtractive human ciliary body cDNA library. It has been shown that PEDF is a secretory glycoprotein with neurite outgrowth activity on Y-79 cells and neuronal survival effects on cerebellar granule cell neurons in culture. The amino acid sequence of PEDF identifies it as a new member of the serine protease inhibitor family. However, recent structure-function studies indicate that PEDF behaves as a substrate rather than as an inhibitor of numerous proteases. This biochemical behavior is shared by noninhibitory and dysfunctional serpins. Pigment epithelium-derived factor lacks the serpin conformational change on cleavage at its serpin reactive center, placing PEDF within the subgroup of noninhibitory serpins—for example, ovalbumin, angiotensinogen, maspin.

In this article, we describe the expression, synthesis, and secretion of PEDF by ciliary epithelial cells. Pigment epithelium-derived factor protein is readily detected intracellularly in the ciliary epithelial cells (NPE and PE) and appears to accumulate in the extracellular domain of the plasma membrane of NPE cells facing the aqueous humor (Figs. 5, 6). These observations, together with Northern, Western, and metabolically labeling experiments, strongly support the idea that PEDF is synthesized and secreted by the ciliary epithelial cells in the aqueous humor under physiological conditions. Human ocular tissues of the anterior...
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FIGURES. Localization of pigment epithelium-derived factor (PEDF) in the human ocular ciliary epithelium. Paraffin-embedded sections from the ciliary body of a donor eye were stained by Ab-rPEDF antisera using a biotin-streptavidin assay. (A) Pars plicata region of the ciliary epithelium. (B) Pars plana region of the ciliary epithelium. Insets in A and B are shown in higher magnification in C and D, respectively. (E,F) Controls; sections were stained with serum preadsorbed with rPEDF protein. NPE = nonpigmented cell layer; PE = pigmented cell layer. Magnifications: X100 (A,B,E,F), X600 (C,D).

segment, including the ciliary body, express PEDF mRNA (Fig. 1A). Other ocular tissues in the anterior segment, such as cornea, iris, and trabecular meshwork, also might contribute to PEDF protein accumulation in aqueous humor. Pigment epithelium-derived factor has been purified from bovine interphotoreceptor matrix and vitreous and detected by Western blot analysis in aqueous humor (Fig. 4). However, a higher level of protein accumulation is found in vitreous than in the other extracellular spaces (Fig. 4). Compared to the vitreous, components of the aqueous humor are removed constantly from the aqueous chamber by drainage from the eye. Thus, the relative lower levels of PEDF in aqueous humor are limited to the rate of drainage, whereas the low degree of protein turnover in the vitreous may allow higher accumulation of PEDF protein. Because there is no cell barrier between posterior and anterior segments of the eye, some degree of diffusion of components from vitreous to aqueous humor is possible. The fact that retinal cells express relative low amounts of PEDF transcripts (Fig. 1) suggests the retina as a source of the PEDF protein that accumulates in vitreous.

Another route of possible diffusion of PEDF protein between the posterior segment and the anterior segment of the eye is at the cellular transition zone between RPE and the ciliary epithelium, that is, the ora serrata region. However, this is unlikely because PEDF mRNA and metabolically labeled PEDF have been detected in the pars plicata, which is the most distant region from the RPE. Furthermore, cell lines established from the PE and NPE in culture still maintain the transcriptionally active PEDF gene, further supporting the idea that PEDF gene expression in ciliary epithelium is independent of the RPE.

The embryologic relationship between RPE and the PE cells supports the idea that these two cell types may share some similar biochemical and physiological properties. There is evidence, however, that PE and RPE cells exhibit unique differentiated properties. For example, the cell polarity in these cells is different. In PE cells, which lack tight junctions, the Na+-pump is distributed mainly along the basolateral domain, whereas in RPE cells, which contain tight junctions, the Na+-pump is localized at the apical plasma membrane facing the photoreceptors. One of the main functions of RPE cells is to support and maintain the photoreceptors. It has been suggested that PE cells in the ciliary epithelium have a role in supporting NPE cells. In this regard, the cellular interaction between NPE and PE cells occurs preferentially through gap junctions located along their apical membranes, opposite each other. The cell communication between PE and NPE cells suggests that the ciliary epithelium functions as a syncytium. This configuration does not exclude the existence of differential gene expression.
between PE and NPE cells, which, based on a few examples, determines a decreasing gradient of expression from the most anterior region of the ciliary epithelium toward the posterior region near the ora serrata. Whether PEDF synthesis and secretion by the ciliary epithelium in the aqueous humor perform under different mechanisms of regulation than in the RPE or retina is unknown.

It has been reported that enzymatic deglycosylation of bovine PEDF of 50 kDa purified from extracellular interphotoreceptor matrix yields a 46-kDa PEDF polypeptide form. The size of the PEDF polypeptides derived from the ciliary body (Fig. 4) is consistent with a PEDF nonglycosylated precursor of 46 kDa synthesized in ciliary body cells that, when secreted to the aqueous humor, matures to the glycosylated form of 50 kDa. The size of the intracellular PEDF protein species in agreement with the size of the open reading frame (418 amino acids) in the PEDF cDNA. The fact that no 50 kDa accumulates intracellularly suggests that glycosylation of the 46-kDa protein occurs immediately before or during secretion into the aqueous humor. In contrast to our data and to the size of the PEDF coding sequence, Tombran-Tink et al have reported that the predominant PEDF protein species found intracellularly in RPE cells migrates as a 36-kDa protein. We have detected de novo synthesized proteins of several sizes, including a 36-kDa protein, that are related to PEDF but that do not accumulate in the ciliary body cells (Figs. 4A, 4B). Because we have used purified antiserum to PEDF to perform these experiments, detection of these proteins might have been accomplished based on their affinity for PEDF rather than the antibody to PEDF. Thus, the 46-kDa protein appears to be the genuine PEDF product within the ciliary body cell.

It is interesting that the aqueous humor contains proteinases such as cathepsin D. Recently, it was observed that PEDF is cleaved by novel serpinases found in vitreous. Proteinases in aqueous humor may act on the endogenous PEDF protein as serpinases. Although further work is needed to understand the PEDF neurotrophic effects in the anterior segment of the eye, the data presented here demonstrate that PEDF gene expression in ciliary body is independent of other ocular tissues, including RPE.

Finally, these studies have revealed distinct expression patterns of PEDF between human and bovine eye tissues. Our simplest explanation of this species difference in the expression of PEDF is that it may reflect evolutionary adaptation. We have found a number of genes restricted to one tissue in the bovine eye but less restricted in the human eye. For example, the antioxidant enzyme plasma selenium-dependent glutathione peroxidase is restricted to the ciliary body in the bovine eye, but it is highly expressed in other tissues, including the ciliary body, iris, RPE, and retina in the human eye. Alternatively, this difference between species may reflect different mechanisms under which PEDF is regulated.

**Key Words**

- aqueous humor fluid
- ciliary epithelium
- gene expression
- neurotrophic factor
- pigment epithelium-derived factor (PEDF)

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