Figure S2. (a) BRECs secrete PDGF or a PDGF-like substance. Fibroblasts expressing the WT PDGFR (Andrews et al., 1999) were grown to 70-80 % confluence and starved in DMEM and 0.25 %fetal bovine serum for 16-18 h. PDGF (0.1, 1, and 5 ng/ml) or conditioned medium, or fresh media was added for 5 min, the cells were lysed, immunoprecipitated with anti-PDGFR antibody, and subjected to anti-phosphotyrosine (PY) Western blot. The blot was then stripped and re-probed with an anti-PDGFR antibody. The medium was "conditioned" by incubating it in a collagen gel assay for 24 h. **(b) Diagram of the putative autocrine loop.** Introduction of PDGFR into PDGF-producing BRECs establishes an autocrine loop. **(c, d) Neutralizing PDGF abolished spontaneous tube formation in BRECs expressing the WT PDGFR.** BRECs expressing the WT PDGFR were subjected to a tube assay supplemented with different concentrations of anti-PDGF neutralizing antibody. This experiment was done in the absence of VEGF-A (Buffer) or in the presence 2.5 ng/ml VEGF-A (c). Fibroblasts expressing the WT PDGFR were grown to 70-80 % confluence and starved in DMEM and 0.25% fetal bovine serum for 16-18 h. PDGF (10 ng/ml) with 10 μg/ml anti-PDGF neutralizing antibody or IgG control or buffer was added for 5 min. Total cell lysates were immunoprecipitated with anti-PDGFR antibody, and subjected to Western blot analysis followed by anti-phosphotyrosine (PY). The blot was then stripped and re-probed with an anti-PDGFR antibody. In addition, a part of lysates was subjected to Western blot analysis followed by anti-phospho-Erk antibody. The blot was then stripped and re-probed with an anti-Erk antibody (d). **(e) A VEGF-A trap did not alter the tube response in PDGFR-expressing cells.** Parental (BRECs) or PDGFR-expressing (WT) cells were subjected to the tube assay under the indicated experimental conditions. "VEGF" indicates 2.5 ng/ml VEGF-A, whereas "Soluble R2" is a VEGF trap consisting of the extracellular domain of VEGFR2. The total tube length was measured after 24 h incubation. **(f) Expression of the WT PDGFR did not elevate VEGF-A mRNA level.** Parental or PDGFR-expressing BRECs were plated in a collagen gel and incubated for 24 h. Following extraction of the cells, the total RNA was isolated and amplified by PCR for 20 or 30 cycles for semi-quantification of PCR. The RT-PCR products were separated in 1.0 % agarose gels and visualized with Ethidium Bromide. Bovine β-actin was amplified simultaneously in a separate set of tubes under the same conditions. **(g) Recruitment of SHP-2 or RasGAP to the Y40/51 receptor failed to destabilize tubes.** BRECS expressing the indicated PDGFRs were subjected to the tube assay. The results at the indicated days are shown in the bar graph. Unlike PLCγ, RasGAP or SHP-2 did not promote tube regression.

References

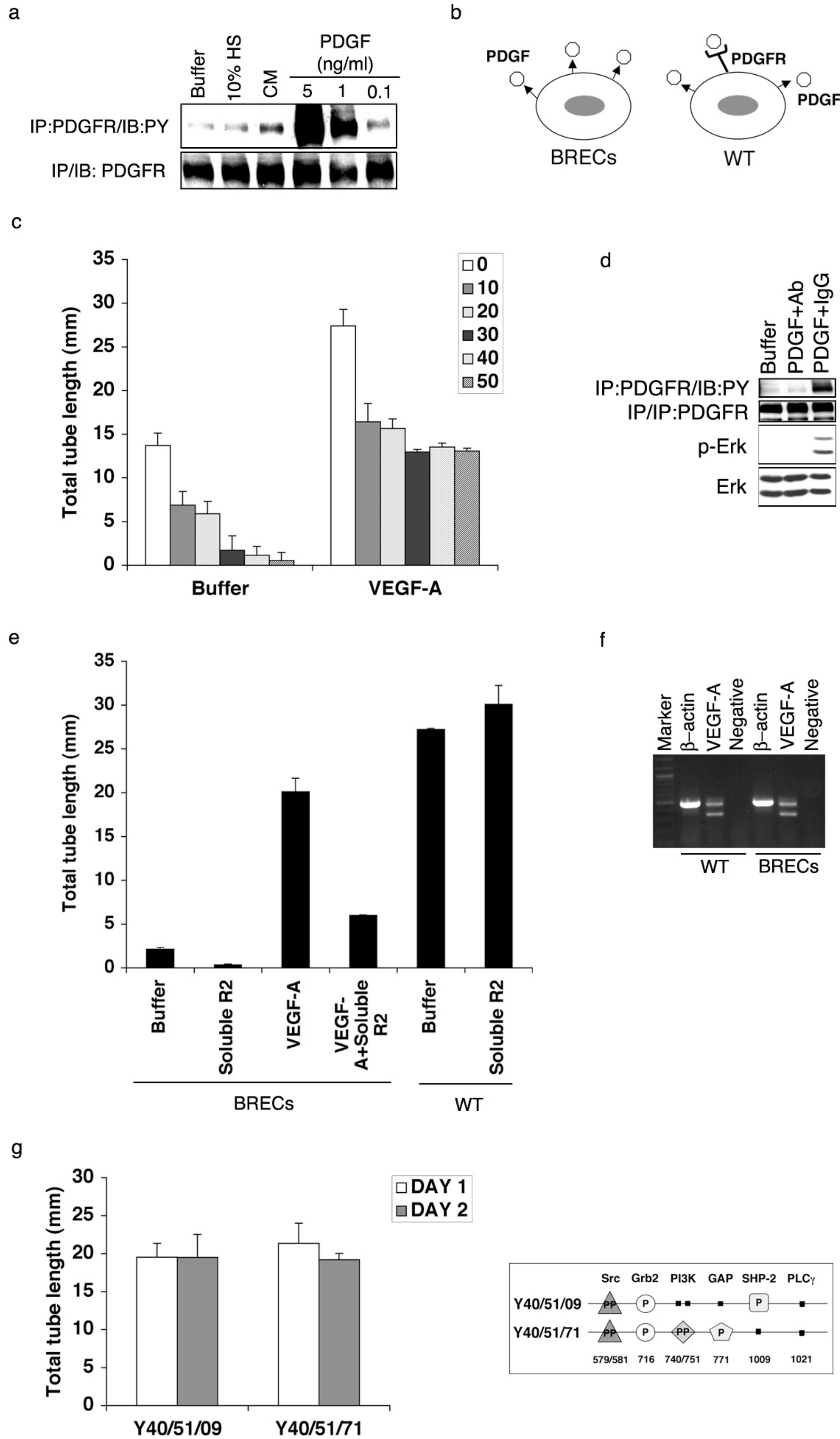
Andrews, A., Balciunaite, E., Leong, F.L., Tallquist, M., Soriano, P., Refojo, M. and Kazlauskas, A. (1999) Platelet-derived growth factor plays a key role in proliferative vitreoretinopathy. *Invest Ophthalmol Vis Sci* **40**, 2683-2689.

Supplementary Materials and Methods

Antibodies and reagents. Rabbit polyclonal anti-phospho-p44/42 MAP kinase antibody, and anti-p44/42 MAP kinase antibody were obtained from Cell Signaling Technology. Goat polyclonal anti-PDGF, neutralizing antibody was purchased form Upstate Biotechnology Inc.

RT-PCR. Cells were plated in a collagen gel for the desired length of time, the cells were recovered from the gel and then total RNA was isolated with RNAgents Total RNA Isolation System (Promega, Madison, WI) according to the manufacturer's protocol. The

RT-PCR reactions were performed using the Onestep RT-PCR kit (QIAGEN, Valencia, CA). Two oligonucleotides, corresponding to nucleotides at the 5' site GAAGGAGGGCAGAAACCCCACGAAGTGG and at the 3' site CCATGAATGCTTC TGCCGGAGCCTCACGC were used as primers for VEGF-A. The first-strand cDNA was reverse-transcribed from total RNA and the cDNA product was amplified by PCR for 20 or 30 cycles for 30 seconds at 94 °C, 1 minute at 55 °C, and 1 minutes at 72 °C (for final extension, 10 minutes at 72 °C). The RT-PCR products were separated in 1.0 % agarose gels and visualized with Ethidium Bromide. Bovine β-actin was amplified simultaneously in a separate set of tubes under the same conditions. The 5' and 3' primers for β-actin were GCTCAGAGCAAGAGAGGCATCCTGACC and GCAGAGCTTCTCCTTGATGTCACGG, respectively.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Buffer
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CM
PDGF (ng/ml)
5
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0.1
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IP/IB: PDGFR
b
PDGF
PDGFR
PDGF
BRECs
WT
c
Total tube length (mm)
Buffer
VEGF-A
d
Buffer
PDGF+Ab
PDGF+IgG
IP:PDGFR/IB:PY
IP/IP:PDGFR
p-Erk
Erk
e
Total tube length (mm)
Buffer
Soluble R2
VEGF-A
VEGF-A+Soluble R2
Buffer
Soluble R2
BRECs
WT
f
Marker
β–actin
VEGF-A
Negative
β–actin
VEGF-A
Negative
WT
BRECs
g
Total tube length (mm)
DAY 1
DAY 2
Y40/51/09
Y40/51/71
Src
Grb2
PI3K
GAP
SHP-2
PLCγ
579/581
716
740/751
771
1009
1021