Table S1. Peptide mass fingerprinting matched the ~60-kDa protein to F1-ATP synthase β-subunit[a]

MS-Fit Search Results

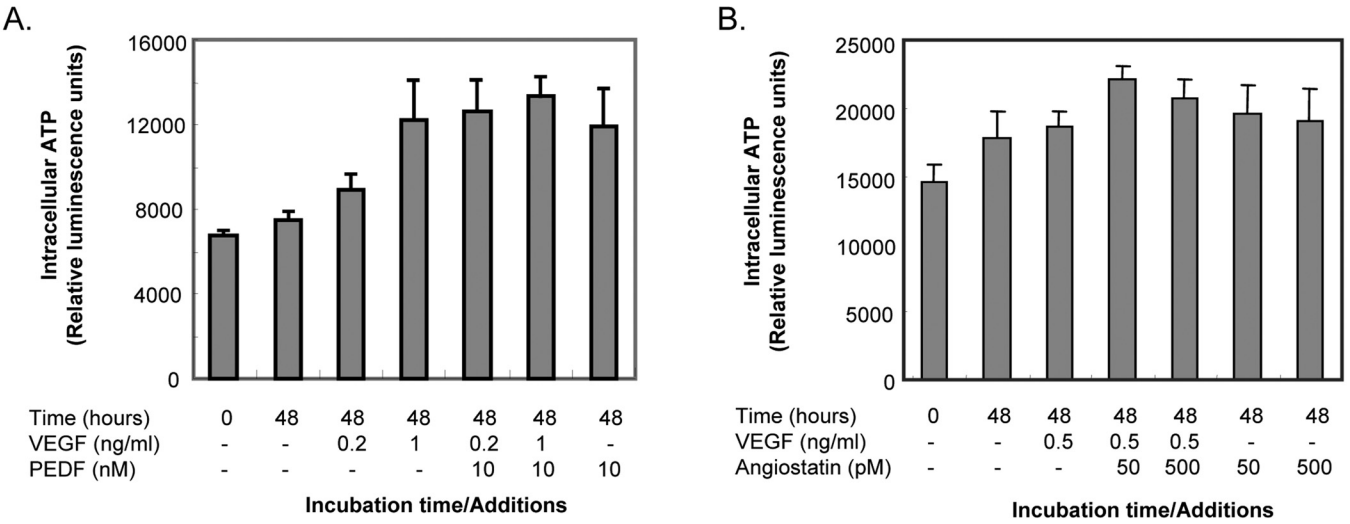
Result Summary

Rank	MOWSE Score	# (%) Masses Matched	Protein MW (Da)/pI	Species	NCBI Accession #	Protein Name
1	9.6e+007	17/23 (73%)	56283.8 / 5.15	BOS TAURUS	114543	(M20929) F-1-ATPase β-subunit precursor
2	4.41e+007	16/23 (69%)	51202.8 / 4.91	RATTUS NORVEGICUS	1374715	(M19044) ATP synthase β subunit
3	4.01e+007	16/23 (69%)	56353.8 / 5.18	RAT	114562	ATP SYNTHASE β CHAIN, MITOCHONDRIAL PRECURSOR
4	3.99e+007	16/23 (69%)	56560.2 / 5.26	HOMO SAPIENS	114549	(M27132) ATP synthase β subunit precursor
5	1.98e+006	14/23 (60%)	57955.9 / 5.80	HOMO SAPIENS	28940	ATPase β, F1

[a]To identify the PEDF-binding site components, the affinity-purified binding proteins from bovine retina (as in Aymerich et al (4)) were analyzed by mass spectrophotometry, and peptide mass fingerprinting. PEDF binding proteins were resolved by SDS-PAGE, the proteins in the gel were stained with Coomassie blue, the ~60-kDa and ~85-kDa protein bands were dissected. The proteins were digested with trypsin, and the resulting peptides were extracted to determine their masses by mass spectroscopy to provide a unique signature by which to identify the protein by peptide mass searches using a MS-Fit peptide mass fingerprinting tool (Protein Prospector). This approach did not yield identification of sequence for the 85-kDa protein band. However, the other band matched the 60-kDa protein(s) to *Bos taurus* F1-ATP synthase β -subunit (Table S1).

Effect of PEDF on intracellular ATP levels.

PEDF does not decrease intracellular ATP levels. The intracellular ATP was measured in wells with HMVECs treated with PEDF, VEGF and PEDF/VEGF combinations for 48 hours. In contrast to extracellular ATP production, PEDF or PEDF/VEGF did not decrease the intracellular ATP levels relative to those in cells treated without factors, if any they slightly increased them, e.g., 1.6-fold for 10 nM PEDF, and 10 nM PEDF/1 ng/ml VEGF combinations; and 1.2-fold for 50 pM and 500 pM angiostatin K1-5 (Fig. S1A and S1B). In a separate experiment, treatments of PEDF and PEDF/VEGF combinations for 48h inhibited the extracellular ATP production, and VEGF did not affect it (Fig. S1C).



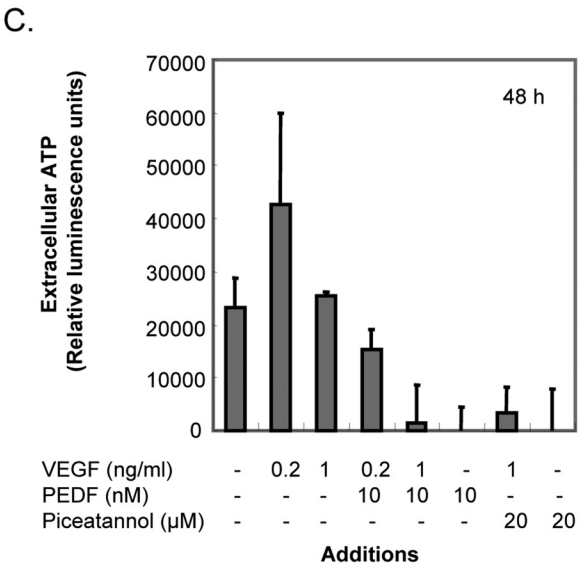


Fig. S1. Effect of PEDF on intracellular ATP levels. HMVECs were cultured in the presence of inhibitors, VEGF or combinations as indicated in the X-axis for 48 h. Cells were incubated with ATP synthase substrates ADP and inorganic phosphate and the addition of inhibitors as indicated, for 30 s. Intracellular pools (A and B) and extracellular medium (C) were separated, and the ATP content in those pools was determined.

PEDF-binding proteins in BRECs and human retinoblastoma Y-79 cells

PEDF-affinity column chromatography of plasma membrane extracts of BRECs revealed the cell-surface proteins involved in binding of PEDF. Spyro Ruby protein staining of gels revealed a ~60-kDa doublet proteins in the lane with BRECs proteins that bound and eluted from the PEDF-resin (Fig. S2) and additional minor ~40-kDa and ~22-kDa proteins. Chromatography of human retinoblastoma Y-79 cells revealed 85-kDa and 60-kda PEDF binding proteins. However, the ~85-kDa PEDF-binding protein was not the major band detected in samples from BRECs, suggesting a different set of PEDF-binding proteins in endothelial than in neuronal cells, which could be used to distinctly to trigger angiostatic vs. neurotrophic activities. The results with Y-79 are in agreement with previous reports for human retinoblastoma Y-79 cells and normal bovine retina showing two distinct bands for PEDF-binding proteins, a major one migrating like a ~85 kDa protein and a minor doublet of about 60-kDa proteins (21,22).

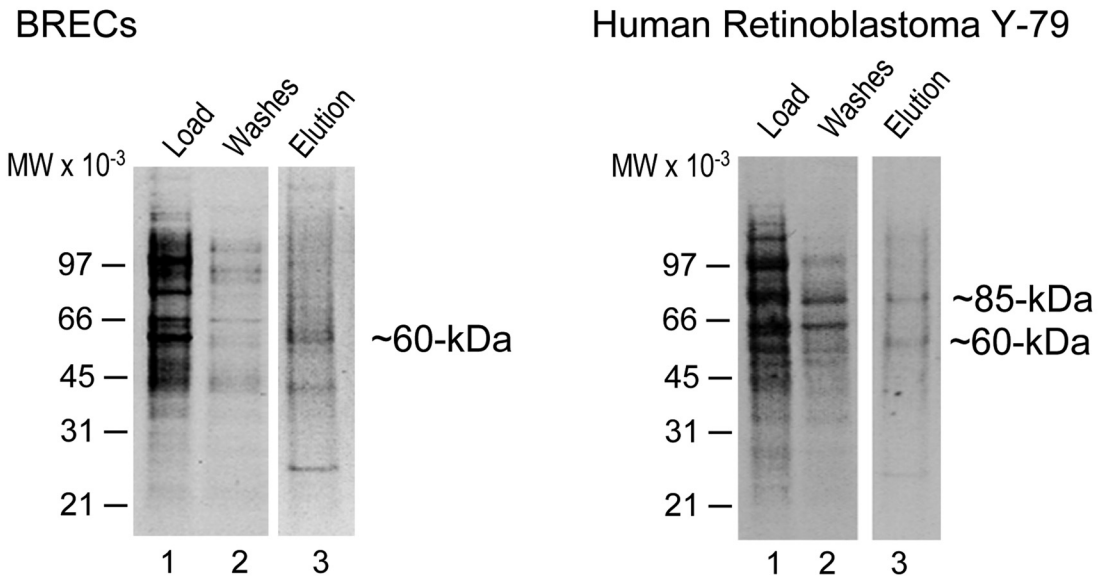


Fig. S2. PEDF-affinity column chromatography. The protocol followed was described before (21,22). Plasma membranes of BRECs (81 x 10^6 cells) and human retinoblastoma Y-79 cells (104 x 10^6 cells) were isolated and proteins were solubilized with CHAPS. Detergent-soluble plasma membrane extracts (lane 1) were applied to a control column without PEDF and the flow-through collected into a PEDF-affinity column. The unbound material to the PEDF column was washed with binding buffer (lane 2), and then with low pH buffer and 1M NaCl in low pH buffer. Finally, the bound proteins were eluted with high pH buffer (lane 3). Samples were concentrated with centricon C-10 and proteins were resolved by SDS-PAGE followed by Sypro protein stain to be visualized under UV light. A negative image is shown. Migration positions for ~60-kDa and ~85-kDa protein bands are indicated.

SUPPLEMENTARY INFORMATION

ACKNOWLEDGEMENTS

We thank Marisol Aymerich for performing PEDF affinity chromatography with bovine retina, Al Yergey and Peter Backlund for Mass Spec preparations, analyses and interesting discussions.

Complex formation assay between soluble fluorescein-conjugated PEDF (Fl-PEDF) and F_1-ATPase

Fluorescein conjugation modifies primary amines of PEDF similar to the chemical modification that occurs during immobilization of PEDF on SPR chips. The figure shows that Fl-PEDF formed complexes only when F1 was present (compare lanes 1 and 2) . Levels of retained PEDF were ~40% of the input (compare lanes 2 and 3).

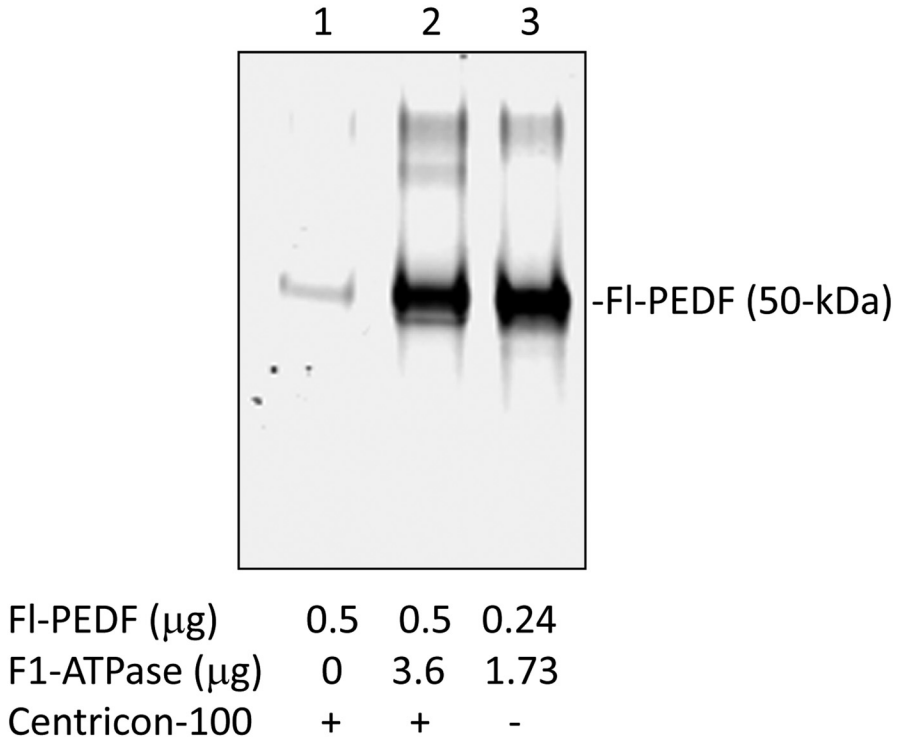


Fig. S3. Binding of Fluorescein-conjugated PEDF (Fl-PEDF) to F1-ATPase was as in figure 1. Proteins were incubated for 1 h at room temperature and then the mixtures were subjected to size-exclusion ultrafiltration using membranes with size exclusion limits of 100-kDa. The amounts of each component in reaction mixtures are indicated. The total protein complexes retained by the membrane for each reaction were applied to 10-20% polyacrylamide gel and resolved by SDS-PAGE. A fraction of the reaction corresponding to lane 2 before subjected to ultrafiltration was applied to lane 3 of the same gel. The gel was scanned by using Typhoon 9410 Laser-based scanner.

Competition of Fl-PEDF binding to F1-ATPase with PEDF and Angiostatin
The figure shows that both PEDF and angiostatin decreased the complex formation of Fl-PEDF with F1-ATPase. These observations indicate that the chemically modified PEDF binds F1 similarly as the unmodified one. The results also indicate that angiostatin competed with Fl-PEDF for F1 binding, implying that Fl-PEDF bound to an angiostatin binding site on F1.

Fl-PEDF (μg)	0.5	0.5	0.5	0.5	0.5	0.5
F1-ATPase (μg)	3.6	3.6	3.6	3.6	3.6	3.6
PEDF (μg)	0.05	0.5	5	0	0	0
Angiostatin (μg)	0	0	0	0.05	0.5	5

Fig. S4. Binding reactions of Fl-PEDF to F1-ATPase in the presence of increasing amounts of PEDF and angiostatin were as in figure 1. Proteins were incubated for 1 h at room temperature and then the mixtures were subjected to size-exclusion ultrafiltration using membranes with size exclusion limits of 100-kDa. The amounts of each component in reaction mixtures are indicated. The total protein complexes retained by the membrane for each reaction were applied to 10-20% polyacrylamide gel and resolved by SDS-PAGE. The gel was scanned by using Typhoon 9410 Laser-based scanner.