Platelet-derived growth factor D, Tissue-specific Expression in the Eye, and a Key Role in Control of Lens Epithelial Cell Proliferation*

Received for publication, December 2, 2004, and in revised form, December 10, 2004 Published, JBC Papers in Press, December 16, 2004, DOI 10.1074/jbc.M413570200

Sugata Ray, Chun Gao, Keith Wyatt, Robert N. Fariss, Amanda Bundek, Peggy Zelenka, and Graeme Wistow‡

From the NEI, National Institutes of Health, Bethesda, Maryland 20892-0703

Platelet-derived growth factor D (PDGF-D), also known as Iris-expressed growth factor, is a member of the PDGF/vascular endothelial growth factor family. The expression of PDGF-D in the eye is tissue-specific. In the anterior segment, it is localized to iris and ciliary body, whereas in the retina, PDGF-D is restricted to the outer plexiform layer. PDGF-D is present in aqueous humor but is not detectable in mature lens or in mouse lens-derived αTN-1 cells. However, it is expressed in rabbit lens-derived N/N1003A cells. N/N1003A cell-conditioned medium stimulates proliferation in rat lens explants, and this is blocked by immunodepletion of PDGF-D. Immunopurified PDGF-D also stimulates cell proliferation in rat lens explants and in NIH 3T3 cells. In organ culture of rat eye anterior segments, anti-PDGF-D strongly inhibits lens epithelial cell proliferation. This finding suggests a major in vivo role for PDGF-D in the mechanisms of coordinated growth of eye tissues. Intervention in the PDGF-D pathway in the eye, perhaps by antibody or blocking peptide, could be useful in the treatment of certain cataracts, including post-operative secondary cataract.

Coordinated growth of different tissues of the eye is essential for normal eye development and for normal vision (1, 2). In the anterior segment of the eye, the lens has important effects on the normal development of surrounding tissues (3), whereas the optical properties of the eye at all ages depend on maintaining the correct size and shape of the lens for the growing eye. Continuing growth of the lens past maturity has deleterious consequences for visual acuity with age (4).

Because of its highly organized structure and regulated cell growth, the lens is also one of the classic systems for studying growth and differentiation (5–10). Lens cells in different states of proliferation and differentiation are spatially segregated in specific zones (7, 11). An anterior monolayer of relatively undifferentiated epithelium contains a central region of quiescent cells surrounded by zones of cells that proliferate and migrate toward the lens equator. The proliferative zone is demarcated by the posterior chamber of the aqueous humor bounded by the lens, the lens zonules, the ciliary body, and the iris. As migrating epithelial cells reach the equator of the lens and leave the posterior chamber, they come under the influence of factors secreted into the vitreous (12). In this differentiation zone, migration and proliferation cease and the epithelial cells reorganize and elongate to form layers of fiber cells that reach half-way around the lens, express high levels of crystallins, and make up most of the refractive structure of the lens. FGF1 family growth factors play important roles in the control of the differentiation process (12–14). The control of proliferation is not so well understood. Early work implicated insulin and epidermal growth factor (15, 16). More recently, it has been shown that both PDGF-A and PDGF-B are expressed in the iris in the newborn mouse eye and that, in combination with FGF-2, PDGF-A affects proliferation and differentiation in lens epithelial explants (17, 18). VEGF-A has also been detected in lens epithelium, and the level of this factor, usually associated with angiogenesis, actually increases with age in the avascular lens (19).

In the course of the NEIBank project for ocular genomics (20), a new member of the PDGF/VEGF family of growth factors was discovered in the human iris and named iris-expressed growth factor (IEGF) (21). The same protein was independently identified by other groups and named spinal cord-derived growth factor B or PDGF-D (22–26). The latter has become the accepted designation, although it may overemphasize a fairly distant superfamly relationship with the familiar PDGF-A and PDGF-B (see family tree in Ref. 21). The closest sequence relative of PDGF-D is another protein variously known as fallotein, spinal cord-derived growth factor A, or PDGF-C (27–29). Both of these newly identified proteins have an N-terminal CUB domain, an immunoglobulin-like protein interaction module (30), and a C-terminal PDGF-like growth factor domain (31). Recombinant PDGF-D has been studied in cell culture systems, and both proteolytically cleaved and full-length proteins have been shown to have activity, including proliferative, transforming, and angiogenic activities, mediated through PDGFRα and PDGFRβ receptors (24–26). Here we describe the localization of PDGF-D in the eye and its effects on lens epithelial cell proliferation.

MATERIALS AND METHODS

Antibody Production—A peptide from human PDGF-D (IEGFp1, KKVDLDRLLNDADKRYSC) was synthesized at BIOSOURCE/QCB (Hopkinton, MA), conjugated to carrier, and used to immunize rabbits. Antiserum was tested on Western blots. The most potent bleed was affinity-purified at BIOSOURCE.

Western Blots—Proteins were extracted from rat eye tissues in TE buffer (10 mM Tris-HCl, pH 7.4, 1 mM EDTA). For cell-conditioned medium, cells were grown overnight at 37 °C, 5% CO2, washed three times.

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Section on Molecular Structure and Functional Genomics, NEI, National Institutes of Health, Bldg. 7, Rm. 201, National Institutes of Health, Bethesda, MD 20892-0703. Tel.: 301-402-3452; Fax: 301-496-0078; E-mail: graeme@helix.nih.gov.
times with serum-free medium, and grown overnight in serum-free medium. Conditioned medium was collected at 0 and 21 h and concentrated using Amicon YM10 filters (Millipore, Billerica, MA). Bovine aqueous humor was extracted from 12–18-month-old bovine eyes using a Hamilton syringe. The eyes were obtained through J. W. Treuhaft & Sons (Baltimore, MD).

For Western blots, 10-μg protein samples (or 30 μg for aequorin hormone) were run in 10% SDS-PAGE and transferred to polyvinylidene difluoride membranes. Western blots were processed following the protocols of the Western Breeze chemiluminescent Western blot kit (Invitrogen). Blots were incubated with anti-PDGF-D primary antibody (IEGF1) diluted 1:1000 for 1 h at room temperature. Primary antibody was also biotinylated using EZ-Link sulfo-NHS-LC-biotin (Pierce). The biotinylated antibody was used at 1:5000 dilution in Western blots and visualized with streptavidin-horseradish peroxidase conjugate (Pierce) and Supersignal West Pico substrate (Pierce) following the manufacturer’s protocols.

**Antigen-antibody complex at 4 °C for 1 h. Complexes were precipitated using EZ-Link sulfo-NHS-LC-biotin (Pierce). The biotinylated anti-PDGF-D antibody was eluted.**

To test for PDGF-D in the eye and its role in lens cell proliferation, explants were dissected from newborn rat eyes (14). Five explants were placed in each well of a 96-well plate in medium from N/N1003A cells, conditioned medium was concentrated to 1 ml by YM10 filtration (Millipore) and immunoprecipitated using biotin-labeled anti-PDGF-D to avoid secondary antibody detection of any contaminating rabbit IgG heavy chains. No rabbit serum, the cells were washed three times, transferred to serum-free medium, and tested by Western blot at 21 h for 37 °C, 5% CO₂ overnight. They were changed to medium with 1% fetal bovine serum and penicillin/streptomycin and grown overnight again. Cells were treated for 21 h with 100 ng/ml PDGF-A, PDGF-B (Roche Applied Science), and IP-purified rabbit PDGF-D with or without the addition of the appropriate antibody (PDGF-A and PDGF-B antibodies from R&D, Benicia, CA) at dilutions of 1:200, 1:100, and 1:50. Proliferation was assayed using the BrdUrd labeling kit III.

**Antigen Segment Organ Culture—Whole antigen segments were dissected from newborn rat eyes. Five antigen segments were placed in each well of a 96-well culture plate and incubated with DMEM/F-12 medium containing 20 ng/ml insulin/100 ng/ml ascorbic acid, and 1% fetal bovine serum. After 21 h of serum starvation, 1 ml of conditioned media from N/N1003A cells (32) were positive. Because the rabbit cells are grown in media from N/N1003A cells (32) were cultured in DMEM with L-glutamine containing 10% fetal bovine serum and penicillin/streptomycin, and treated with two different concentrations of IP-purified PDGF-D. Cell survival and proliferation were assayed in triplicate as before.**

**Proliferation on NIH 3T3 Cells—NIH 3T3 Cells were cultured in DMEM (1% fetal bovine serum/penicillin/streptomycin), and treated with two different concentrations of IP-purified PDGF-D. Cell survival and proliferation were assayed in triplicate as before.**

**RESULTS**

**Expression of PDGF-D in Eye Tissues and Cultured Cells—A peptide antibody (designated IEGF1) was designed to target a region of the growth factor-like domain of the human PDGF-D sequence that was predicted to be both antigenic and specific and to represent a core functional region in case of a loss of other regions by post-translational processing (Fig. 1). In Western blots of adult rat eye tissues, the anti-PDGF-D antibody detected protein in the iris, cornea, and retina (Fig. 2A). In contrast, the expression in lens was very low or absent. Although there was some minor size heterogeneity among different eye tissues, the immunoreactive protein was consistent in size with full-sized glycosylated PDGF-D (≈50 kDa), rather than with proteolytically cleaved fragments. To test for the presence of PDGF-D in the aqueous humor, bovine eyes were used to overcome the difficulty in obtaining this fluid cleanly from rodent eyes. The antibody retained its activity across species, and apparently full-sized PDGF-D was also detected in bovine aqueous humor (Fig. 2B). As a control, the antibody was also used to test the aqueous sample for a known aqueous marker, macrophage migration inhibitory factor (34).**

**Conditioned media from two lens-derived cell lines were also tested for PDGF-D (Fig. 2C). Mouse lens-derived αTN4-1 cells, which are transformed with SV40 T-antigen (33), gave no reaction, but non-transformed rabbit lens-derived N/N1003A cells (32) were positive. Because the rabbit cells are grown in the presence of rabbit serum, the cells were washed three times, transferred to serum-free medium, and tested by Western blot at 0 and 24 h after transfer to serum-free conditions using biotin-labeled anti-PDGF-D to avoid secondary antibody detection of any contaminating IgG heavy chains. No PDGF-D was detected at 0 h, but full-sized PDGF-D was detected in the medium after 24 h. To confirm the expression of rabbit PDGF-D in N/N1003A cells, intracrossing PCR primers were designed from the conserved regions of human and mouse sequences and used to amplify PDGF-D transcripts by RT-PCR from mouse lens and from the lens-derived cells. Consistent with previous results, no product was obtained from mouse lens or αTN4-1 cells but a 900-bp cDNA fragment for rabbit PDGF-D was obtained from N/N1003A cells (data not shown). This was cloned and sequenced to confirm the identity and to provide partial sequence for the rabbit protein (GenBankTM accession AY347260).**
The anti-PDGF-D antibody was used in immunofluorescence analysis of sections of rodent and monkey eye tissues (Figs. 3–5). In newborn rat anterior segment (Fig. 3), specific staining was seen in the iris and ciliary body but not in the lens, whereas limbal cells of the corneal epithelium also gave a positive reaction. Staining was intense in the stroma of both ciliary body and iris but was also present in the epithelial cell layers. To see whether similar expression is present in primate eyes, adult monkey anterior segment was also examined by immunofluorescence. In ciliary body (Fig. 4A), there was again intense stain in the muscle layer and significant staining throughout the epithelium. Intense staining was also seen in the sphincter muscle of the iris and in an apparently connected layer basal to the pigmented epithelium (Fig. 4B). There was no obvious stain in the intensely pigmented cells themselves. This could either reflect the absence from these cells in the adult monkey iris or possibly the quenching of a weaker signal by melanin. Overall, these results show that PDGF-D is differentially expressed in the anterior segment in both rodent and primate eye in a pattern that is consistent with a role in lens growth through secretion into the posterior chamber.

Localized staining was also seen in the retina. In the mature adult rat retina (Fig. 5), this expression localizes intensely to the outer plexiform layer, which contains photoreceptor axons and the synaptic layer between photoreceptors and second-order neurons.

Expression of Receptors for PDGF/VEGF Family Members in Lens—Previous results have shown that PDGF-D, in contrast to PDGF-A, acts mainly through the PDGFRβ receptor (24–26). Rat eye tissues were examined for the expression of PDGFRβ and for several other receptors for members of the VEGF/PDGF family using RT-PCR (Fig. 6). Primers were designed for rat sequences taken from GenBank™ and dbEST for PDGFRα, PDGFRβ, FLT1 (VEGFR1), FLT3, and FLK1 (VEGFR2) receptors. The products of the expected size for all five receptors were detected in total RNA extracted from newborn rat lens epithelium, lens fibers, and iris, whereas no RT controls were blank. Bands were sequenced to confirm their identity. The same primers were also used on RNA from the rabbit lens-derived cell line. Positive results were obtained for PDGFRβ and for FLT3 (Fig. 6). The failure of the other primers to work may reflect differences in the (unknown) rabbit sequences for these receptors.

Effect of PDGF-D on Cell Survival and Lens Cell Proliferation in Explants—Given the pattern of expression of PDGF-D in both newborn and adult anterior segment, the presence of secreted PDGF-D in the aqueous humor of the bovine eye, and
the presence of appropriate receptor expression in lens epithelium, the effects of PDGF-D on lens epithelial cell proliferation were examined. N/N1003A cell-conditioned medium was used as a source of native PDGF-D. Newborn rat lens epithelial explants were dissected and cultured. Cell survival was examined by trypan blue staining in explants at 0 and 21 h after incubation in N/N1003A-conditioned medium, conditioned medium immunodepleted using the anti-PDGF-D antibody, mock-depleted medium, and conditioned medium from cTN4-1 cells (Fig. 7A). Lens epithelial cells in culture have previously been shown to produce an unidentified cell survival factor and to be able to survive in minimal medium (35). After 21 h, both conditioned and depleted media retained a 75–80% number of live cells seen at 0 h with an increased number of dead cells. However, there is a distinct enhancement of cell survival in N/N1003A-conditioned medium compared with depleted medium (essentially serum-free medium) or cTN4-1 medium, suggesting that PDGF-D has survival activity in this system.

Proliferative activity in the same system was examined by
BrdUrd incorporation, normalized by the number of live cells (Fig. 7B). Significant cell proliferation was detected for explants in N/N1003A-conditioned medium. This was substantially reduced by immunodepletion of PDGF-D, whereas mock depletion with total rabbit IgG had no effect. In addition, αTN4-1-conditioned medium, which lacks significant amounts of PDGF-D, was much less effective in promoting cell proliferation. These results show that PDGF-D is strongly mitogenic in the lens explant system. The proliferative effect of PDGF-D seems to be greater than its survival effects. Although immunodepletion of PDGF-D reduced cell survival by ~20% in this system, proliferation was reduced >5-fold.

In addition to using N/N1003A-conditioned medium, PDGF-D protein was purified by immunoprecipitation from the medium. The IP material was tested on isolated rat lens explants for its effect on cell viability (Fig. 8A) and BrdUrd incorporation (Fig. 8B). The IP material promoted cell proliferation, confirming the activity of the protein recognized by the antibody. Quantification of PDGF-D in this preparation is uncertain because of contaminating antibody heavy chain that is present despite cross-linking the antibody to beads. However, two concentrations of the IP material (simply 2- and 4-μl samples of the preparation) were tested on explants and both induced a 2-fold increase in cell proliferation.

The active IP material was then used to test the ability of the anti-PDGF-D antibody to act as a blocking activity. The IP-purified PDGF-D was tested on NIH 3T3 cells alongside recombinant PDGF-A and PDGF-B. The anti-PDGF-D antibody and commercial blocking antibodies for the other two growth factors were tested in blocking cell proliferation. In this qualitative system, all three antibodies exhibited blocking activity and had their maximum effect at 1:100 dilution (Fig. 8C). Higher
concentrations of antibody were actually less effective at blocking proliferation. These antibodies were then used for blocking activity in organ culture.

**FIG. 8.** Activity of immunopurified PDGF-D and blocking antibody effect of anti-PDGF-D. A, effect of immunopurified PDGF-D on cell viability in lens epithelial explants measured by trypan blue assay for groups of five explants after 21 h with no added IP PDGF-D and with 2- and 4-μl samples of IP PDGF-D preparation. B, effect of immunopurified PDGF-D on cell proliferation in lens epithelial explants. BrdUrd incorporation in explants cultured as in part a. BrdUrd incorporation is measured by absorbance at 405 nm after subtraction of blank control value and normalized by live cell content from A. C, effect of PDGF family growth factors and their blocking antibodies on NIH 3T3 cells. BrdUrd incorporation was measured as above.

**Effect of PDGF-D on Lens Cell Proliferation in Organ Culture**—PDGF-D is mitogenic in the lens epithelial explant system. To determine whether it plays a similar role in the eye
itself, complete anterior segments with cornea, lens, ciliary body, and iris intact were dissected from newborn rat eyes, maintaining the native environment for lens cell proliferation (Fig. 9A). The anterior segments were maintained in culture for 21 h in DMEM with no added growth factors, relying on endogenous factors from the anterior segment tissues. To test the involvement of specific growth factors, the anterior segments were also incubated in DMEM supplemented with anti-PDGF-D antibody or blocking antibodies for PDGF-A, PDGF-B, or FGF-2. After 21 h, lens epithelia were dissected and assayed for cell survival (Fig. 9B). Under all of the conditions tested, there was significant cell survival at a higher level (2–3-fold) than was seen for isolated explants cultured for 21 h. This was not surprising, reflecting the more native environment of the epithelia during culture. Blocking antibody for PDGF-D had the largest effect on cell survival, with a 15% reduction in the number of surviving cells compared with incubation in DMEM alone.

BrdUrd incorporation by lens epithelia was measured in the same system (Fig. 9C). As measured in this assay, epithelial cell proliferation, normalized for the number of live cells, was reduced by 75% by treatment with the antibody to PDGF-D. In contrast, blocking antibodies to PDGF-A and PDGF-B reduced lens epithelia cell proliferation by 15% or less. Anti-FGF-2 (FGF-2 is associated with lens cell differentiation) had no significant effect on proliferation. These results suggest that PDGF-D is not only a factor capable of inducing or maintaining lens cell proliferation but is a major component of this process.

**DISCUSSION**

The complex architecture of the vertebrate eye is often cited as one of the most remarkable products of evolution (36–38). Normal vision depends on the tightly coordinated growth of several highly differentiated tissues of different embryological origins (1). A key mechanism for control of growth among the different tissues of the eye is the exchange of growth factors as each tissue influences the growth and development of its neighbors. This is illustrated particularly clearly in experiments that show that the lens plays a central role in the development of the rest of the anterior segment, whereas growth and differentiation of the lens itself depend on gradients of growth factors from the aqueous to the vitreous compartments (2, 3, 5, 6, 12, 14). A model has emerged with FGF family members secreted into the vitreous having a major role in the control of lens cell differentiation, whereas growth factors in the posterior chamber of the aqueous humor control lens cell proliferation. Recently, it has been shown that PDGF-A can stimulate epithelial cell proliferation (17, 18). Here we show that a new addition to the repertoire of growth factors, PDGF-D, has a major role in this part of the control mechanism for lens growth.

PDGF-D, which is related to both PDGF and VEGF growth factors, has mitogenic, angiogenic, and transforming activities in various cell types in culture and has been shown to act as a homodimer (22–26). Originally named “iris-expressed growth factor,” ocular PDGF-D was discovered by expressed sequence tag analysis of the human iris (21). As shown here, PDGF-D is expressed with striking specificity in the eye. In both rodents and primates, the protein is found principally in the tissues bounding the anterior surface of the posterior chamber of the aqueous humor and full-sized secreted PDGF-D is present in bovine aqueous humor. In rodents and primates, there is in-
tense immunoreactivity in the stroma and muscles of the iris and ciliary body and significant but lower values in the ciliary epithelium. In the newborn rat, there is clear immunostaining throughout the epithelial layers of both ciliary body and iris. In the adult monkey, the pattern is very similar in ciliary body but it is not so clear whether there is any PDGF-D in the pigmented epithelium of the iris, although there is a layer of strong staining basal to these cells. The factors secreted from ciliary body/iris into the aqueous humor can bathe the epithelial cells of the proliferative zone of the lens. The strong staining for PDGF-D in the muscles of the ciliary body and iris is intriguing. It remains to be seen whether this is a principal site of synthesis with possible transport to the epithelia through the blood/eye barrier or whether there is a specific role for this protein in these ocular muscles. However, PDGF-D is present in aqueous humor from bovine eye and, as such, can have effects on lens growth.

Mature lens does not express PDGF-D, although preliminary results (data not shown) suggest that the protein may be expressed in the lens early in development at stages when the growth rate is at its highest. This is under further investigation.

Similar to mature lens, the cTn4-1 cell line derived from transgenic mouse lens transformed by SV40 T-antigen (33) does not express detectable PDGF-D. However, the untransformed rabbit lens N/N1003A line (32) does express this growth factor. The possibility that the expression of PDGF-D may play a role in the immortalization of these cells is also under examination. Interestingly, in these cells as well as in native eye tissues, PDGF-D seems to be full-sized with no evidence for the proteolytic cleavage that has been observed in some cultured tissues, PDGF-D seems to be full-sized with no evidence for the proteolytic cleavage. The factors secreted from ciliary body/iris into the aqueous humor can bathe the epithelial cells of the proliferative zone of the lens. The strong staining for PDGF-D in the muscles of the ciliary body and iris is intriguing. It remains to be seen whether this is a principal site of synthesis with possible transport to the epithelia through the blood/eye barrier or whether there is a specific role for this protein in these ocular muscles. However, PDGF-D is present in aqueous humor from bovine eye and, as such, can have effects on lens growth.

Mature lens does not express PDGF-D, although preliminary results (data not shown) suggest that the protein may be expressed in the lens early in development at stages when the growth rate is at its highest. This is under further investigation. Similar to mature lens, the cTn4-1 cell line derived from transgenic mouse lens transformed by SV40 T-antigen (33) does not express detectable PDGF-D. However, the untransformed rabbit lens N/N1003A line (32) does express this growth factor. The possibility that the expression of PDGF-D may play a role in the immortalization of these cells is also under examination. Interestingly, in these cells as well as in native eye tissues, PDGF-D seems to be full-sized with no evidence for the proteolytic cleavage that has been observed in some cultured cells and which seems to be required for activation in some experimental systems (26).

The principal receptor for PDGF-D is the PDGFRβ receptor (26). Rat lens epithelium was examined for expression of PDGFRα and PDGFRβ and also for the VEGF receptors FLT1 (VEGFR1) and FLK1 (KDR or VEGFR2) and for FLT3, another tyrosine kinase receptor associated with angiogenesis. All five were detected in the epithelial cells by RT-PCR. In addition, the PDGFRβ and FLT3 receptors were identified in the rabbit lens N/N1003A cells. The expression of receptors usually associated with angiogenesis in the avascular lens and in lens-derived cells suggests that these factors may have additional roles in this tissue. PDGFRα receptor has been identified in lens by other groups (17, 39), and VEGFR2 (FLK1) receptor been shown to be expressed in mouse lens epithelium (19). The wide variety of receptors expressed in the lens suggests that many pathways are available for growth factor-mediated communication among ocular tissues. The presence of PDGFRβ in the iris raises the likelihood that PDGF-D and related proteins expressed in this tissue may exert autocrine or paracrine effects that remain to be examined.

N/N1003A conditioned medium is a convenient source for native PDGF-D, avoiding possible processing issues that may arise for recombinant protein in heterologous systems. The conditioned medium stimulates epithelial cell proliferation in rat lens explants, and this is efficiently abolished by immunodepletion of the growth factor. PDGF-D protein purified by immunoprecipitation from the conditioned medium is also able to stimulate proliferation in lens explants and in NIH 3T3 cells.

Although there are also some effects on cell survival, the proliferative effects of the conditioned medium in the explant system are at least an order of magnitude greater.

Antibody to PDGF-D is able to block lens epithelial cell proliferation efficiently in intact rat eye anterior segments in organ culture. Complete anterior segments (cornea, iris, ciliary body, lens, and attached tissues) can be maintained in culture, and in this system, proliferation of lens epithelial cells continues for at least 21 h. Even in the presence of the surrounding tissues that provide the native sources of growth factors, incubation with anti-PDGF-D significantly reduces proliferation of lens epithelial cells. Blocking antibodies to PDGF-A, PDGF-B, and FGF-2 have a much less effect. The potent effect of the anti-PDGF-D antibody suggests that PDGF-D has a major role in the anterior segment of the eye and may indeed be the main factor controlling growth of the lens in vivo.

This observation could have direct clinical value. Post-operative secondary cataract is a major complication of standard cataract surgery (40). Residual epithelial cells attached to lens capsule can proliferate and cause a new opacity that must be addressed by laser treatment or other measures. Conceivably, intervention in the PDGF-D signaling pathway, perhaps by antibody fragments or blocking peptides, could be used at the time of intraocular lens implantation to reduce the occurrence of post-operative secondary cataract. Intervention in the PDGF-D related proliferative pathways might also be therapeutically useful in addressing some forms of posterior subcapsular cataract where cell proliferation may be involved (41).

Continued lens growth with age is also a general problem for the aging eye and contributes to age-related problems including presbyopia (4, 8). Slowing the growth of the mature lens could help delay some of these problems.

In addition to this role in the anterior segment of the eye, PDGF-D also shows striking localization to the outer plexiform layer of the retina in the adult rat. This is a region that contains photoreceptor axons and the synaptic connections among photoreceptor, bipolar, and horizontal cells. The significance of the expression of this growth factor in the retina is not yet known and is under further investigation. One interesting possibility is that PDGF-D might be one of the factors involved in survival of photoreceptor cells (42) and, as such, might be useful in therapeutic approaches to retinal degeneration.

Acknowledgment—We thank Dr. Paul Russell (NEI, National Institutes of Health) for providing bovine aqueous humor.

REFERENCES

1. Walls, G. L. (1967) The Vertebrate Eye and Its Adaptive Radiation. Facsimile of 1942 Edition, Hafner, New York, NY.
2. Coulombre, A. J., and Coulombre, J. L. (1975) Int. Ophthalmol. Clin. 15, 7–18.
3. Beebe, D. C., and Coats, J. M. (2000) Dev. Biol. 220, 424–431.
4. Glasser, A., Croft, M. A., and Kaufman, P. L. (2001) Int. Ophthalmol. Clin. 41, 1–15.
5. Coulombre, J., and Coulombre, A. (1963) Science 142, 1489–1494.
6. Coulombre, J. L., and Coulombre, A. J. (1969) Invest. Ophthalmol. 8, 251–257.
7. Piatijski, G. (1981) Differentiation 19, 134–153.
8. Bron, A. J., Vrensen, G. F., Koretz, J., Maraini, G., and Harding, J. J. (2000) Ophthalmologica 214, 86–104.
9. Jeffery, W. R. (2001) Dev. Biol. 231, 1–12.
10. Beebe, D. C. (1986) Trans. Ophthalmol. Soc. U. K. 105, 123–130.
11. Harding, J. J., and Crabbe, M. C. (1984) in The Eye (Dawson, H., ed) Vol 1B, pp. 207–492, Academic Press, New York.
12. Mcavoy, J. W., Chamberlain, C. G., de, I. R. U., Hales, A. M., and Lovicu, F. J. (1999) Eye 13, 425–437.
13. Hyatt, G. A., and Beebe, D. C. (1993) Development 117, 701–709.
14. Chamberlain, C. G., and Mcavoy, J. W. (1989) Growth Factors 1, 125–134.
15. Reddan, J. R., and Wilson-Dziedzic, D. (1983) Invest. Ophthalmol. Vis. Sci. 24, 499–410.
16. Maidment, J. M., Duncan, G., Tamiya, S., Collison, D. J., Wang, L., and Wormstone, I. M. (2004) Invest. Ophthalmol. Vis. Sci. 45, 1427–1435.
17. Reneker, L. W., and Overbeck, P. S. (1990) Dev. Biol. 190, 554–565.
18. Kok, A., Lovicu, F. J., Chamberlain, C. G., and Mcavoy, J. W. (2002) Growth Factors 20, 37–34.
19. Shui, Y. B., Wang, X., Hu, J. S., Wang, S. P., Garcia, C. M., Potts, D. J., Sharma, Y., and Beebe, D. C. (2005) Invest. Ophthalmol. Vis. Sci. 44, 3911–3919.
20. Wistow, G. (2002) Mol. Vis. 8, 161–163.
21. Wistow, G., Bernstein, S. L., Ray, S., Wyatt, M. K., Behal, A., Touchman, J. W., Bouffard, G., Smith, D., and Peterson, K. (2002) Mol. Vis. 8, 185–195.
22. Hamada, T., Uti-Teri, K., Imaki, J., and Miyata, Y. (2001) Biochem. Biophys. Res. Commun. 290, 733–737.
23. Bergsten, E., Uutela, M., Li, X., Pietras, K., Ostman, C. H., Alitalo, K., and Eriksson, U. (2001) Nat. Cell Biol. 3, 512–516.
24. LaRochelle, W. J., Jeffers, M., McDonald, W. F., Chilakuru, R. A., Giese, N. A., Lokker, N. A., Sullivan, C., Boldog, F. L., Yang, M., Vernet, C., Burgess, C. A., and Locovei, R. (1996) J. Mol. Biol. 260, 802–810.
25. Li, H., Fredriksson, L., Li, X., and Eriksson, U. (2003) Oncogene 22, 1501–1510
26. Li, X., and Eriksson, U. (2003) Cytokine Growth Factor Rev. 14, 91–98
27. Tsai, Y. J., Lee, B. K., Lin, S. P., and Chen, Y. H. (2000) Biochim. Biophys. Acta 1492, 196–202
28. Gilbertson, D. G., Duff, M. E., West, J. W., Kelly, J. D., Sheppard, P. O., Hofstrand, P. D., Gao, Z., Shoemaker, K., Bukowski, T. R., Moore, M., Feldhaus, A. L., Humes, J. M., Palmer, T. E., and Hart, C. E. (2001) J. Biol. Chem. 276, 27406–27414
29. Li, X., Punten, A., Aase, K., Karlsson, L., Abramsson, A., Uutela, M., Backstrom, G., Hellstrom, M., Bostrom, H., Li, H., Soriano, P., Betsholtz, C., Heldin, C. H., Alitalo, K., Ostman, A., and Eriksson, U. (2000) Nat. Cell Biol. 2, 392–399
30. Bork, P., and Beckmann, G. (1993) J. Mol. Biol. 231, 539–545
31. Westermark, B., Claesson-Welsh, L., and Heldin, C. H. (1990) CIBA Found. Symp. 150, 6–22
32. Reddan, J. R., Chepelinsky, A. B., Dziedzic, D. C., Piatigorsky, J., and Goldemberg, E. M. (1986) Differentiation 33, 168–174
33. Yamada, T., Nakamura, T., Westphal, H., and Russell, P. (1990) Curr. Eye Res. 9, 31–37
34. Apte, R. S., Sinha, D., Mayhew, E., Wistow, G. J., and Niederkorn, J. Y. (1998) J. Immunol. 160, 5693–5696
35. Ishizaki, Y., Veyvodie, J. T., Burne, J. F., and Raff, M. C. (1993) J. Cell. Biol. 121, 899–908
36. Darwin, C. R., and Darwin, F. (1866) The Autobiography of Charles Darwin and Selected Letters, Murray, London
37. Nilsson, D. E., and Pelger, S. (1994) Proc. R. Soc. Lond. B Biol. Sci. 256, 53–58
38. Dawkins, R. (1994) Nature 368, 690–691
39. Potts, J. D., Bassnett, S., Kornacker, S., and Beebe, D. C. (1994) Investig. Ophthalmol. Vis. Sci. 35, 3413–3421
40. Kappelhof, J. P., and Vrensen, G. F. (1992) Acta Ophthalmol. Suppl. 13–24
41. Eshagian, J. (1982) Trans. Ophthalmol. Soc. U. K. 102, 364–368
42. Chaum, E. (2003) J. Cell. Biochem. 88, 57–75
Platelet-derived Growth Factor D, Tissue-specific Expression in the Eye, and a Key Role in Control of Lens Epithelial Cell Proliferation
Sugata Ray, Chun Gao, Keith Wyatt, Robert N. Fariss, Amanda Bundek, Peggy Zelenka and Graeme Wistow

J. Biol. Chem. 2005, 280:8494-8502.
doi: 10.1074/jbc.M413570200 originally published online December 16, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M413570200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 33 references, 7 of which can be accessed free at http://www.jbc.org/content/280/9/8494.full.html#ref-list-1