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Platelet-Derived Growth Factor Gene Expression in Cultured Human Retinal Pigment Epithelial Cells

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Summary: Gene expression of platelet-derived growth factor (PDGF) and its receptors in cultured human retinal pigment epithelial (RPE) cells was studied by using semiquantitative polymerase chain reaction. The RPE cells were found to express PDGF A- and B-chain genes as well as α- and β-receptor genes with dominant expression of B-chain and β-receptor isoforms. Phorbol myristate acetate (PMA) and thrombin increased the expression of PDGF B-chain gene to 19.8 ± 1.75 and 15.9 ± 1.84 fold (n=3) of the control without affecting β-receptor gene expression. PDGF produced by the RPE cells may play an important role in the pathogenesis of some ocular proliferative diseases.

Platelet-derived growth factor (PDGF) is a growth factor of multiple biological activities (1,2). Although this growth factor was first purified from human platelet, production of PDGF in other types of cell is known (1,2). PDGF is highly growth promoting and chemotactic to the retinal pigment epithelial (RPE) cells, and is also known to induce intraocular proliferation (3,4). Thus, intraocular production of PDGF is an important phenomenon possibly explain pathogenesis of ocular proliferative diseases. No report is available, however, to show the gene expression of PDGF in the RPE cells although occurrence of possible PDGF-like immunoreactivities in the conditioned culture medium of the cell is reported (5). Herein we report that RPE cells in culture express genes for PDGF and its receptor isomers. We will also show that phorbol myristate acetate (PMA), thrombin, and interleukin-1 (IL-1) increase gene expression of PDGF B-chain in the cells without affecting gene expression of PDGF β-receptor.

Materials and Methods

Cell Culture and Synthesis of cDNA Human RPE cells were obtained and cultured as previously described (6). Cells from the 3rd to the 5th passage were used in the experiments. RPE cells were cultured to near confluency in

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Table 1. Primers used in the experiment

	5' primer	3' primer	length
A-chain	CTGGAGATAGACTCCGT	CCTGACGTATTCCACCT	336 bp
B-chain	CCCGGAGTCGGCATGAA	TTTCTCACCTGGACAGGTCG	476 bp
α-receptor	CTGTAACTGGCGGATTC	GCTGAAGTTCTGTCGCA	352 bp
β-receptor	ACGAGATCCGATGGAAG	GCAGAGCATTGCTGTAG	477 bp

100-mm culture dishes, then the media was replaced with serum-free medium containing insulin (1 μg/ml), transferrin (5 μg/ml), and bovine serum albumin (0.5 mg/ml) for 16-20 hr. Total cellular RNA was prepared by the acid guanidinium thiocyanate-phenol-chloroform extraction method (7). Polyadenylated (Poly A^+) RNA was prepared by using a Micro-Fast Track (Invitrogen, San Diego, CA). First strand complementary DNA (cDNA) was prepared using a First-strand cDNA synthesis kit (Pharmacia-LKB, Uppsala, Sweden).

Polymerase Chain Reaction (PCR) and Nucleotide Sequencing of the PCR Products PCR was carried out by the method of Saiki et al. with a slight modification (8). A DNA thermal cycler, Taq DNA polymerase and other reagents for PCR experiments were purchased from Perkin-Elmer Cetus (Norwalk, CT). The primers used in this experiment (shown in Table 1) were designed so that at least one intron was located in the corresponding genomic sequence in order to detect possible amplification of any contaminating genomic DNA. PCR products were subcloned into the pBluescript II vector (Stratagene, La Jolla, CA) and the nucleotide sequencing was determined according to the dideoxynucleotide chain termination method (9).

Northern Blot Analysis Poly A^+ RNA (1 μg) was electrophoresed in a 1% agarose gel under denaturing conditions, then transferred to a nylon filter (Genescreen Plus, NEN Du Pont, Boston, MA) by the capillary transfer method with 20x standard saline citrate (SSC). The RNA was fixed to the filter by baking at 80°C for 2 hr. DNA fragments corresponding to the target sequences were labeled with 5'α-[^{32}P]dCTP(Amersham Japan, Tokyo, Japan) by the Multiprime DNA labeling system (Amersham, Little Chalfont, UK). Hybridization and washing was done after the method of Thomas (10).

Semiquantification of PDGF Gene Expression by PCR Changes in PDGF gene expression induced by the stimulants were estimated by using PCR. The cDNA concentration was first normalized by the expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The primers used were TGCACCACCAACTGCTT (sense strand) and TACTCCTTGGAGGCCAT (antisense strand) (11), which yielded a 554 bp product. For quantification, 2 μCi of dCTP was added in the reaction mixture and cDNA concentrations of different batches were compared. In detail, cDNA derived from 150 ng of total RNA was prepared and diluted 1:2, 1:4, 1:8, 1:16, 1:32 in water. The PCR products were electrophoresed in a 2% agarose gel, the 554-bp bands were excised and the radioactivity incorporated into the DNA was measured by Cerenkov scintillation counting. A standard curve was drawn from the radioactivities. The relative quantity of the expressed GAPDH gene in the cDNA was thus calculated and normalized by this standard curve. Similar experiments were done using specific primers of PDGF B-chain and PDGF β-receptor in order to determine relative expression of the two genes.

Results

PCR, Nucleotide Sequencing and Southern Blot Analysis PCR using specific primers for PDGF A-chain, B-chain, α-receptor, and β-receptor showed products of expected lengths of DNA (12-16) (Fig. 1). Although the primers gave quite specific amplification of the target genes, nucleotide sequencing of the amplified DNA was performed to ensure that the bands represent amplified sequences of the target genes. Digestion with restriction endonucleases and Southern blot analysis of the PCR products also confirmed that RPE cells express PDGF A-and B-chain isomers as well as α- and β-receptors (graphic data not shown).

Semiquantification of the PDGF Gene Expression in Stimulated RPE Cells We next tried to quantitate the levels of expression of PDGF and PDGF receptor genes following exposure of the cells to biochemical stimuli that may elicit some intraocular diseases. We tried to quantitate the expression of the four genes, but expression of PDGF A-chain and α-receptor genes were too small to yield reproducible data. Therefore, relative amounts of PDGF B-chain and PDGF β-receptor genes were calculated. As shown in Figure 2, PMA and thrombin dramatically increased expression of PDGF B-chain gene. The relative amount was 19.8 ± 1.75 (mean ± S.E.M.) fold of the control by PMA (n=3), and 15.9 ± 1.84 fold by thrombin (n=3). In contrast, IL-1β induced only a moderate increase (4.43 ± 1.20, n=3). TGF-β and PDGF-BB itself were not effective in increasing gene expression of PDGF B-chain. Same experiments were carried out for the PDGF β-receptor gene. In this case, no stimulant used in this study was effective to change the expression of PDGF β-receptor gene.

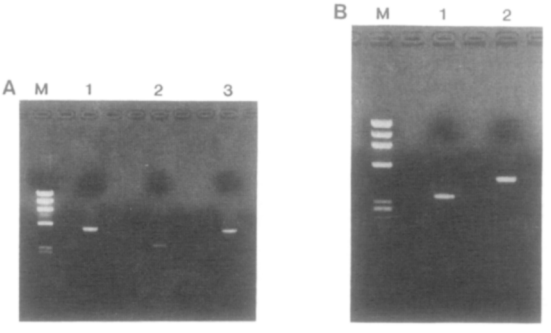

Figure 1. Agarose gel electrophoresis of the PCR products using specific primers for PDGF A-chain, B-chain, α-receptor, β-receptor and GAPDH.
A: Lane M, marker (ΦX174 *HaeIII* digest); Lane 1, GAPDH (554 bp); Lane 2, PDGF A-chain (336 bp); Lane 3, PDGF B-chain (476 bp).
B: Lane M, marker (ΦX174 *HaeIII* digest); Lane 1, PDGF α-receptor (352 bp); Lane 2, PDGF β-receptor (477 bp).

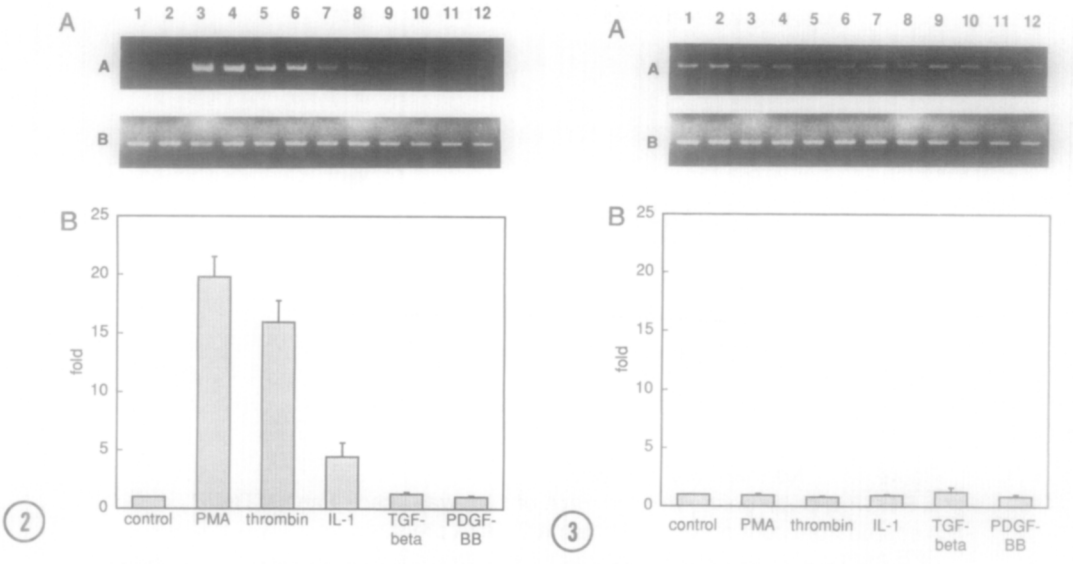

Figure 2. Semiquantitative PCR analysis of PDGF B-chain gene expression.
A: Agarose gel electrophoresis of the PCR products using conditioned templates. Top (A), PDGF B-chain; bottom (B), GAPDH. Lanes 1 and 2, control; Lanes 3 and 4, PMA (100 ng/ml, Sigma, St. Louis, MO); Lanes 5 and 6, thrombin (3 U/ml, Sigma); Lanes 7 and 8, IL-1β (20 ng/ml, Ohtsuka Pharmaceutical, Tokushima, Japan); Lanes 9 and 10, TGF-β (5 ng/ml, Wako Pure Chemicals, Osaka, Japan); Lanes 11 and 12, PDGF-BB (10 ng/ml, Genzyme, Boston, MA). Duplicate experiments for three different batches of cDNA were done. Stimulants were added after a 16-20 hr serum depletion and RNA was extracted 4h after the stimulation. PMA (1-100 ng/ml), thrombin (0.3-3 U/ml) and IL-1β (2-20 ng/ml) showed dose-dependent increase in the expression of PDGF-B chain gene expression and maximal increase was observed between 2-8 hr after stimulation.
B: Quantitative data of Fig. 2A.

Figure 3. Semiquantitative PCR analysis of PDGF β-receptor gene expression.
A: Agarose gel electrophoresis of the PCR products using conditioned templates. Top (A), PDGF β-receptor; bottom (B), GAPDH. Lanes 1 and 2, control; Lanes 3 and 4, PMA (100 ng/ml); Lanes 5 and 6, thrombin (3 U/ml): Lanes 7 and 8, IL-1β (20 ng/ml); Lanes 9 and 10, TGF-β (5 ng/ml); Lanes 11 and 12, PDGF-BB (10 ng/ml). Duplicate experiments for three different batches of cDNA were done.
B: Quantitative data of Fig. 3A.

Expression of PDGF β-receptor gene was 0.8 to 1.2 fold of the control (Fig. 3). Thus PMA, thrombin, and IL-1β were found to increase PDGF-B chain transcripts without affecting the expression of PDGF β-receptor gene. Because Northern blot analysis is a well established method to quantify changes in the expression of genes, we tried to analyze the expression of PDGF B-chain gene by this means. Our initial attempt to detect the message of PDGF B-chain by using RPE cells in the resting state failed. However, a transcript of about 3.5 kbp was detected in poly A^+ RNA of PMA-stimulated RPE cells (Fig. 4A). The

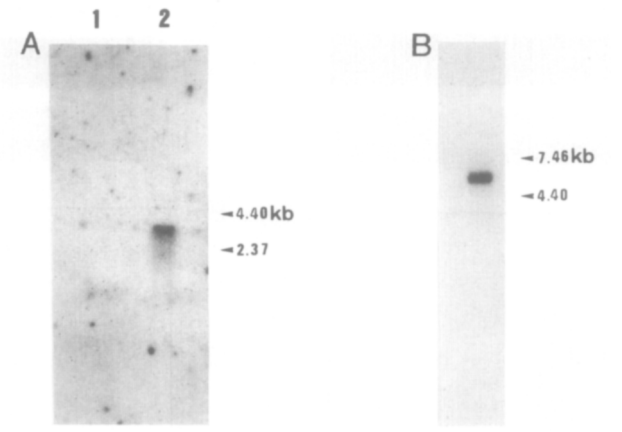

Figure 4. Northern blot analysis.
A: PDGF B-chain gene. Poly A^+ RNA of 1 μg was used. Lane 1, RNA prepared from control RPE cells; Lane 2, RNA prepared from PMA-treated RPE cells.
B: PDGF β-receptor gene. Poly A^+ RNA of 1 μg was used. Lane 1, RNA prepared from control RPE cells; Probes used were a 476 bp DNA fragment for the PDGF B-chain and a 477 bp DNA fragment for the PDGF β-receptor.

size of this transcript is similar to that of human PDGF B-chain, and increased transcript of PDGF B-chain in PMA-treated RPE cells is consistent with our PCR study. Northern blotting of PDGF β-receptor gene was also carried out, and a 5.3 kbp message was detected (Fig. 4B).

Discussion

Contribution of cytokines and growth factors in the pathogenesis of intraocular proliferative diseases such as proliferative vitreoretinopathy and proliferative diabetic retinopathy is proposed (17). Among the growth factors, PDGF is one of the most important candidates that induce intraocular proliferation because this growth factor is known to be a most potent stimulator of RPE cell migration and proliferation (3). Serum-derived PDGF may be an important factor that induce intraocular proliferation. However, because of its rather short half-life in the vitreous cavity, importance of locally-produced PDGF should be considered (2,5). Campochiaro et al. have shown that cultured RPE cells produce PDGF-like immunoreactivities in the culture media (5). However, PDGF gene expression in the RPE cells has been unknown. In this study, using a sensitive PCR method, we have clearly shown that RPE cells express messages of both PDGF A-chain and B-chain. Also gene expression of α- and β-receptors was shown. In our analyses, PDGF B-chain and β-receptor genes were expressed more dominantly than A-chain and α-receptor genes in the cells. Increased expression of PDGF B-chain gene in the RPE cells by PMA, thrombin and also by IL-1β without affecting the expression

of β-receptor gene shown in this study is an interesting finding. Although we failed to show production of PDGF B-chain protein in the culture media of the RPE cells possibly because of the low sensitivity of the available radioimmunoassay system, our semiquatitative PCR data support an idea that once activated by inflammatory stimuli, RPE cells produce more PDGF to stimulate proliferation and migration of RPE cells in an autocrine or paracrine fashion and also to induce recruitment and proliferation of other types of cells in the process of intraocular proliferative diseases.

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