Lens-Specific Expression of PDGF-A in Transgenic Mice Results in Retinal Astrocytic Hamartomas

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**Purpose.** To investigate the possibility that platelet-derived growth factor (PDGF) might regulate aspects of mouse retinal development in vivo.

**Methods.** In situ hybridization was used to study the expression patterns of PDGF-A and PDGF-B and their receptors during normal mouse eye development. Transgenic mice that express human PDGF-A in the lens under the control of αA-crystallin promoter were generated by pronuclear microinjection. The effects of PDGF overexpression on eye development were analyzed by ocular histology, immunohistochemistry and in situ hybridizations.

**Results.** The PDGF genes are expressed by cells in close contact with retinal astrocytes. The PDGF-A messenger RNA is upregulated in the retinal ganglion neurons after birth, and PDGF-B is expressed by the blood vessel cells in the hyaloid vasculature. The authors found that lens-specific expression of PDGF-A in the eye can induce hyperplasia of retinal astrocytes, which express PDGF-α receptor (PDGF-αR) during development. The retinal alterations in the PDGF-A transgenic mice closely resemble the retinal astrocytic hamartomas found in human tuberous sclerosis (TSC) disease.

**Conclusions.** These findings suggest that proliferation of retinal astrocytes is regulated by PDGF during normal eye development. The authors speculate that proliferation of retinal astrocytes is mediated through a PDGF signaling pathway, which may involve the TSC gene product.


Platelet-derived growth factor (PDGF) is a disulfide-linked heterodimer or homodimer of two subunits (A and B chains). There also are two types of PDGF receptors, PDGF-α receptor (PDGF-αR) and PDGF-β receptor (PDGF-βR). The former can bind all forms of PDGF, whereas the latter can bind only those forms containing a B chain (i.e., AB or BB). Both PDGF-A and B-chains as well as their receptors are widely distributed in the developing and mature central nervous system (CNS), suggesting that interaction between PDGF and its receptor may play an important role in the normal development of CNS. Studies in rat optic nerve have shown that PDGF-A can promote the proliferation and survival of glial progenitor cells (O-2A progenitors), which later differentiate into oligodendrocytes and type-2 astrocytes. It is believed that PDGF-A can be provided by the type I astrocytes that also are present in rat optic nerve. These findings indicate that synthesis of PDGF by type I astrocytes can exert a major mitogenic influence on oligodendrocyte development. However, it is unclear whether this hypothesis can be generalized to other types of glial cells and to other parts of the mammalian CNS.

During gliogenesis in the CNS, glial progenitor cells retain significant plasticity. They continue to divide during migration and even after they reach their final destinations. This observation suggests that glial cell proliferation may be regulated by local environmental cues. It was reported that neurons, not astrocytes, synthesize most of the PDGF in neonatal and adult brain, implying that neurons may serve as a source of mitogenic stimulation for the surrounding glial cells.

The retina is one of the simplest and best-characterized derivatives of the CNS. It contains two types of neuroglial cells: Müller cells, which span the entire thickness of the retina, and astrocytes, which are confined to the inner surface of the retina. The glial cells of the retina and of the CNS appear to be architectur-
**FIGURE 1.** Schematic drawing of the platelet-derived growth factor-A (PDGF-A) minigene used for microinjection. Human PDGF-A complementary DNA (cDNA) was inserted into the polylinker site of the crystallin promoter vector (CPV2). SacII fragment contains the mouse aA-crystallin promoter, human PDGF-A cDNA, and simian virus 40 (SV40) early region intron and polyadenylation sequences.

ally and developmentally similar, suggesting that studies on retinal glial cells may provide information about gliogenesis and putative roles of PDGF in the CNS. Additionally, PDGF-A has been found in retinal neurons and PDGF-αR is expressed by the neighboring glial cells, hinting at a role for retinal neurons in regulation of retinal glial cell development. In this study, we show that overexpression of PDGF-A in transgenic eyes can induce abnormal proliferation of retinal astrocytes derived from the optic nerve. Our results support the hypothesis that PDGF-A produced by retinal ganglion cells normally promotes the proliferation of developing retinal astrocytes.

**MATERIALS AND METHODS**

**Construction of DNA for Microinjection**

Clone D1 mph was obtained from Dr. Stuart Aaronson to generate a PDGF-A minigene for microinjection. To release an approximately 900 bp human PDGF-A complementary DNA (cDNA) fragment, the plasmid was digested with Sac II, blunt ended by T4 DNA polymerase, and then cut with HindIII. The cDNA fragment was inserted into the polylinker site of the aA-crystallin promoter vector (CPV2), which had been digested with Bam HI blunt ended by T4 DNA polymerase, and then digested with HindIII. The CPV2 vector contains an intron and polyadenylation sequences from simian virus 40 (SV40). An approximately 2.1-kb DNA fragment containing the PDGF-A minigene was released from the plasmid hPDFGA/CPV2 (Fig. 1) by digestion with Sac II, then separated by agarose gel electrophoresis and purified for microinjection using a Qiaex gel extraction kit (Qiagen, Chatsworth, CA).

**Generation of Transgenic Mice**

The use and treatment of mice in this study conformed to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmology and Vision Research and the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

The purified PDGF-A minigene DNA fragment was microinjected into pronuclei of inbred FVB/N embryos at a concentration of 2 ng/μl. Injected embryos were transferred into pseudopregnant ICR females (Harlan Sprague-Dawley). Potential transgenic mice were screened by using the polymerase chain reaction (PCR) on genomic DNA isolated from tails after the procedure described previously, and using primers that were specific for SV40 sequences in the microinjected DNA.

**Histologic Analysis and Immunohistochemistry**

FVB mice are homozygous for a recessive mutation in the rd (retinal degeneration) gene. To assess the effects of the PDGF-A transgene on retinal morphology, transgenic (rd−/−) mice were mated to C57BL/6j (rd+/+) mice to generate offspring (rd+/−) that did not undergo retinal degeneration. Eyes or embryo heads were fixed overnight in 10% phosphate-buffered formaldehyde solution (pH 7.0), dehydrated in ethanol, cleared in xylene, and embedded in paraffin wax for sectioning. Sections (5 μm) were stained with hematoxylin and eosin.

For immunohistochemical analyses, tissue sections were prepared as described above. For detection of DNA replication, incorporation of bromodeoxyuridine (BrdU) was analyzed by immunohistochemistry as described by Fromm et al. For detection of myelin basic protein (MBP), goat antibody raised against rabbit MBP was obtained from Dr. David Shine and diluted 1:1000 in a solution of 0.5 M Tris-HCl, pH 7.5, 0.2% Triton X-100, and 2% normal horse serum. For detection of glial fibrillary acidic protein (GFAP), S-100, and von Willebrand factor (or factor VIII), primary antibodies were purchased from Dako (Carpinteria, CA), and diluted at 1:500 (GFAP) or 1:200 (S-100 and factor VIII) in phosphate-buffered saline (PBS) (10 mmol NaPO₄ [pH 7.4], 0.9% sodium chloride) containing 2% normal horse serum. Incubation with primary antibodies was carried out at 4°C overnight. Immunostaining was performed by the avidin-biotin peroxidase complex method (Vector Laboratories). Peroxidase was visualized with diaminobenzidine-H₂O₂ (SK-4100 kit; Vector, Burlingame, CA). Sections were counterstained with hematoxylin.

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In Situ Hybridization

In situ hybridizations were performed after the procedure described previously, using \(^{35}\text{S}-\text{UTP}\) labeled probes. To examine the expression pattern of endogenous mouse PDGF-A and B-chains, clones in pGEM-3Zf(+) were obtained from Dr. Daniel F. Bowen-Pope (University of Washington, Seattle, WA).

To generate a riboprobe that hybridizes to the endogenous mouse PDGF-\(\alpha\)-R, we obtained a cDNA clone from Dr. Phil Soriano. The plasmid was digested with PstI and an 815-bp DNA fragment corresponding to nucleotide sequences from 1 to 815 (Genbank accession M57683) was isolated and inserted into the polylinker site of plasmid Bluescript KS(-). Antisense and sense riboprobes were generated from linear plasmids that had been cut with HindIII and Xba I, respectively.

Riboprobes for endogenous mouse cyclin A, cyclin D1, and cyclin D2 were prepared as described by Fromm and Overbeek.

RESULTS

Transgenic Mice That Express Human Platelet-Derived Growth Factor-A in the Lens

Transgenic mice that express a human PDGF-A cDNA under the control of the mouse lens-specific \(\alpha\)-A-crystallin promoter were generated. Four transgenic founder mice were obtained, and they all displayed cataracts. One female founder mouse was infertile, so three transgenic lines were established, designated as lines OVE460, OVE486, and OVE487.

Expression of transgenic messenger RNA (mRNA) was detected by reverse transcriptase (RT)-PCR using primers that span the small intron in the SV40 segment of the transgene (data not shown). Both spliced and unspliced forms of transgenic transcripts were present in the ocular RNA samples, which is consistent with
Abnormal Development of Retinal Astrocytes in Transgenic Mice

The PDGF-A transgenic mice all displayed cataracts associated with pathologic alterations of the lens fiber cells in the anterior subepithelial region. The lens defects are under further investigation. The PDGF-A overexpression in the lens also induced alterations in retinal glial cells. Although the transgene is expressed highly at embryonic stages (Figs. 2B, 2D), we did not detect any phenotypic changes in E15 retinas of transgenic mice by routine histologic analysis. Abnormalities become apparent after birth. During normal eye development, astrocytes start to appear at the optic nerve head around birth (Fig. 3A, small arrows). As the retina develops, the astrocytes migrate onto the retina and spread across the nerve fiber layer toward the periphery of the retina, in association with the development of retinal blood vessels. At postnatal day 7 (P7) (Figs. 3C, 3E), the inner surface of the non-transgenic retina is covered mainly by two cell types, the retinal astrocytes that have round or oval-shaped nuclei (Fig. 3E, arrows) and blood vessel cells with elongated nuclei (Fig. 3E, arrowhead). In the transgenic mice, more cells are present at the optic nerve head at birth (compare Fig. 3B to 3A). During postnatal development, these cells continue to accumulate and migrate slowly toward the peripheral retina. At day P7, there is a thick layer of cells on the vitreous surface of the retina (Fig. 3D, arrows). At higher magnification (Fig. 3F), it is apparent that most of these cells have round or oval-shaped nuclei, a characteristic of retinal astrocytes. The growth of this cell layer ceases after day P14 (data not shown). Although extra cells are present along the inner surface of the retina in these transgenic eyes, the retinal neural cell layers seem to develop normally.

The developmental pattern and morphology of the cells accumulating on the inner surface of the retina in the PDGF-A transgenic mice suggested that they might be retinal astrocytes. To test this hypothesis, we assayed for expression of the astrocytic marker GFAP using immunohistochemistry (Fig. 4). In non-transgenic mice, GFAP+ cells are found in the optic nerve region as well as on the inner surface of the retina (Figs. 4A, 4C). In the transgenic mice, almost all the cells on the inner surface of the retina express GFAP (Figs. 4B, 4D), suggesting that they indeed are astrocytes. A small population of GFAP-negative cells are found at the center of the retina near the optic nerve head in transgenic mice (Figs. 4D, 4F). These probably are blood vessel cells. We also examined expression of another glial cell marker, protein S-100. The S-100 protein also is present in almost all the cells on the inner surface of the retina in transgenic mice.

FIGURE 4. Immunohistochemistry to detect glial fibrillary acidic protein (GFAP) and S-100 proteins. Distribution of GFAP in postnatal day 7 (P7) retinas of nontransgenic (A,C) and transgenic (B,D) mice was determined by immunohistochemistry. Enlargements of the boxed regions at the optic nerve head in (A,B) are shown in (C,D), respectively. The distribution of S-100 in P7 retinas of nontransgenic (E) and transgenic (F) mice also was assayed by immunohistochemistry. Brown color indicates the presence of GFAP (A to D) or S-100 (E,F). GFAP and S-100 are present in the retinal astrocytes (arrows) but not in the blood vessel cells (arrowheads). The astrocytes have undergone a substantial expansion in the transgenic eyes. Scale bars = 100 μm. R = retina; V = vitreous; ON = optic nerve; as = retinal astrocytes; gcl = ganglion cell layer; inl = inner nuclear layer.

FIGURE 5. Immunohistochemistry to detect myelin basic protein (MBP) and factor VIII proteins in 7-day-old eyes. The distribution of MBP (A,B) and factor VIII (C,D) in the eyes of nontransgenic (A,C) and transgenic (B,D) mice was assayed by immunohistochemistry. Brown color indicates the detection of MBP (A,B) or factor VIII (C,D). The MBP positive cells (oligodendrocytes) are restricted to the optic nerve. Factor VIII positive cells (arrowheads) are associated with the blood vessels in the hyaloid and retinal vasculature. The retinal astrocytes (arrows, C) do not express factor VIII. V = vitreous; ON = optic nerve; as = retinal astrocytes; nfl = nerve fiber layer; gcl = ganglion cell layer. Scale bars = 100 μm.
FIGURE 4.

FIGURE 5.
(Figs. 4F). The cells located at the center of the retina that do not express GFAP (Fig. 4D) also are S-100 negative. These results together strongly suggest that overexpression of PDGF-A in the eye results in an abnormal and substantial increase in retinal astrocytes, i.e., formation of hamartoma, reflecting either enhanced migration or proliferation of these cells.

It has been reported that PDGF-A can stimulate the proliferation of oligodendrocyte lineage progenitor cells (O-2A progenitors) in the developing rat optic nerve. The O-2A progenitor cells give rise to two distinctive cell types, oligodendrocytes and type-2 astrocytes. To exclude the possibility that the cells on the inner surface of the retina in transgenic mice are a mixture of oligodendrocytes and astrocytes, we assayed for the presence of oligodendrocytes in postnatal day 16 (P16) eyes. Immunohistochemistry using an antibody against MBP, a specific marker for mature oligodendrocytes, showed that MBP+ cells are confined to the optic nerve at P16 in both nontransgenic and transgenic mice (Figs. 5A, 5B). No MBP+ cells were found in the retinas of transgenic mice, indicating that no mature oligodendrocytes are present among the expanded retinal astrocytes. In addition, no phenotypic changes were detected in the Müller cells of the transgenic retinas.

Because the development of retinal astrocytes is tightly associated with the retinal vasculature, we also assayed for the presence of extra blood vessel cells in the retina by immunohistochemistry using an antibody against factor VIII, a marker for mature vessel endothelial cells. Factor VIII+ retinal endothelial cells are found in both nontransgenic and transgenic mice (Figs. 5C, 5D, arrowheads). Figure 5D shows extra cells on the inner surface of the retina near the optic disc in the transgenic mice. This is the region where the hyaloid vasculature enters the eye. No extra factor VIII positive cells were detected peripheral to the optic disc region on the surface of the transgenic retina (data not shown). Hyaloid vessel cells in the vitreous cavity also express factor VIII.

Expression of Platelet-Derived Growth Factor-α Receptor Messenger RNA in Retinal Astrocytes

To test the prediction that the hyperplasia of the retinal astrocytes in PDGF-A transgenic mice is mediated through a PDGF receptor, we examined the expression of PDGF-αR in newborn and P7 retinas by in situ hybridization. In neonatal retinas, PDGF-αR mRNA is localized mostly at the optic nerve head in both nontransgenic and transgenic mice (Figs. 6A, 6B, 6C, 6D). By P7, the PDGF-αR expressing cells have expanded in the transgenic eyes and have extended toward the peripheral retina in both transgenic and nontransgenic mice (Figs. 6E, 6F, 6G, 6H). The PDGF-αR mRNA is not found in the blood vessel cells of the hyaloid vasculature (Fig. 6F, arrowheads). The spatial distribution of PDGF-αR+ cells coincides with the pattern of GFAP staining, suggesting that most of the cells that express PDGF-αR are retinal astrocytes. This finding is consistent with previous observations that PDGF-αR is expressed in rat retinal astrocytes during eye development.11 The finding that PDGF-αR mRNA is expressed in retinal astrocytes is consistent with the following model. In the transgenic mice, PDGF-A would be produced and secreted by the lens, followed by diffusion through the vitreous, and activation of receptors on the retinal astrocytes, thereby inducing increased numbers of these cells in the transgenic mice. We have not yet shown molecularly that there is a quantitative change in PDGF-αR activity.

Mitogenic Effect of Platelet-Derived Growth Factor-α in Retinal Astrocytes

To determine whether PDGF-A induces increased proliferation of retinal astrocytes in the transgenic mice, we analyzed DNA synthesis in 4-day-old retinas by BrdU incorporation (Figs. 7A, 7B). At P4, many cells on the inner surface of the transgenic retina with round or oval-shaped nuclei are labeled with BrdU (dark brown-staining nuclei, Fig. 7B), suggesting that PDGF-A stimulates retinal astrocytes to enter S-phase. We calculated the percentage of BrdU-positive nuclei in the astrocytic cluster of cells on the inner surface of the P4 retinas. The average percentage of BrdU-positive nuclei was 20% in the astrocytic expansion of the transgenic retinas. Overall, an average of 14% of the astrocytes were BrdU-positive in nontransgenic retinas. Therefore, the percentage of retinal astrocytes in S-phase is elevated in the transgenic retina. Within the neural retina, we did not notice any consistent differences in BrdU incorporation between the nontransgenic and transgenic mice (compare Fig. 7A and 7B). We did not detect any BrdU-labeled astrocytes after day P14 in either nontransgenic or transgenic eyes (data not shown), suggesting that retinal astrocytes exit from cell cycle by that age, presumably reflecting either terminal differentiation or a decline in the strength of the PDGF-A signal.

To further analyze the mitogenic effect of PDGF-A on retinal astrocytes, we examined the expression of endogenous cyclins (cyclin A, cyclin D1, and cyclin D2) in the retinas of both nontransgenic and transgenic mice. In neonatal retinas of both nontransgenic (Figs. 8A, 8C) and transgenic (Figs. 8B, 8D) mice, cyclin A is expressed in the cells at the center of the retina near the optic nerve head, suggesting that the retinal astrocytes that are migrating into the retina from the optic nerve are in the cell cycle. Cyclin A mRNA also is expressed in the neonatal retinas of nontransgenic mice (Figs. 8A, 8B, 8C, 8D). Cyclin D1 is expressed highly in all neural layers of the neonatal retina (data not shown). In contrast, cyclin D2 (Figs. 8E, 8F, 8G, 8H) is expressed in the cells on the inner and outer
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FIGURE 6. Expression of platelet-derived growth factor α-receptor (PDGF-αR) messenger RNA (mRNA) during retinal development. PDGF-αR mRNA in neonatal retinas (A to D) and in postnatal day 7 (P7) retinas (E to H) of nontransgenic (A,C,E,G) and transgenic (B,D,F,H) mice was detected by in situ hybridization. Sections are displayed with bright-field (A,B,E,F) and dark-field (C,D,G,H) illumination, respectively. Astrocytes express PDGF-αR in transgenic and nontransgenic neonatal and P7 retinas. The cells in the hyaloid vasculature do not express PDGF-αR (arrowheads, F). Open arrows indicate the location of the retinal pigment epithelium (A to D). Scale bars = 100 μm.

DISCUSSION

We have established three transgenic mouse lines that express human PDGF-A in the lens using the αA-crystallin promoter. Both lenticular and retinal defects develop in transgenic mice characterized as cataracts and retinal astrocytic hamartomas, respectively. Here we focus on the alterations in retinal astrocyte development.

Mitogenic Effects of Platelet-Derived Growth Factor-A on Retinal Astrocytes

There are two types of neuroglial cells in the mouse retina: astrocytes and Müller cells. Retinal astrocytes are associated with blood vessels and are immigrants from the optic nerve.25,26 They migrate into the retina at around birth and spread across the inner surface of the retina during development. Müller cells are believed to differentiate from the retinal neuroepithelium.25 These cells span the entire retina from external- to internal-limiting membrane. Here we present in vivo evidence that PDGF-A can stimulate retinal astrocyte proliferation. Transgenic mice that express human PDGF-A in the lens under the control of the

Expression of Mouse Endogenous Platelet-Derived Growth Factor Genes During Retinal Development

To examine the possibility that expression patterns of endogenous mouse PDGF-A and PDGF-B might be altered in the transgenic eyes, we compared the expression patterns in nontransgenic and transgenic eyes by in situ hybridization (Fig. 9). In nontransgenic eyes, PDGF-A mRNA was not detected in the neonatal retina (data not shown); however, the signal was found in the retinal ganglion cell layer at day P7 (Figs. 9A, 9C, open arrows). Expression of PDGF-B was detected in the cells of the hyaloid vasculature during prenatal eye development (data not shown). These cells continue to express PDGF-B during postnatal development (Figs. 9E, 9G, arrowheads). PDGF-B mRNA also was found in cells on the inner surface of the retina (Figs. 9E, 9G, arrows). These probably are a subset of astrocytes or blood vessel cells in the retinal vasculature. A low level of PDGF-B mRNA also was found in the neural retina, including retinal ganglion cell layer (Figs. 9E, 9G, open arrows). In the eyes of the transgenic mice, although there is an increase in the number of astrocytes, the expression level of endogenous PDGF-A in the retinal ganglion cell layer does not seem to be altered (compare Fig. 9B, 9D to Fig. 9A, 9C). However, a significant number of cells on the inner surface of the retina in transgenic mice express endogenous PDGF-B, suggesting either that there are extra blood vessel cells or that the extra retinal astrocytes may produce PDGF-B (Figs. 9F, 9H). Alternatively, the heterogeneous patterns of the in situ hybridization, combined with the heterogeneous staining patterns for GFAP and S-100, suggest the possibility that other cells are being stimulated to proliferate along with the retinal astrocytes.
FIGURE 7. Incorporation of bromodeoxyuridine (BrdU) in 4-day-old retinas. The 4-day-old (P4) mice were injected with BrdU and killed 1 hour later. Eyes were removed and processed for BrdU immunohistochemistry. Examples of BrdU labeled cells (dark nuclei) in non-transgenic (A) and transgenic (B) retina are indicated by the arrows. Scale bars = 100 μm.

αA-crystallin promoter exhibit a dramatic increase in the number of cells lying along the inner surface of the retina. The fact that most of these cells express high levels of astrocytic cell markers (GFAP and S-100) strongly suggests that they primarily are retinal astrocytes. The cells on the surface of the transgenic retina do not stain for type III β-tubulin, a marker for neuronal cells (Campochiaro P, Reneker L, and Overbeek P, unpublished observations, 1995). The migration pattern of these cells follows that of the retinal astrocytes; they initially appear at the optic disc at birth and migrate toward the peripheral region of the retina during development. In addition, we found that retinal astrocytes express the receptor for PDGF-A in both nontransgenic and transgenic mice. These results together suggest that PDGF-A produced from the lens...
can be secreted and can act as a mitogen to induce a transient hyperplasia of retinal astrocytes in these transgenic mice.

An alternative (or additional) explanation for the astrocytic expansion is that overexpression of PDGF-A in the lens may induce extra astrocytic cells to migrate onto the retina from the optic nerve during development. However, we do not think that a chemotactic effect of PDGF-A alone causes the expansion of the retinal astrocytes in the transgenic mice. For example, although the PDGF-A transgene is expressed as early as E12, extra astrocytes in the transgenic mice do not appear until birth. The extra astrocytes remain attached to the retina and do not migrate through the vitreous to attach to the lens. Another possible function for PDGF-A could be to act as a survival factor for retinal astrocytes. However, a TUNEL assay28 for apoptotic cells did not show any evidence of astrocytic cell death in nontransgenic retinas at either postnatal

FIGURE 8. Expression of endogenous cyclins A and D2 in neonatal retina. In situ hybridizations were done to detect the expression of cyclin A (A to D) and cyclin D2 (E to H) in the neonatal retinas of nontransgenic (A,C,E,G) and transgenic (B,D,F,H) mice. Sections are shown with bright-field (A,B,E,F) and dark-field (C,D,G,H) illumination. Both cyclin A and cyclin D2 are found in the cells of the optic disc and inner surface of the retina. Cyclin A also is found in the neonatal neural retina. Cyclin D2 is expressed in the cells of the hyaloid vasculature (arrowheads) and in the peripapillary mesenchyme surrounding the retina (E to H). Scale bars = 100 μm.

FIGURE 9. Expression of platelet-derived growth factor (PDGF) messenger RNA (mRNA) in 7-day-old retinas. In situ hybridizations were done to detect the expression of platelet-derived growth factor-A (PDGF-A) (A to D) and platelet-derived growth factor-B (PDGF-B) (E to H) in nontransgenic (A,C,E,G) and transgenic retinas from line OVE487 (B,D,F,H). Sections are shown with bright-field (A,B,E,F) and corresponding dark-field (C,D,G,H) illumination, respectively. PDGF-A mRNA is present at similar levels in the retinal ganglion neurons of transgenic and nontransgenic mice (open arrows, A to D). The expression of PDGF-B mRNA is associated with the cells in the hyaloid vasculature (arrowheads, E to H) and in the retinal blood vessels (arrows, E to H). The signal in the retinal pigment epithelium (rpe) is not because of silver grains but because of pigmentation. No signals were found in the eye when hybridizations were done with sense strand probes. V = vitreous; ON = optic nerve; gc = ganglion cell layer; inl = inner nuclear layer; onl = outer nuclear layer; rpe = retinal pigment epithelium. Scale bars = 100 μm.
day 2 or 5 (Ash J, Overbeek P, unpublished observations, 1995). Additionally, the BrdU incorporation study (Fig. 7) shows that a high percentage of the extra astrocytes are undergoing cellular proliferation in the transgenic eyes. Therefore, it is unlikely that the expansion of the retinal astrocytes in the transgenic eyes is because of prevention of programmed cells death by PDGF-A.

To further analyze the mitogenic effect of PDGF-A on astrocytes, we examined the expression of cyclins in these cells at different stages. We found that in both nontransgenic and transgenic mice, cyclin A and cyclin D2 are expressed highly in the astrocytes that are migrating into the retina around birth. These results suggest that astrocytes still are proliferative in the neonatal retina. In 7-day-old retinas of nontransgenic mice, cyclin A is only found in a few cells at the retinal periphery (data not shown), suggesting that most of the astrocytes have stopped cell division and exited from the cell cycle. However, cyclin A still is expressed highly in the retinal astrocytes in the PDGF-A transgenic mice. Our hypothesis is that the transgenic mice have an elevated level of PDGF-A in the vitreous, causing extra activation of the PDGF-αR on the astrocytes, thereby stimulating cell proliferation, and delaying exit from the cell cycle. The growth of retinal astrocytes in the PDGF-A transgenic mice ceases after approximately 2 weeks of age as indicated by BrdU labeling experiments as well as decreased cyclin A expression (data not shown). The most likely explanation is that a lesser amount of PDGF-A is available to stimulate the PDGF-αR on the astrocytes because the transgene expression is turned down by day P14.

The other glial cells in the retina, the Müller cells, have been reported to respond to PDGF by proliferation and chemotaxis in vitro. However, in PDGF-A transgenic mice, there are no apparent phenotypic changes in the Müller cells. Perhaps the level of PDGF-A released from the lenses in transgenic mice is not high enough to stimulate the receptors on the Müller cells. It also is possible that Müller cells do not respond well to PDGF-A in vivo.

Based on the fact that astrocytes express a wide range of neurotrophic factors in vitro, it has been hypothesized that astrocytes may play a role in the maintenance and survival of their partner neurons. However, in our PDGF-A transgenic mice, although the number of astrocytes in the retina is increased significantly, we did not notice any changes in the number of retinal neurons. Current evidence suggests that expression and release of neurotrophic factors by astrocytes in adult CNS may occur primarily as a response to traumatic events, such as disease or mechanical injury. The mechanisms of such induction still is unknown. It will be interesting to test whether retinal wound healing is affected by the presence of extra astrocytes in the PDGF-A transgenic mice. Our transgenic mice may become a useful model to understand the role of astrocytes during trauma in the CNS.

**Platelet-Derived Growth Factor Signaling in Normal Retinal Glial Cell Development**

During normal development, retinal astrocytes express PDGF-αR and start to migrate from the optic disc around the time of birth. The migration of astrocytes onto the nerve fiber layer coincides with the appearance of PDGF-A mRNA in the cell bodies of the retinal ganglion neurons (see Fig. 9A, 9C). Meanwhile, the retinal vasculature develops radially along the inner surface of the retina in close association with the astrocytes. In situ hybridization experiments indicate that PDGF-B is expressed by cells on the inner surface of the retina (Figs. 9E, 9G). The expression patterns of PDGF (both A- and B-chains) and PDGF-αR strongly suggest paracrine interactions between neighboring neurons and astrocytes. It has been proposed previously that during normal retinal development, proliferation and migration of retinal astrocytes are mediated by PDGF-A produced by retinal ganglion cells. The PDGF-A and PDGF-αR also have been shown to be present in the ganglion neurons and astrocytes, respectively, of the adult retina, leading to the hypothesis that PDGF may be required for the long-term survival of astrocytes in the eyes.

Our model of neuronal–glial interaction through PDGF-A signaling in the eye also may have general implications for gliogenesis of the CNS. Glial progenitors are active mitogenically after leaving the germinative zone. They continue to divide during migration and even after, they reach their ultimate destination. It was reported that PDGF-αR is expressed by glial progenitors (O-2A progenitors) after they migrate away from the germinative zone in the subventricular layer. It also was found that PDGF-A is expressed by almost all the neurons of the central and peripheral nervous systems. Neurons synthesize PDGF-A as early E15, before the differentiation of most types of glial cells. Synthesis occurs at very high levels between E18 and 1 week after birth, the period when glial progenitors migrate to their final locations and differentiate. The temporal and spatial distributions of PDGF-A suggest that neurons may be a major source of PDGF-A for glial cell proliferation and migration.

**Retinal Astrocytic Hamartoma in Humans**

Excess growth of retinal astrocytes has been observed in human patients with tuberous sclerosis (TSC). Tuberous sclerosis is an autosomal dominant disease characterized by the development of benign tumors classified as hamartomas in numerous tissues and organs, such as brain, eyes, skin, kidneys, heart, lung, and skeleton. Retinal astrocytic hamartoma in
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TSC is characterized as a collection of fusiform astrocytes with small and oval nuclei. The tumors are confined to the nerve-fiber layer of the retina and do not destroy or infiltrate other layers of the retina. Our transgenic studies show that elevated levels of PDGF in the eye can cause hyperproliferation of retinal astrocytes and result in a phenotype that resembles that found in patients with TSC. Recently, one of the loci for TSC (TSC2) has been identified by the European Chromosome 16 Tuberous Sclerosis Consortium. Intriguingly, the TSC2 product has a region of homology with the GTPase activating protein GAP3. It is known that binding of PDGF to its receptor can lead to the activation of Ras, and inactivation of Ras is stimulated by GTPase activating proteins. Although it may be premature, it is tempting to speculate that the TSC2 gene product could modulate the PDGF-signaling pathway by converting activated GTP-Ras to its inactive form. When the TSC2 gene is mutated, PDGF receptor signaling might be enhanced, leading to an increase in cellular proliferation and hamartoma formation.

Key Words
- cell proliferation, eye, platelet-derived growth factor, platelet-derived growth factor-A, retinal astrocytes, transgenic mice

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