

Involvement of Pigment Epithelial derived Factor (PEDF) in Angiogenesis

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Decrease of pigment epithelium-derived factor in aqueous humor with increasing age.

Ogata N, Matsuoka M, Imaizumi M, Arichi M, Matsumura M.

PURPOSE: To determine whether the level of pigment epithelium-derived factor (**PEDF**) in the aqueous humor is altered with age. **DESIGN:** Observational case series. **METHODS:** The PEDF concentration in the aqueous humor was measured by enzyme-linked immunosorbent assay in 152 eyes of 121 patients who underwent cataract surgery. **RESULTS:** The mean aqueous level of PEDF was 0.86 +/- 0.04 microg/ml (mean +/- standard error, 70.7 +/- 1.0 years). The PEDF levels decreased with increasing age, and the decrease was significantly correlated with age (Pearson product moment correlation coefficient, $r = -0.22$, $P = .006$). The mean PEDF level in the men (0.76 +/- 0.06 microg/ml, 53 eyes, 71.5 +/- 2.0 years) was significantly lower than that in women (0.91 +/- 0.04 microg/ml, 99 eyes, 70.2 +/- 1.1 year, $P = .03$). **CONCLUSIONS:** The negative correlation of PEDF level and age should be considered in age-related eye diseases, especially those associated with angiogenesis.

Lab Invest. 2004 May 3

Abnormal retinal vascular development in IL-18 knockout mice.

Qiao H, Sonoda KH, Sassa Y, Hisatomi T, Yoshikawa H, Ikeda Y, Murata T, Akira S, Ishibashi T.

Recent studies have indicated that interleukin 18 (IL-18) might act as either an angiogenic or an angiostatic factor, but the true function of this protein in vascular development is unclear. We therefore investigated the role of IL-18 in the formation of retinal vessels. Development of the retinal vasculature was compared in IL-18 knockout (KO) and wild-type (WT) mice at several different time points. The formation of vessels was evaluated using angiography of flat-mounted retinal samples after inoculation with fluorescein dextran. Retinal samples from both groups were also evaluated through histological examinations, and the expression of angiogenic factors was examined using the reverse-transcription-polymerase chain reaction. The capillary retinal vessels in both WT and IL-18 KO mice had reached the peripheral retina by postnatal day (P) 7. However, IL-18 KO mice showed angiectasis and vascular leakage at P7, especially in the mid-peripheral retina. These symptoms were not observed in WT mice at any stage. Histopathological analysis confirmed abnormal vascular formation in IL-18 KO mice at P14. Interestingly, these abnormalities regressed over time and had disappeared by P84. Several angiogenesis-associated factors, including vascular endothelial growth factor (VEGF), basic fibroblast-growth factor (bFGF), platelet-derived growth factor (PDGF) and pigment epithelium-derived factor (**PEDF**), were overexpressed in the retinas of IL-18 KO mice compared with those of WT mice at P14. Interferon-gamma was detected only in WT mouse retinas at P14. These results provide new evidence for the role of IL-18 in retinal vascular development. Laboratory

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Vitreous levels of pigment epithelium-derived factor and vascular endothelial growth factor: implications for ocular angiogenesis.

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PURPOSE: Pigment epithelium-derived factor (**PEDF**) has been demonstrated to suppress ocular angiogenesis in several animal models. In this study, we sought to measure the levels of PEDF and vascular endothelial growth factor (VEGF) in the vitreous of patients with and without ocular neovascular disorders. **DESIGN:** Case-control study of patients undergoing intraocular surgery for a variety of neovascular and nonneovascular conditions. **METHODS:** Vitreous samples were collected from 65 eyes of 65 patients with no neovascular disorder ($n = 24$), choroidal neovascularization ($n = 9$), active proliferative diabetic retinopathy ($n = 16$), and inactive proliferative diabetic retinopathy ($n = 16$). The levels of VEGF and PEDF in these vitreous samples were determined by enzyme-linked immunosorbent assay. **RESULTS:** The VEGF levels were at or below the level of detectability in the reference and choroidal neovascularization groups. The VEGF levels were significantly elevated in both the active and inactive PDR

groups, and significantly higher in the active PDR group as compared with the inactive PDR group. The PEDF levels, which were present at relatively high concentrations in all groups, were higher in patients with active PDR compared with the control and choroidal neovascularization groups. **CONCLUSIONS:** High levels of immunoreactive PEDF are present in the vitreous of individuals with or without ocular neovascularization, but PEDF levels are significantly higher in patients with active PDR compared with patients with choroidal neovascularization or nonneovascular retinal diseases. Although these results do not preclude the possibility that endogenous PEDF helps to modulate ocular neovascularization, they do not support ischemia-induced downregulation of PEDF as a mechanism for such modulation.

Cancer Gene Ther. 2004 May;11(5):325-32.

Inhibition of glioma invasion by overexpression of pigment epithelium-derived factor.

Guan M, Pang CP, Yam HF, Cheung KF, Liu WW, Lu Y.

Pigment epithelium-derived factor (**PEDF**) is a potent inhibitor of angiogenesis and an inducer of neural differentiation. We previously reported the loss of PEDF expression in glioma progression. In this study, we investigated whether PEDF overexpression could suppress glioma growth and invasion. Glioma cell line U251 was stably transfected with a full-length human PEDF expression vector. The expression and release of various cytokines and angiogenic factors into the medium were analyzed by real-time reverse transcription-polymerase chain reaction, enzyme-linked immunosorbent assay, and gelatin zymography. Apoptosis was checked by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling. Growth inhibition was evaluated by using the *in vitro* Matrigel invasion. Tumorigenicity was examined *in vivo* by subcutaneous xenotransplantation into severe combined immunodeficient mice. In U251 cells overexpressing PEDF, thrombospondin-1 protein was upregulated (5.3-fold more), but the production of vascular endothelial growth factor (VEGF) (1.8-fold less) and basic fibroblast growth factor (2.5-fold less) was lower than in cells transfected with the vector only. PEDF also downregulated the production of matrix metalloproteinase-9. Conditioned medium collected from the PEDF-transfected U251 cells showed a significant reduction of VEGF expression. *In vitro* invasiveness was reduced by approximately 40%. PEDF expression prevented the growth of transfected cells and caused a significant increase in the percentage of cells undergoing apoptosis (50.4% in PEDF-transfected cells). Furthermore, the size of xenotransplants was significantly smaller. In conclusion, PEDF overexpression decreased malignancy, and this might be attributed to the promotion of apoptosis and the regulation of expression of angiogenic effectors. Thus, treatment with PEDF may be useful in patients with malignant gliomas. However, the mechanism of apoptosis induction needs to be investigated.

Am J Pathol. 2004 Apr;164(4):1225-32.

Overexpression of pigment epithelium-derived factor decreases angiogenesis and inhibits the growth of human malignant melanoma cells *in vivo*.

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Pigment epithelium-derived factor (**PEDF**) has recently been shown to be the most potent inhibitor of angiogenesis in the mammalian eye, and is involved in the pathogenesis of angiogenic eye disease such as proliferative diabetic retinopathy. However, a functional role for PEDF in tumor growth and angiogenesis remains to be determined. In this study, we have investigated both the *in vitro* and *in vivo* growth characteristics of human malignant melanoma G361 cell lines, stably transfected to overexpress human PEDF. Expression levels of PEDF proteins in melanoma cell lines G361 and A375 were comparable with that of human cultured melanocytes, whereas vascular endothelial growth factor levels in two tumor cell lines were much stronger than that in normal melanocytes. Overexpression of PEDF was found to significantly inhibit tumor growth and vessel formation in G361 nude mice xenografts. Furthermore, *in vitro* proliferation rates of G361 cells were decreased in PEDF-transfected cells. PEDF proteins showed dose-dependent induced growth retardation and apoptotic cell death in nontransfected G361 cells, which were completely prevented by treatment with antibodies against the Fas ligand. Our present study highlights two beneficial effects of PEDF treatment on melanoma growth and expansion; one is the suppression of tumor angiogenesis, and the other is induction of Fas ligand-dependent apoptosis in tumor cells. PEDF therefore might be a promising novel therapeutic agent for treatment of patients with melanoma.

Biochem Biophys Res Commun. 2004 Apr 2;316(2):573-9.

Osteoblasts and osteoclasts express PEDF, VEGF-A isoforms, and VEGF receptors: possible mediators of angiogenesis and matrix remodeling in the bone.

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Pigment epithelial derived factor (**PEDF**) is one of the most effective inhibitors of angiogenesis described so far, especially in controlling the growth of blood vessels in the eye. We now describe the localization of PEDF in regions of active bone formation in the mid-gestation mouse embryo and its specific and high levels of secretion by osteoblasts. PEDF is detected to a lesser extent in osteoclasts as well. The proangiogenic factors, VEGF and its receptors VEGF-R1 and VEGF-R2, are also expressed by both osteoblasts and osteoclasts. These findings suggest that bone angiogenesis and matrix remodeling may be mediated both by PEDF and by VEGF.

Invest Ophthalmol Vis Sci. 2004 Feb;45(2):441-7.

Mouse strain-dependent heterogeneity of resting limbal vasculature.

Chan CK, Pham LN, Chinn C, Spee C, Ryan SJ, Akhurst RJ, Hinton DR.

PURPOSE: Heterogeneity of the extent of angiogenesis induced by exogenous growth factors may be determined by genetic influences. Because angiogenesis is the formation of new vessels from preexisting ones, strain-related influences on naive resting limbal vessel phenotype and gene expression were determined in mice having divergently low and high angiogenic responses. **METHODS:** Resting limbal vessel surface area and density and extent of bFGF-induced corneal angiogenesis were determined in C57BL/6J, BALB/cJ, F1 intercross identical with C57BL/6J X 129S3/SvIM, and 129S3/SvIM mouse strains by quantitative three-dimensional reconstruction confocal microscopy. Strain-related influences on pro- and antiangiogenic gene expression in naive cornea were determined by quantitative real-time RT-PCR. **RESULTS:** The strain-dependent rank order of resting limbal vessel surface area and resting vessel density paralleled bFGF-induced neovascularization: 129S3/SvIM > BALB/cJ, F1 > C57BL/6J (P < 0.0006). Pigment epithelium-derived factor (**PEDF**) was increased more than 67-fold compared to Ang-2 in resting cornea of both C57BL/6J and 129S3/SvIM strains (P < 0.0001; P < 0.0001), suggesting a strongly antiangiogenic environment. The corneas of the C57BL/6J mice demonstrated 1.8-, 1.5-, and 1.7-fold increased mRNA levels for Flt-1, VEGF, and bFGF, respectively (P < 0.02; P < 0.04; P < 0.02); however, TSP-1 expression was increased 2.4-fold compared with 129S3/SvIM (P < 0.0004). **CONCLUSIONS:** Strain-dependent differences in the resting limbal vessel surface area and density correlated with heterogeneity in the extent of bFGF-induced angiogenesis. Differences in pro- and antiangiogenic gene expression levels in resting cornea may influence vascular limbal phenotype during quiescence and may predict susceptibility to angiogenesis-dependent diseases.

Graefes Arch Clin Exp Ophthalmol. 2004 Jan 14

Differential expression of angioregulatory factors in normal and CNV-derived human retinal pigment epithelium.

Martin G, Schlunck G, Hansen LL, Agostini HT.

BACKGROUND. Choroidal neovascularization (CNV) causes loss of vision in age-related macular degeneration (AMD). In CNV, choroidal capillaries penetrate Bruch's membrane and the retinal pigment epithelium (RPE). Angiogenic factors produced by RPE cells are suspected as major contributors to CNV development. We therefore studied the differential expression of angioregulatory factors in normal and CNV-derived RPE. **METHODS.** Cultures of normal (ARPE-19) and CNV-derived RPE (CNV-RPE) were compared by quantitative PCR. Differential expression was verified on the protein level by immunohistochemistry in tissue samples. **RESULTS.** The angioregulatory factors VEGF-A, VEGF-B, VEGF-C, Angiopoietin-1 (Ang-1) and Angiopoietin-2, Semaphorin-3A, **PEDF**, HIF-1, FGF-2, and the receptors VEGF-R2, Neuropilin-1 and Neuropilin-2 were detected in both, ARPE-19 and CNV-RPE. Transcription of PEDF, FGF-2, Neuropilin-2, Ang-1 and Ang-2 was significantly upregulated in CNV-RPE. EphA7, VEGF-R1 and leptin were transcribed exclusively in CNV-RPE and Eph-A7 and VEGF-R1 proteins were present exclusively in CNV specimens. **CONCLUSIONS.** A set of common factors controlling angiogenesis was detected in both, ARPE-19 cells and CNV-RPE cells. Surprisingly, PEDF and other factors inhibiting angiogenesis are strongly upregulated in CNV-RPE; thus, at least in later stages, the RPE has a potential to control angiogenesis in age-related macular degeneration.

Graefes Arch Clin Exp Ophthalmol. 2004 Jan;242(1):91-101. Epub 2003 Dec 18.

Role of growth factors and the wound healing response in age-related macular degeneration.

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Growth factors (GF) are important in several stages of the pathogenesis of age-related macular disease (AMD). In choroidal neovascularization (CNV) in exudative AMD, the GF involved are similar to those involved in wound healing of the skin. Like granulation tissue of skin, CNV is characterized by clotting, inflammation, angiogenesis and fibrosis, and like in skin wounds, members of the VEGF, angiopoietin, PDGF and TGF-beta families of GF are expressed. However, several of these GF may also serve physiological functions in the normal eye, where the retinal pigment epithelium (RPE) employs them to provide trophic support to the neuroretina and choriocapillaris, in addition to maintaining an anti-angiogenic state. Derangement of these physiological functions may underlie the initiation of CNV in AMD. Basolateral secretion of VEGF-A by the RPE maintains the choriocapillaris, and is enhanced by hypoxia. Age-related changes in Bruch's membrane lead to impairment of this trophic function and choriocapillaris atrophy, as well as to decreased diffusion of oxygen towards the neuroretina. The resulting outer retina hypoxia may be an important driving force of CNV formation, by stimulating VEGF overexpression by the RPE, in addition to the effects of increased oxidative stress and low-grade inflammation. RPE senescence and hypoxia may also decrease expression of angiogenesis inhibitors such as **PEDF**, further shifting the balance to a pro-angiogenic state in the aging eye.

Nippon Ganka Gakkai Zasshi. 2003 Nov;107(11):657-73.

[Molecular mechanism for choroidal neovascularization in age-related macular degeneration]

[Article in Japanese]

Ohno-Matsui K.

Choroidal neovascularization (CNV) in age-related macular degeneration (AMD) is the most common cause of severe visual loss in patients over age 60 years in developed countries. While much is unknown about the underlying pathogenesis of CNV, the increased production of vascular endothelial growth factor (VEGF) by retinal pigment epithelium (RPE) is thought to play a central role in the development of this condition. However, recent studies using gene-manipulated mice question the importance of VEGF alone in promoting CNV. Angiogenesis is thought to result from the balance between angiogenesis stimulation and inhibition. A potent antiangiogenic factor recently has been identified in the retina and shown to be secreted by RPE cells. The inhibitor, pigment epithelium-derived factor (**PEDF**) is considered the key factor associated with avascularity of the cornea, vitreous, and outer retinal layer of the eye. We recently demonstrated that an imbalance between PEDF and VEGF in RPE cells caused by aging and oxidative stress may contribute to the dysregulation of endothelial cell proliferation in CNV. In this review, we also discuss the angiogenic role of inflammatory cells in CNV, age-related changes in Bruch's membrane, and the possibility of the development of animal models reflecting CNV in AMD.

Exp Eye Res. 2003 Oct;77(4):433-45.

Expression of pigment epithelium-derived factor (PEDF) and vascular endothelial growth factor (VEGF) in sickle cell retina and choroid.

Kim SY, Mocanu C, McLeod DS, Bhutto IA, Merges C, Eid M, Tong P, Luty GA.

Pigment epithelium-derived factor (**PEDF**) has been shown to be an inhibitor of angiogenesis as well as a multipotent neurotrophic factor in the mammalian eye. Changes in PEDF levels have been correlated with development of retinal neovascularization in oxygen-induced retinopathy. The purpose of this study was to determine the localization and relative level of PEDF in human retinas and choroids using immunohistochemistry and evaluate the changes in PEDF and vascular endothelial growth factor (VEGF) localization and their relation to the progression of proliferative sickle cell retinopathy. Cryopreserved tissues from eyes of normal subjects and subjects with non-proliferative or proliferative sickle cell retinopathy were used with streptavidin peroxidase immunohistochemistry. A rabbit polyclonal antibody was made against recombinant human PEDF. Binding of the antibody was blocked by preincubation of the antibody with excess human recombinant PEDF. Relative levels of immunoreactivity were scored with a seven-point grading system and by microdensitometric analysis. The most prominent sites of PEDF localization in the normal eye were the vitreous condensed at the internal limiting membrane and RPE-Bruch's membrane-choriocapillaris complex. PEDF was also prominent in choroidal stroma. There was limited immunoreactivity in some cells of the neural retinas, in blood vessels and in the interphotoreceptor matrix (IPM). There was no difference in ratio (1.47 vs. 1.44) of **PEDF/VEGF** or the

relative levels of either growth factor in the retinal vasculatures of the control subjects and perfused area of non-proliferative sickle cell retinas. The ratio was increased in the non-perfused area of the non-proliferative sickle cell retinas (2.24). In eyes with proliferative sickle cell retinopathy, elevated PEDF and VEGF immunostaining was present in viable vessels of sea fan neovascular formations as well as feeder vessels of sea fans. The PEDF/VEGF ratio in sea fans was 1.0. Immunoreactivity for PEDF was prominent in retinal vessels in non-perfused regions and in atrophic sea fans, while VEGF immunoreactivity was weak or absent in these structures. In conclusion, PEDF and VEGF were both significantly elevated in viable sea fan formations in sickle cell disease ($p < 0.05$) but only PEDF was present in non-viable sea fans. The highest levels of PEDF in all eyes were associated with extracellular matrices (vitreous, choroidal stroma, IPM, and walls of blood vessels). PEDF might play an important role in inhibiting angiogenesis and inducing the regression of sea fans. Progression of angiogenesis may be dependent on the ratio of PEDF/VEGF.

EMBO J. 2003 Aug 15;22(16):4091-102.

Contrasting effects of VEGF gene disruption in embryonic stem cell-derived versus oncogene-induced tumors.

Viloria-Petit A, Miquerol L, Yu JL, Gertsenstein M, Sheehan C, May L, Henkin J, Lobe C, Nagy A, Kerbel RS, Rak J.

Previous gene targeting studies have implicated an indispensable role of vascular endothelial growth factor (VEGF) in tumor angiogenesis, particularly in tumors of embryonal or endocrine origin. In contrast, we report here that transformation of VEGF-deficient adult fibroblasts (MDF528) with ras or neu oncogenes gives rise to highly tumorigenic and angiogenic fibrosarcomas. These aggressive VEGF-null tumors (528ras, 528neu) originated from VEGF(-/-) embryonic stem cells, which themselves were tumorigenically deficient. We also report that VEGF production by tumor stroma has a modest role in oncogene-driven tumor angiogenesis. Both ras and neu oncogenes down-regulated at least two endogenous inhibitors of angiogenesis [pigment epithelium derived factor (**PEDF**) and thrombospondin 1 (TSP-1)]. This is functionally important as administration of an antiangiogenic TSP-1 peptide (ABT-526) markedly inhibited growth of VEGF(-/-) tumors, with some ingress of pericytes. These results provide the first definitive genetic demonstration of the dispensability of tumor cell-derived VEGF in certain cases of 'adult' tumor angiogenesis, and thus highlight the importance of considering VEGF-independent as well as VEGF-dependent pathways when attempting to block this process pharmacologically.

O J Nucl Med. 2003 Sep;47(3):149-61.

Angiogenic and angiostatic factors in the molecular control of angiogenesis.

Distler JH, Hirth A, Kurowska-Stolarska M, Gay RE, Gay S, Distler O.

The vascular system that ensures an adequate blood flow is required to provide the cells with sufficient supply of nutrients and oxygen. Two different mechanisms of the formation of new vessels can be distinguished: vasculogenesis, the formation of the first primitive vascular plexus de novo and angiogenesis, the formation of new vessels from preexisting ones. Both processes are regulated by a delicate balance of pro- and anti-angiogenic factors. Physiologically, angiostatic mediators outweigh the angiogenic molecules and angiogenesis does not occur. Under certain conditions such as tumor formation or wound healing, the positive regulators of angiogenesis predominate and the endothelium becomes activated. Angiogenesis is initiated by vasodilatation and an increased permeability. After destabilization of the vessel wall, endothelial cells proliferate, migrate and form a tube, which is finally stabilized by pericytes and smooth muscle cells. Numerous soluble growth factors and inhibitors, cytokines and proteases as well as extracellular matrix proteins and adhesion molecules strictly control this multi-step process. The properties and interactions of angiogenic molecules such as VEGFs, FGFs, angiopoietins, PDGF, angiogenin, angiotropin, HGF, CXC chemokines with ELR motif, PECAM-1, integrins and VE-cadherin as well as angiostatic key players such as angiostatin, endostatin, thrombospondin, CXC chemokines without ELR motif, **PEDF** are discussed in this review with respect to their molecular impact on angiogenesis.

J Thorac Cardiovasc Surg. 2003 Jul;126(1):28-38.

In vivo gene transfer of pigment epithelium-derived factor inhibits tumor growth in syngeneic murine models of thoracic malignancies.

Mahtabifard A, Merritt RE, Yamada RE, Crystal RG, Korst RJ.

OBJECTIVE: Pigment epithelium-derived factor is known to be an inhibitor of angiogenesis. We hypothesized that in vivo gene transfer of pigment epithelium-derived factor may inhibit tumor angiogenesis and growth in syngeneic models of thoracic malignancies. **METHODS:** An adenovirus vector encoding the human pigment epithelium-derived factor cDNA (AdPEDF) was used to transduce human lung cancer cells in vitro. Transgene expression was assessed using Western analysis. Three different murine flank tumors (2 lung, 1 colon) were then established in syngeneic mice and treated intratumorally with phosphate-buffered saline, AdPEDF, or an empty vector (AdNull). Endpoints measured included transgene expression, tumor size, and animal survival, as well as microvessel density within the tumor. Additionally, a murine pulmonary metastasis model was established by intravenous injection of a syngeneic colon adenocarcinoma cell line expressing a marker gene (beta-galactosidase). One day later, treatment (phosphate-buffered saline, AdNull, or AdPEDF) was administered intrapleurally. Tumor burden (gross and histologic inspection, lung weight, and beta-galactosidase expression) was then evaluated 13 days after vector dosing, and survival was recorded. **RESULTS:** AdPEDF-derived expression of pigment epithelium-derived factor was demonstrated in vitro and in vivo. In syngeneic murine lung cancer flank tumors, intratumoral administration of AdPEDF significantly inhibited tumor growth ($P < .01$), prolonged mouse survival ($P < .01$), and decreased microvessel density ($P < .01$) compared with control groups. In the pulmonary metastasis model, AdPEDF-treated mice exhibited significantly reduced lung lesions, lung weight ($P < .0005$), beta-galactosidase expression ($P < .05$), and animal survival was prolonged ($P < .05$). **CONCLUSION:** Gene transfer of pigment epithelium-derived factor suppresses tumor vascularization and growth, while prolonging survival in syngeneic murine models of thoracic malignancies.

Pathobiology. 2002-2003;70(6):361-7.

Resistance of epiphyseal cartilage to invasion by osteosarcoma is likely to be due to expression of antiangiogenic factors.

Quan GM, Ojaimi J, Nadesapillai AP, Zhou H, Choong PF.

OBJECTIVES: Epiphyseal cartilage is a barrier to osteosarcoma invasion, however the mechanisms behind this resistance remain unclear. The aim of this study was to examine the chronological and spatial patterns of osteosarcoma growth and invasion of local tissue structures including epiphyseal cartilage. **METHODS:** We used an in vivomouse model of osteosarcoma to histologically examine tumors at different stages of disease progression. We compared the pattern of osteosarcoma penetration of epiphyseal cartilage with the expression pattern of two potent mediators of angiogenesis; proangiogenic vascular endothelial growth factor (VEGF) and antiangiogenic pigment epithelium-derived factor (PEDF). **RESULTS:** Epiphyseal cartilage remained intact across its entire length in all sections examined, despite increasing tumor size as well as intra- and extraosseous destruction. In the most advanced cases, only the proangiogenic lowermost layers of the hypertrophic zone of the growth plate were eroded. This corresponded with the growth plate layers which highly expressed the angiogenic factor VEGF. In contrast, the resting, proliferative and upper hypertrophic layers were resistant to osteosarcoma invasion in all cases. This corresponded to the layers with the highest expression of the potent antiangiogenic factor PEDF. **CONCLUSION:** Epiphyseal cartilage is resistant to local invasion by osteosarcoma. The balance of angiogenesis, influenced by pro- and antiangiogenic factors, is likely to play an important role in this resistance.

Cancer Lett. 2003 Jun 30;196(1):77-85.

Regulation of the expression of pigment epithelium-derived factor, an anti-angiogenic factor in human oral squamous cell carcinoma cell lines.

Miyagishi D, Ohno-Matsui K, Amagasa T, Morita I.

Angiogenesis plays an important role in tumor growth and metastasis and is regulated by a balance between angiogenic stimulators and inhibitors. We investigated the gene expression profile of vascular endothelial growth factor (VEGF) and pigment epithelium-derived factor (PEDF), a potent endogenous anti-angiogenic factor, in human oral squamous cell carcinoma (SCC) cell lines. The treatment of SCC cells with hypoxia increased the expression of PEDF as well as VEGF. Moreover, the treatment of SCC cells with VEGF enhanced the expression of PEDF mRNA and secretion of PEDF. In LMF-4, a SCC clone producing abundant VEGF and PEDF, the addition of neutralizing VEGF antibody substantially blocked PEDF expression. These data suggest that human oral squamous cell carcinoma cells produce VEGF, which in turn regulates PEDF production, and this balance may be contributing to neovascularization in tumors.

Mol Ther. 2003 Jul;8(1):72-9.

Suppression of angiogenesis and tumor growth by adenoviral-mediated gene transfer of pigment epithelium-derived factor.

Wang L, Schmitz V, Perez-Mediavilla A, Izal I, Prieto J, Qian C.

Pigment epithelium-derived factor (**PEDF**) was identified from retinal pigment epithelial cells and has been shown to display neurotrophic effects. In addition it has been found to induce a potent inhibition of angiogenesis. In this study we have explored whether overexpression of PEDF by a gene transfer approach can block tumor angiogenesis and reduce tumor growth. We found that cells infected with an adenovirus encoding PEDF under the control of the CMV promoter (AdPEDF) secreted PEDF protein into the medium that exhibited strong inhibitory effects on migration and tube formation of endothelial cells cultured in the presence of vascular endothelial growth factor. Moreover, the systemic administration of AdPEDF was able to inhibit angiogenesis in Matrigel assay *in vivo*, and treatment with this adenovirus of established hepatocellular carcinoma tumor in nude mice resulted in strong suppression of tumor growth. This anti-tumor effect could also be seen in a mouse lung carcinoma model by systemic administration of vector. In that model, treatment of tumor by intratumoral injection of AdPEDF also caused significant inhibition of tumor growth. The anti-tumor effect was related to a decrease in density of microvessels in tumors after treatment with AdPEDF. These data suggest that the antiangiogenic properties of PEDF can be exploited to inhibit the establishment of tumor neovasculature and reduce tumor growth.

Int J Clin Pharmacol Res. 2002;22(3-4):67-71.

Pigment epithelium-derived factor Met72Thr polymorphism in patients with diabetic microangiopathy.

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Pigment epithelium-derived factor (**PEDF**) has recently been shown to be the most potent inhibitor of angiogenesis in the mammalian eye. We, along with others, have very recently found that loss of PEDF is involved in the development and progression of diabetic retinopathy. However, there are no studies on the allelic effects of PEDF gene polymorphism in diabetic retinopathy. In this study, we investigated whether a functional amino acid change, a methionine to threonine polymorphism (Met72Thr polymorphism) of the PEDF gene, is associated with microangiopathy in 143 patients with diabetes. We found that there were no significant associations between PEDF Met72Thr gene polymorphism and diabetic microangiopathy. These observations suggest that these genetic variants might not be involved in the mechanism of diabetic microangiopathy in patients with diabetes.

Trends Mol Med. 2003 Jun;9(6):244-50.

Therapeutic prospects for PEDF: more than a promising angiogenesis inhibitor.

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Blood vessel growth and stability are under the exquisite control of a network of pro- and anti-angiogenic factors. Disruption of the balance between these factors is a characteristic of tumor growth and many vascular diseases. Endogenous angiogenesis inhibitors, particularly those that act broadly at the earliest stages, could be excellent pharmacological tools in combating pathogenic vessel growth. Pigment-epithelium-derived factor (**PEDF**) is a natural angiogenesis inhibitor that (1) targets only new vessel growth, (2) can be administered therapeutically as a soluble protein or by viral-mediated gene transfer, (3) is stable and nontoxic when injected, and (4) is more potent than other well-characterized angiogenesis inhibitors. Because PEDF also has differentiating and neuroprotective activities, it has additional benefits for use in the nervous system. The production of PEDF by many tissues suggests its therapeutic potential should be explored in a much wider range of diseases, including tumor proliferation and metastasis.

J Periodontal Res. 2003 Aug;38(4):405-10.

Mechanical stress induces production of angiogenic regulators in cultured human gingival and periodontal ligament fibroblasts.

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BACKGROUND: As periodontal tissues are constantly exposed to mechanical stress during mastication, the relationship between mechanical stimulation and biochemical phenomena has been extensively investigated. **OBJECTIVES:** The aim of the present study was to assess the change in the production of angiogenic regulators produced by human gingival fibroblasts (HGF) and periodontal ligament fibroblasts (HPLF), cultured on a flexible substrate, before and after application of cyclic tensile stretching. **MATERIALS AND METHODS:** Both cell types were stretched in a Flexercell Strain Unit to 7, 14 and 21% elongation, at a frequency of 12 cycles/min. Medium cultured with HGF or HPLF was examined by enzyme-linked immunosorbent assay (ELISA) for vascular endothelial growth factor (VEGF), Western blotting of pigment epithelium-derived factor (**PEDF**) and in vitro angiogenesis assay. The residual cells were analyzed by reverse transcription-polymerase chain reaction (RT-PCR) for both VEGF and PEDF mRNA expression. **RESULTS:** Stretching increased the VEGF mRNA level and VEGF secretion in both HGF and HPLF. The concentration of VEGF in the conditioned medium of the stretched HPLF was almost the same as that of stretched HGF. In the in vitro angiogenesis assay, the conditioned medium of HPLF after stretching showed a dramatic increase in tube formation. In contrast, stretched HGF did not show enhanced tube formation, despite the increase in VEGF secretion by stretched HGF. The mRNA levels of PEDF, an inhibitor of angiogenesis, were higher in HGF than HPLF. The protein level of PEDF in HGF was also higher than that in HPLF. **CONCLUSION:** These findings suggest that under mechanical stress HPLF promotes angiogenesis via expression of VEGF, whereas under the same conditions angiogenesis is not promoted in HGF, due to the expression of PEDF.

Int J Tissue React. 2002;24(4):137-42.

Up-regulation of vascular endothelial growth factor and down-regulation of pigment epithelium-derived factor messenger ribonucleic acid levels in leptin-exposed cultured retinal pericytes.

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Leptin, a circulating hormone secreted mainly from adipose tissues, is involved in the control of body weight. Recently, leptin was found to be an angiogenic factor and its vitreous levels were shown to be elevated in patients with angiogenic eye diseases such as proliferative diabetic retinopathy. However, the role of leptin in diabetic retinopathy is not fully understood. Since pericyte loss and dysfunction have been considered to be one of the characteristic changes of the early phases of diabetic retinopathy, we investigated the effects of leptin on the growth and function of bovine cultured retinal pericytes. Although it did not affect cell growth, leptin significantly up-regulated pericyte messenger ribonucleic acid levels of an endogenous angiogenic stimulator, vascular endothelial growth factor (VEGF). Leptin was also found to significantly inhibit gene expression of pigment epithelium-derived factor (**PEDF**), the most potent angiogenesis inhibitor in the mammalian eye, in pericytes. The present study suggests that leptin might elicit angiogenesis through VEGF induction as well as PEDF suppression in pericytes and could thus be involved in the development and progression of diabetic retinopathy, especially in obese insulin-resistant patients.

Nat Med. 2003 Jun;9(6):774-80. Epub 2003 May 12.

Pigment epithelium-derived factor regulates the vasculature and mass of the prostate and pancreas.

Doll JA, Stellmach VM, Bouck NP, Bergh AR, Lee C, Abramson LP, Cornwell ML, Pins MR, Borensztajn J, Crawford SE.

Angiogenesis sustains tumor growth and metastasis, and recent studies indicate that the vascular endothelium regulates tissue mass. In the prostate, androgens drive angiogenic inducers to stimulate growth, whereas androgen withdrawal leads to decreased vascular endothelial growth factor, vascular regression and epithelial cell apoptosis. Here, we identify the angiogenesis inhibitor pigment epithelium-derived factor (**PEDF**) as a key inhibitor of stromal vasculature and epithelial tissue growth in mouse prostate and pancreas. In PEDF-deficient mice, stromal vessels were increased and associated with epithelial cell hyperplasia. Androgens inhibited prostatic PEDF expression in cultured cells. In vivo, androgen ablation increased PEDF in normal rat prostates and in human cancer biopsies. Exogenous PEDF induced tumor epithelial apoptosis in vitro and limited in vivo tumor xenograft growth, triggering endothelial apoptosis. Thus, PEDF regulates normal pancreas and prostate mass. Its androgen sensitivity makes PEDF a likely contributor to the anticancer effects of androgen ablation.

Biochem J. 2003 Aug 15;374(Pt 1):199-206.

Pigment-epithelium-derived factor (PEDF) occurs at a physiologically relevant concentration in human blood: purification and characterization.

Petersen SV, Valnickova Z, Enghild JJ.

Pigment epithelium-derived factor (**PEDF**) inhibits the formation of blood vessels in the eye by inducing apoptosis in actively dividing endothelial cells. The activity of PEDF equals or supersedes that of other anti-angiogenic factors, including angiostatin, endostatin and thrombospondin-1. In addition, PEDF has the potential to promote the survival of neurons and affect their differentiation. Here we show that PEDF is present in plasma at a concentration of approx. 100 nM (5 microg/ml) or twice the level required to inhibit aberrant blood-vessel growth in the eye. Thus the systemic delivery of PEDF has the potential to affect angiogenesis or neurotrophic processes throughout the body, significantly expanding the putative physiological role of the protein. A complete map of all post-translational modifications revealed that authentic plasma PEDF carries an N-terminal pyroglutamate blocking group and an N-linked glycan at position Asn266. The pyroglutamate residue may regulate the activity of PEDF analogously to the manner in which it regulates thyrotropin-releasing hormone.

Microvasc Res. 2003 May;65(3):186-90.

Pigment epithelium-derived factor inhibits leptin-induced angiogenesis by suppressing vascular endothelial growth factor gene expression through anti-oxidative properties.

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Leptin, a circulating hormone secreted mainly from adipose tissues, is involved in the control of body weight. Recently, leptin was found to be an angiogenic factor, and its vitreous levels are associated with angiogenic eye diseases such as proliferative diabetic retinopathy. However, the molecular mechanism for leptin-elicited angiogenesis remains to be elucidated. Pigment epithelium-derived factor (**PEDF**) has been shown to be the most potent natural inhibitor of angiogenesis in the mammalian eye, and its levels in the vitreous were decreased in angiogenic eye diseases. In this study, we investigated whether and how PEDF could inhibit the leptin-induced DNA synthesis in microvascular endothelial cells (EC), a key step of angiogenesis. Leptin significantly increased intracellular reactive oxygen species (ROS) generation in microvascular EC. PEDF was found to inhibit the leptin-induced ROS generation in EC. An antioxidant, N-acetylcysteine, or PEDF completely prevented the leptin-induced upregulation of vascular endothelial growth factor (VEGF) mRNA levels as well as any increase in DNA synthesis in microvascular EC. Polyclonal antibodies against human VEGF were also found to completely inhibit DNA synthesis in leptin-exposed EC. The present study suggests that leptin could elicit angiogenesis through autocrine VEGF production via intracellular ROS generation. PEDF may block the angiogenic effects of leptin through its anti-oxidative properties.

Diabetologia. 2003 Mar;46(3):394-400. Epub 2003 Mar 01.

Low content of the natural ocular anti-angiogenic agent pigment epithelium-derived factor (PEDF) in aqueous humor predicts progression of diabetic retinopathy.

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AIMS/HYPOTHESIS: Retinopathy is the most common microvascular complication of diabetes. Our aim was to address the predictive value of pro-angiogenic and anti-angiogenic markers for progression of retinopathy. METHODS: Aqueous humor was collected at cataract surgery from 32 diabetic patients who had no or very mild retinopathy (ETDRS stage \leq 20) and 33 normoglycaemic control subjects. Content of pro-angiogenic vascular endothelial growth factor and angiogenic inhibitor pigment epithelium-derived factor were determined. ANGIOGENIC activity was quantified by measuring its effect on the migration of capillary endothelial cells. The predictive value of the initial level of these markers for progression of retinopathy was studied by following the probands for a maximum of 75 months. RESULTS: In the aqueous fluid content of vascular endothelial growth factor was increased in diabetic patients (mean values 492 versus 292 pg/ml; $p=0.0052$), and **pigment epithelium-derived factor** values were decreased (mean values 1740 versus 3680 ng/ml; $p=0.0058$) compared to control subjects. Of the diabetic patients ten progressed during follow-up (ETDRS stage $>$ 47B). This subgroup showed lower pigment epithelium-derived factor content when compared to non-progressors and control subjects. Migratory activity in samples of patients from the control group and in diabetic patients without progression was generally inhibitory due to pigment epithelium-derived factor. Inhibition was blocked by neutralizing antibodies to pigment epithelium-derived factor. In diabetic patients initial angiogenic activity was

higher in those who later developed retinopathy (vs. controls $p=0.00005$; vs. no progressors $p=0.0003$). Both pigment epithelium-derived factor and migratory response predicted progression. **CONCLUSION/INTERPRETATION:** Pigment epithelium-derived factor is an important negative regulator of angiogenic activity of aqueous humor. Its content in the aqueous humor of diabetic patients strongly predicts who among them will develop progression of retinopathy.

Biochem Biophys Res Commun. 2003 Apr 11;303(3):962-7.

Vascular endothelial growth factor upregulates pigment epithelium-derived factor expression via VEGFR-1 in human retinal pigment epithelial cells.

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We previously demonstrated that differentiated retinal pigment epithelial (RPE) cells express high levels of vascular endothelial growth factor (VEGF) and pigment epithelium-derived factor (PEDF), and a critical balance between VEGF and PEDF is important to prevent the development of choroidal neovascularization. We report here that VEGF secreted by RPE cells upregulates PEDF expression via VEGFR-1 in an autocrine manner. PEDF mRNA and protein expression was downregulated by neutralizing antibody against VEGF in differentiated human RPE cells. VEGFR-1 neutralization decreased PEDF mRNA and protein expression whereas anti-VEGFR-2 antibody had no effect. Addition of placenta growth factor (PIGF) restored PEDF expression in the presence of anti-VEGF antibody. These results demonstrate a regulatory interaction between angiogenesis stimulators and inhibitors to maintain homeostasis in normal human retina.

J Clin Pathol. 2003 Apr;56(4):277-82.

Loss of pigment epithelium derived factor expression in glioma progression.

Guan M, Yam HF, Su B, Chan KP, Pang CP, Liu WW, Zhang WZ, Lu Y.

BACKGROUND: Pigment epithelium derived factor (PEDF) was first isolated from medium conditioned by human fetal retinal pigment epithelial cells. PEDF was detected in a broad range of human fetal and adult tissues including almost all brain areas. It can also inhibit the proliferation of cultured rat astrocytes. Recent studies have implicated PEDF in activities that are inhibitory to angiogenesis. **AIMS:** To investigate the expression of PEDF in gliomas to assess its "gliastatic" effects and its role in anti-angiogenesis. **METHODS:** PEDF mRNA values were measured by quantitative real time reverse transcription polymerase chain reaction (RT-PCR) analysis of normal brain tissue and tumour specimens from both low and high grade gliomas. In addition, immunohistochemical staining for PEDF and vascular endothelial growth factor (VEGF) was performed on 32 paraffin wax embedded glioma samples, 10 of them grade IV, 10 grade III, seven grade II, and five grade I. **RESULTS:** RT-PCR showed that PEDF mRNA values were 5.0 ($p < 0.001$) and 15.4 ($p < 0.001$) times higher in normal human brain specimens ($n = 5$) than in tumour tissue specimens of low grade glioma (grades I and II; $n = 15$) and high grade glioma (grades III and IV; $n = 10$), respectively. VEGF was strongly positive in 90% of grade IV, 70% of grade III, 43% of grade II, and 20% of grade I cases. In contrast, PEDF was positive in none of grade IV, 20% of grade III, 43% of grade II, and 60% of grade I tumours. There was an inverse correlation between VEGF and PEDF expression, and a lack of PEDF in advanced grade gliomas. **CONCLUSIONS:** It is possible that the absence of PEDF expression is a potent factor for the enhancement of angiogenesis in glioma.

J Pediatr Surg. 2003 Mar;38(3):336-42; discussion 336-42.

Wilms' tumor growth is suppressed by antiangiogenic pigment epithelium-derived factor in a xenograft model.

Abramson LP, Stellmach V, Doll JA, Cornwell M, Arensman RM, Crawford SE.

BACKGROUND/PURPOSE: Pigment epithelium-derived factor (PEDF), a potent endogenous inhibitor of angiogenesis, is highly expressed in the kidney. The authors postulated that systemic administration of PEDF would decrease Wilms' tumor growth in a xenograft model, and increased renal vascularity would result in a mouse null for PEDF. **METHODS:** Tumors were induced in athymic mice using human anaplastic Wilms' tumor cells. Purified PEDF protein or vehicle was administered for 7 days beginning 2 to 3 weeks after inoculation. Tumors were stained with anti-PEDF and anti-Factor VIII antibodies. Mitoses and microvascular density (MVD) were counted per high-power field

(hpf). PEDF-null mice were generated on a SV129/C57Bl6 background. Wild-type and null kidneys were assessed for MVD. RESULTS: Mean tumor weight in the 2-week group was 60% less than controls ($P < .05$). The MVD and mitotic count in treated tumors were significantly less than controls ($P < .05$). PEDF stained strongly in normal kidneys but was minimal to absent in Wilms' tumor. PEDF-null kidneys had increased MVD compared with wild-type ($P < .05$). CONCLUSIONS: PEDF is expressed strongly in normal murine kidney, and loss of its angiostatic activity may contribute to pathologic angiogenesis in Wilms' tumor. Systemic PEDF suppresses WT growth by targeting both the tumor cells and its associated vasculature.

Hepatology. 2003 Mar;37(3):696-704.
Comment in: Hepatology. 2003 Mar;37(3):505-6.

Antiangiogenic gene therapy for hepatocellular carcinoma using angiostatin gene.

Ishikawa H, Nakao K, Matsumoto K, Ichikawa T, Hamasaki K, Nakata K, Eguchi K.

Recent studies have reported that antiangiogenic gene delivery into cancer cells inhibits growth of certain tumors in vivo. Hepatocellular carcinoma (HCC) is a hypervascular cancer, and antiangiogenic gene therapy might be suitable for HCC. In the present study, we investigated the antiangiogenic effects of angiostatin gene transduction into HCC both in vitro and in vivo. Angiostatin gene was cloned into a pSecTag2B mammalian expression vector to construct pSecTag2B-ANG. pSecTag2B or pSecTag2B-ANG were transfected into an HCC cell line, PLC/PRF/5, and then stable transfectants were obtained by Zeocin selection. pSecTag2B or pSecTag2B-ANG transfection did not alter the expression of vascular endothelial growth factor (VEGF), a potent angiogenic stimulator, or pigment epithelium-derived factor (PEDF), an angiogenic inhibitor, in PLC/PRF/5 cells. However, conditioned media (CM) derived from pSecTag2B-ANG-transfected PLC/PRF/5 cells (CM-ANG) suppressed the proliferation and migration of human umbilical vein endothelial cells (HUVEC) by 35% and 50%, respectively, relative to their effects on nontransfected cells. In in vivo experiments, pSecTag2B-ANG stable transfected (CM-Mock) and nontransfected cells (CM-N) were mixed at various proportions and the mixed cells were subcutaneously implanted into athymic mice. Suppression of tumor growth was noted in mice implanted with angiostatin gene-transfected cells, and such suppression was proportional with the percentage of transfected cells. Analysis of the vascular density in these tumors showed that the tumor growth suppression effect of angiostatin gene correlated with suppression of tumor vascularity. In conclusion, antiangiogenic gene therapy using angiostatin gene is potentially suitable for the treatment of patients with HCC.

Biol Chem. 2002 Nov;383(11):1677-82.

Pigment epithelium-derived factor (PEDF), a serpin with potent anti-angiogenic and neurite outgrowth-promoting properties.

Gettins PG, Simonovic M, Volz K.

Pigment epithelium-derived factor is a member of the serpin superfamily of proteins, but one that lacks inhibitory properties against either serine or cysteine proteinases. Nevertheless it possesses a number of physiological properties that make it a potentially important protein in regulation of angiogenesis, in neuronal cell survival and in protection of neurons from neurotoxic agents. It is also a protein that is highly up-regulated in the G0 phase of early-passage cells compared with rapidly proliferating cells or senescent cells, and so is also linked to both the cell cycle and cell senescence. The determination of a high resolution X-ray crystal structure of native PEDF provides insight into regions of the protein that may be involved in one or more of these functions.

Mol Ther. 2002 Oct;6(4):490-4.

Inhibition of retinal neovascularization by intraocular viral-mediated delivery of anti-angiogenic agents.

Auricchio A, Behling KC, Maguire AM, O'Connor EM, Bennett J, Wilson JM, Tolentino MJ.

Neovascularization characterizes diabetic retinopathy and choroidal neovascularization associated with age-related macular degeneration, the most common causes of severe visual loss in the developed world. Gene transfer to the eye using adeno-associated viral (AAV) vectors is a promising new treatment for inherited and acquired ocular diseases. We used an AAV vector with rapid onset and high levels of gene expression in the retina to deliver three anti-angiogenic factors (pigment epithelium-derived factor, tissue inhibitor of metalloproteinase-3, and endostatin) to the eyes of mice in a mouse model of retinopathy of prematurity. All three vectors inhibited ischemia-induced neovascularization.

Am J Ophthalmol. 2002 Sep;134(3):348-53.

Unbalanced vitreous levels of pigment epithelium-derived factor and vascular endothelial growth factor in diabetic retinopathy.

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PURPOSE: To determine the levels of pigment epithelium-derived factor (**PEDF**) and vascular endothelial growth factor (VEGF) in the vitreous of patients with diabetic retinopathy (DR). **DESIGN:** Experimental study of PEDF and VEGF levels in vitreous samples collected during vitrectomy. **METHODS:** The levels of PEDF and VEGF were measured by enzyme-linked immunosorbent assay in the vitreous of 46 eyes of 43 patients who underwent vitrectomy with diabetic retinopathy (DR) (32 eyes of 29 patients) and an idiopathic macular hole (MH) (14 eyes of 14 patients). **RESULTS:** The vitreal concentration of PEDF was significantly lower at 1.11 +/- 0.14 microg/ml (mean +/- standard error) in eyes with DR than in eyes with MH at 1.71 +/- 0.22 microg/ml (P =.021). The VEGF level was 1799 +/- 478 pg/ml in eyes with DR and not detectable in MH. The PEDF level in proliferative DR (PDR) (0.94 +/- 0.12 microg/ml) was lower than that in nonproliferative DR (NPDR) (2.25 +/- 0.32 microg/ml), and that in active DR (0.85 +/- 0.14 microg/ml) was significantly lower than that in inactive DR (1.59 +/- 0.24 microg/ml; P =.01). The VEGF level was 2025 +/- 533 pg/ml in PDR and 215 +/- 201 pg/ml in NPDR and that in active DR (2543 +/- 673 pg/ml) was significantly higher than that in inactive DR (395 +/- 188 pg/ml; P =.0098). **CONCLUSIONS:** These results suggest that lower levels of PEDF and higher levels of VEGF may be related to the angiogenesis in DR that leads to active PDR.

Biochem Biophys Res Commun. 2002 Aug 30;296(4):877-82.

Pigment epithelium-derived factor protects cultured retinal pericytes from advanced glycation end product-induced injury through its antioxidative properties.

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Pigment epithelium-derived factor (**PEDF**) has recently been shown to be the most potent inhibitor of angiogenesis in the mammalian eye, suggesting that loss of PEDF is involved in the pathogenesis of proliferative diabetic retinopathy. However, a protective role for PEDF in pericyte loss in early diabetic retinopathy remains to be elucidated. In this study, we investigated whether PEDF proteins could protect against advanced glycation end product (AGE)-induced injury in retinal pericytes. Ligand blot analysis revealed that pericytes possessed a membrane protein with binding affinity for PEDF. PEDF proteins were found to significantly inhibit AGE-induced reactive oxygen species (ROS) generation and the subsequent decrease in DNA synthesis and apoptotic cell death in pericytes. Further, PEDF proteins completely restored the down-regulation of bcl-2 gene expression in AGE-exposed pericytes. The results demonstrated that PEDF proteins protected cultured pericytes from AGE-induced cytotoxicity through its anti-oxidative properties. Our present study suggests that substitution of PEDF proteins may be a promising strategy in treatment of patients with early diabetic retinopathy.

Am J Ophthalmol. 2002 Aug;134(2):220-7.

Pigment epithelium-derived factor is deficient in the vitreous of patients with choroidal neovascularization due to age-related macular degeneration.

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PURPOSE: Pigment epithelium-derived growth factor (**PEDF**) is a potent inhibitor of angiogenesis that is found in the normal eye. The purpose of this study is to report decreased levels of PEDF in the vitreous of eyes with choroidal neovascularization (CNV) due to age-related macular degeneration (AMD). **DESIGN:** Prospective case-control study. **METHODS:** In a prospective case-control study, undiluted vitreous was collected from nine eyes of nine patients with CNV due to AMD and from an age-matched control group of 12 eyes of 12 patients with retinal disorders not involving neovascularization. Vitreous PEDF and vascular endothelial growth factor (VEGF) concentrations were determined by Western blot analyses and enzyme-linked immunosorbent assay (ELISA), respectively. Angiogenic activities of the

vitreous samples were assessed in vitro using an endothelial cell chemotaxis assay. **RESULTS:** In vitreous samples from nine eyes with CNV due to AMD the mean +/- SD PEDF level was 2.8 ng/microl +/- 1.3 ng/microl. In vitreous samples from 12 age-matched control eyes the mean +/- SD PEDF level was 16.4 ng/microl +/- 7.1 ng/microl. The difference between the two groups was statistically significant ($P = .00003$). No significant difference in vitreous VEGF concentration was seen between NV/AMD samples and control samples ($P = .23$). All CNV/AMD vitreous samples induced endothelial cell migration in vitro. No sample from age-matched non-age-related macular degeneration controls could induce endothelial cell migration, and 11 of 12 were able to block VEGF-induced migration in vitro. This inhibitory activity required active PEDF. **CONCLUSION:** The vitreous of patients with CNV due to AMD contained lower levels of PEDF and lacked the antiangiogenic activity of vitreous from age-matched controls. This suggests that loss of PEDF creates a permissive environment for CNV patients with AMD.

Trends Mol Med. 2002 Jul;8(7):330-4.

PEDF: anti-angiogenic guardian of ocular function.

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Sight-threatening eye diseases can be caused and exacerbated by the aberrant growth of new blood vessels. Recent work indicates that this neovascularization not only is a response to a rise in the local concentration of molecules that induce such angiogenesis but also requires a fall in the levels of endogenous molecules that inhibit angiogenesis. One of the most potent of these endogenous inhibitors is pigment epithelium-derived factor (**PEDF**), which serves as a survival factor for neuronal components of the eye as well as an essential inhibitor of the growth of ocular blood vessels. Its anti-angiogenic activity is selective in that it is effective against newly forming vessels but spares existing ones, and it is reversible. The molecular basis for this delicate control of endothelial cells is beginning to be understood and strategies to test the ability of PEDF to ameliorate or prevent vessel damage in the eye are developing rapidly.

Invest Ophthalmol Vis Sci. 2002 May;43(5):1574-80.

Expression of pigment epithelium-derived factor in experimental choroidal neovascularization.

Renno RZ, Youssri AI, Michaud N, Gragoudas ES, Miller JW.

PURPOSE: To investigate the expression of pigment epithelium-derived factor (**PEDF**) in the rat laser-injury model of choroidal neovascularization (CNV). **METHODS:** Retinas were immunostained for PEDF at different times (1, 2, and 3 weeks) after laser injury. Levels of PEDF protein in the vitreous at 1, 3, 7, 14, and 28 days after laser injury were also assayed by Western blot. **RESULTS:** Protein levels of PEDF in the vitreous were increased during the first 7 days after CNV induction. Immunostaining for PEDF was observed throughout normal nonlasered control retinas, sham-lasered retinas, and areas remote to laser lesions, which were generally more intense in the outer nuclear layer (ONL) and less intense in the internal nuclear layer (INL). Decreased expression of PEDF was observed in flanking areas adjacent to the injury site and was confined mainly to the ONL. In the injury sites, immunostaining within the ONL was either absent or decreased for up to 3 weeks after laser injury (the duration of the study). Preabsorption of the anti-PEDF antibody with the immunizing peptide blocked specific labeling in the retina. **CONCLUSIONS:** These results demonstrate an inverse correlation of expression of PEDF and formation of CNV in the experimental model and suggest that decreased expression of PEDF plays a permissive role in the formation of CNV. PEDF analogues may be a reasonable treatment strategy for CNV.

Nat Med. 2002 Apr;8(4):349-57.

Inducer-stimulated Fas targets activated endothelium for destruction by anti-angiogenic thrombospondin-1 and pigment epithelium-derived factor.

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Natural inhibitors of angiogenesis are able to block pathological neovascularization without harming the preexisting vasculature. Here we show that two such inhibitors, thrombospondin-1 and **pigment epithelium-derived factor**, derive specificity for remodeling vessels from their dependence on Fas/Fas ligand (FasL)-mediated apoptosis to block angiogenesis. Both inhibitors upregulated FasL on endothelial cells. Expression of the essential partner of FasL,

Fas/CD95 receptor, was low on quiescent endothelial cells and vessels but greatly enhanced by inducers of angiogenesis, thereby specifically sensitizing the stimulated cells to apoptosis by inhibitor-generated FasL. The anti-angiogenic activity of thrombospondin-1 and pigment epithelium-derived factor both in vitro and in vivo was dependent on this dual induction of Fas and FasL and the resulting apoptosis. This example of cooperation between pro- and anti-angiogenic factors in the inhibition of angiogenesis provides one explanation for the ability of inhibitors to select remodeling capillaries for destruction.

Invest Ophthalmol Vis Sci. 2002 Apr;43(4):1168-75.

Expression of pigment epithelium-derived factor in normal adult rat eye and experimental choroidal neovascularization.

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PURPOSE: Pigment epithelium-derived factor (**PEDF**) is a protein produced by the retinal pigment epithelial (RPE) cells. Recent studies have implicated PEDF in activities that are inhibitory to angiogenesis. In this study, the expression of PEDF was investigated in normal rat eyes and in eyes with experimentally induced choroidal neovascularization and compared with the expression of vascular endothelial growth factor (VEGF). **METHODS:** Choroidal neovascularization was induced by laser photocoagulation in rat eyes. At intervals of up to 2 weeks after photocoagulation, the eyes were removed and prepared for in situ hybridization and immunohistochemical study. In situ hybridization was performed with digoxigenin-labeled PEDF riboprobes. Protein expression of PEDF and VEGF was studied immunohistochemically. **RESULTS:** In normal adult rat eyes, PEDF mRNA was observed mainly in the corneal epithelial and endothelial cells, lens epithelial cells, ciliary epithelial cells, retinal ganglion cells, and the RPE cells. During the development of choroidal neovascularization, PEDF mRNA, PEDF protein, and VEGF protein were strongly detected in many cells within the laser lesions at 3 days after photocoagulation, after which levels gradually declined. However, PEDF was still expressed in the RPE cells that proliferated and covered the neovascular tissues at 2 weeks, whereas VEGF protein was weakly expressed in endothelial cells in choroidal neovascularization. **CONCLUSIONS:** PEDF is expressed in different cell types of normal rat eyes. The expression of PEDF was detected in the choroidal neovascular tissues induced by photocoagulation, and these findings suggest that PEDF may modulate the process of choroidal neovascularization.

Diabetes. 2002 Apr;51(4):1218-25.

Difference in ischemic regulation of vascular endothelial growth factor and pigment epithelium-derived factor in brown norway and sprague dawley rats contributing to different susceptibilities to retinal neovascularization.

Gao G, Li Y, Fant J, Crosson CE, Becerra SP, Ma JX.

The present study compared susceptibilities of Sprague Dawley (SD) and Brown Norway (BN) rats with ischemia-induced retinal neovascularization. An exposure to constant hyperoxia followed by normoxia induced significant retinal neovascularization in BN rats but not in SD rats, as demonstrated by fluorescein retinal angiography, measurement of avascular area, and count of preretinal vascular cells. These results indicate a rat strain difference in susceptibility to retinal neovascularization. To understand the molecular basis responsible for the strain difference, we have measured the levels of pigment epithelium-derived factor (**PEDF**), an angiogenic inhibitor, and vascular endothelial growth factor (VEGF), a major angiogenic stimulator in the retina. The hyperoxia-treated BN rats showed a significant reduction in retinal PEDF, but they showed a substantial increase of VEGF at both the protein and RNA levels, resulting in an increased VEGF-to-PEDF ratio. Hyperoxia-treated SD rats showed changes in PEDF and VEGF levels that were less in magnitude and of shorter duration than in BN rats. In age-matched normal BN and SD rats, however, there was no detectable difference in the basal VEGF-to-PEDF ratio between the strains. These observations support the idea that different regulation of angiogenic inhibitors and stimulators under ischemia are responsible for the differences in susceptibility to ischemia-induced retinal neovascularization in SD and BN rats.

J Cell Sci. 2001 Dec;114(Pt 24):4421-8.

Pigment epithelium-derived factor (PEDF) in neuroblastoma: a multifunctional mediator of Schwann cell antitumor activity.

Crawford SE, Stellmach V, Ranalli M, Huang X, Huang L, Volpert O, De Vries GH, Abramson LP, Bouck N.

Neuroblastoma is notable for its cellular heterogeneity and unpredictable outcome. Tumors are a variable mixture of primitive malignant neuroblasts, more differentiated ganglionic cells, Schwann and endothelial cells. Although often fatal, neuroblastomas can spontaneously regress, possibly due to favourable autocrine and paracrine interactions among these cells. Here, pigment epithelium-derived factor (**PEDF**), a potent inhibitor of angiogenesis and inducer of neural differentiation, is shown to be produced by ganglionic cells and Schwann cells, but not by more primitive tumor cells. Although undifferentiated neuroblastoma tumor cell secretions were angiogenic primarily due to vascular endothelial growth factor, secretions of Schwann cells were anti-angiogenic due to PEDF. In addition, PEDF was the major factor responsible for Schwann cell's ability to induce tumor cell differentiation in vitro and recombinant PEDF had the same effect in vitro and in vivo. Both the growth and the survival of Schwann cells were enhanced by PEDF. Thus PEDF may serve as a multifunctional antitumor agent in neuroblastomas, inhibiting angiogenesis while promoting the numbers of Schwann cells and differentiated tumor cells that in turn produce PEDF, suggesting that its clinical administration could stimulate a multifaceted antitumor feedback loop with the potential to limit and possibly regress tumor growth.

J Biol Chem. 2002 Mar 15;277(11):9492-7. Epub 2002 Jan 08.

Down-regulation of vascular endothelial growth factor and up-regulation of pigment epithelium-derived factor: a possible mechanism for the anti-angiogenic activity of plasminogen kringle 5.

Gao G, Li Y, Gee S, Dudley A, Fant J, Crosson C, Ma JX.

We have previously shown that intravitreal injection of plasminogen kringle 5 (K5), a potent angiogenic inhibitor, inhibits ischemia-induced retinal neovascularization in a rat model. Here we report that K5 down-regulates an endogenous angiogenic stimulator, vascular endothelial growth factor (VEGF) and up-regulates an angiogenic inhibitor, pigment epithelium-derived factor (**PEDF**) in a dose-dependent manner in vascular cells and in the retina. The regulation of VEGF and PEDF by K5 in the retina correlates with its anti-angiogenic effect in a rat model of ischemia-induced retinopathy. Retinal RNA levels of VEGF and PEDF are also changed by K5. K5 inhibits the p42/p44 MAP kinase activation and nuclear translocation of hypoxia-inducible factor-1alpha, which may be responsible for the down-regulation of VEGF. Down-regulation of endogenous angiogenic stimulators and up-regulation of endogenous angiogenic inhibitors, thus leading toward restoration of the balance in angiogenic control, may represent a mechanism for the anti-angiogenic activity of K5.

J Cell Physiol. 2001 Dec;189(3):323-33.

Novel mechanism for age-related macular degeneration: an equilibrium shift between the angiogenesis factors VEGF and PEDF.

Ohno-Matsui K, Morita I, Tombran-Tink J, Mrazek D, Onodera M, Uetama T, Hayano M, Murota SI, Mochizuki M.
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We investigated gene expression profiles of vascular endothelial growth factor (VEGF) and pigment epithelium-derived factor (**PEDF**) in differentiated and non-differentiated retinal pigment epithelial (RPE) cells during oxidative stress. Human RPE cells were grown in culture on laminin-coated flasks to obtain differentiated features. Cells cultured on plastic were used as non-differentiated controls. After confluence, hydrogen peroxide (H₂O₂) was added for 48 h, then, total RNA was extracted and used for RT-PCR and Northern blot analysis. Medium conditioned by RPE was used for ELISA, Western blotting, and in vitro angiogenesis assay. As a result, differentiated RPE cells expressed significantly higher levels of VEGF protein, as compared to their non-differentiated counterparts. The expression pattern remained consistent even after cellular exposure to H₂O₂. Conversely, while elevated levels of PEDF transcript and protein were seen in differentiated RPE cells, compared to non-differentiated cells, a marked decrease at both PEDF mRNA and protein levels was seen after treatment with H₂O₂. Moreover, this decrease in PEDF expression was dosage dependent. In in vitro angiogenesis assay, conditioned medium from differentiated human RPE cells after exposure to H₂O₂ showed a dramatic increase in tubular formation and migratory activity of microvascular endothelial cells. These data suggest that, in physiological conditions, a critical balance between PEDF and VEGF exists, and PEDF may counteract the angiogenic potential of VEGF. Under oxidative stress, PEDF decreases disrupting this balance. This equilibrium shift may be significant in promoting a pathological condition of RPE cells and contributing to choroidal neovascularization in age-related macular degeneration.

Diabetes. 2001 Dec;50(12):2641-5.

Loss of the antiangiogenic pigment epithelium-derived factor in patients with angiogenic eye disease.

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Retinal neovascularization characterizes proliferative diabetic retinopathy (PDR). Pigment epithelium-derived factor (PEDF) has been shown to be a major antiangiogenic growth factor in the mammalian eye. PEDF expression is suppressed by hypoxia, and changes in PEDF have been correlated to the development of retinal neovascularization in animal models of hypoxic eye disease. However, whether this concept of a reduced angiogenesis inhibitor holds true in humans is as yet unclear. In this study, we analyzed the *in vivo* regulation of PEDF in patients with and without hypoxic eye disease. We used immunoblots to measure PEDF in ocular fluids obtained from 64 nondiabetic and diabetic patients. In addition, immunohistochemistry of PEDF was carried out in specimens of normal human retinas and retinas with various degrees of diabetic retinopathy. The PEDF concentrations in patients with PDR ($P < 0.001$) or extensive nondiabetic retinal neovascularization caused by retinal-vein occlusion ($P < 0.001$) were lower than in control patients. Levels of PEDF were replenished in PDR patients with previous retinal scatter photocoagulation compared with PDR patients without previous photocoagulation ($P = 0.01$). Immunohistochemistry revealed an interstitial staining pattern as expected for a secreted protein, with an intense staining in retinas of patients without proliferative eye disease. However, in patients with PDR, little or no staining was detectable. Our data strongly support the concept that retinal angiogenesis is induced by loss of the major angiogenesis inhibitor in the eye, PEDF, in combination with an increased expression of angiogenic growth factors such as vascular endothelial growth factor. Our findings suggest that substitution of angiogenesis inhibitors may be an effective approach in the treatment of PDR.

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Crystal structure of human PEDF, a potent anti-angiogenic and neurite growth-promoting factor.

Simonovic M, Gettins PG, Volz K.

Pigment epithelium-derived factor (PEDF), a noninhibitory member of the serpin superfamily, is the most potent inhibitor of angiogenesis in the mammalian ocular compartment. It also has neurotrophic activity, both in the retina and in the central nervous system, and is highly up-regulated in young versus senescent fibroblasts. To provide a structural basis for understanding its many biological roles, we have solved the crystal structure of glycosylated human PEDF to 2.85 Å. The structure revealed the organization of possible receptor and heparin-binding sites, and showed that, unlike any other previously characterized serpin, PEDF has a striking asymmetric charge distribution that might be of functional importance. These results provide a starting point for future detailed structure/function analyses into possible mechanisms of PEDF action that could lead to development of therapeutics against uncontrolled angiogenesis.

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Pigment epithelium-derived factor in the vitreous is low in diabetic retinopathy and high in rhegmatogenous retinal detachment.

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PURPOSE: To report the levels of pigment epithelium-derived factor in the vitreous of patients with diabetic retinopathy, rhegmatogenous retinal detachment, and idiopathic macular hole. **METHODS:** Using enzyme-linked immunosorbent assay, we measured the levels of pigment epithelium-derived factor in the vitreous of 34 eyes of 33 patients who underwent vitrectomy for the treatment of diabetic retinopathy (17 eyes of 16 patients), rhegmatogenous retinal detachment (10 eyes), and idiopathic macular hole (seven eyes). **RESULTS:** The vitreal concentration of pigment epithelium-derived factor was 1.15 +/- 0.23 microg/ml (mean +/- standard error) in eyes with diabetic retinopathy, 3.28 +/- 0.69 microg/ml in rhegmatogenous retinal detachment, and 1.71 +/- 0.39 microg/ml in idiopathic macular hole. The pigment epithelium-derived factor level in rhegmatogenous retinal detachment was significantly higher than that in diabetic retinopathy ($P = 0.008$) and idiopathic macular hole ($P = 0.034$). For eyes with diabetic retinopathy, the pigment epithelium-derived factor level was 0.88 +/- 0.21 microg/ml in proliferative diabetic retinopathy and 2.43 +/- 0.37 microg/ml in nonproliferative diabetic retinopathy ($P = 0.0083$). Additionally, the pigment epithelium-derived factor level in active diabetic retinopathy (0.70 +/- 0.22 microg/ml) was significantly lower than the

level in inactive diabetic retinopathy (1.79 +/- 0.35 microg/ml; P =.018). CONCLUSIONS: These results suggest that pigment epithelium-derived factor inhibits angiogenesis and that lower levels of pigment epithelium-derived factor may be related to the angiogenesis in diabetic retinopathy and result in active proliferative diabetic retinopathy. The results also suggest that higher levels of pigment epithelium-derived factor in the eyes with rhegmatogenous retinal detachment may act as a neuroprotective agent for the detached retina.

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Corneal neovascularization.

Chang JH, Gabison EE, Kato T, Azar DT.

Corneal neovascularization (NV) is a sight-threatening condition usually associated with inflammatory or infectious disorders of the ocular surface. It has been shown in the field of cancer angiogenesis research that a balance exists between angiogenic factors (such as fibroblast growth factor and vascular endothelial growth factor) and anti-angiogenic molecules (such as angiostatin, endostatin, or **pigment epithelium derived factor**) in the cornea. Several inflammatory, infectious, degenerative, and traumatic disorders are associated with corneal NV, in which the balance is tilted towards angiogenesis. The pathogenesis of corneal NV may be influenced by matrix metalloproteinases and other proteolytic enzymes. New medical and surgical treatments, including angiostatic steroids, nonsteroidal inflammatory agents, argon laser photocoagulation, and photodynamic therapy have been effective in animal models to inhibit corneal NV and transiently restore corneal "angiogenic privilege."

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Prevention of ischemia-induced retinopathy by the natural ocular antiangiogenic agent pigment epithelium-derived factor.

Stellmach V, Crawford SE, Zhou W, Bouck N.

Aberrant blood vessel growth in the retina that underlies the pathology of proliferative diabetic retinopathy and retinopathy of prematurity is the result of the ischemia-driven disruption of the normally antiangiogenic environment of the retina. In this study, we show that a potent inhibitor of angiogenesis found naturally in the normal eye, pigment epithelium-derived growth factor (**PEDF**), inhibits such aberrant blood vessel growth in a murine model of ischemia-induced retinopathy. Inhibition was proportional to dose and systemic delivery of recombinant protein at daily doses as low as 2.2 mg/kg could prevent aberrant endothelial cells from crossing the inner limiting membrane. PEDF appeared to inhibit angiogenesis by causing apoptosis of activated endothelial cells, because it induced apoptosis in cultured endothelial cells and an 8-fold increase in apoptotic endothelial cells could be detected in situ when the ischemic retinas of PEDF-treated animals were compared with vehicle-treated controls. The ability of low doses of PEDF to curtail aberrant growth of ocular endothelial cells without overt harm to retinal morphology suggests that this natural protein may be beneficial in the treatment of a variety of retinal vasculopathies.

J Cell Biochem. 2000 Sep 7;79(3):442-52.

Regulation of EPC-1/PEDF in normal human fibroblasts is posttranscriptional.

Coljee VW, Rotenberg MO, Tresini M, Francis MK, Cristofalo VJ, Sell C.

The EPC-1 (early population doubling level cDNA-1) gene, also known as **pigment epithelium-derived factor**, encodes a protein belonging to the serine protease inhibitor (serpin) superfamily that has been reported to inhibit angiogenesis and proliferation of several cell types. We have previously reported that the EPC-1 mRNA and the secreted EPC-1 protein are expressed at levels more than 100-fold higher in early passage, G(0), WI-38 cells compared to either proliferating or senescent WI-38 fibroblasts. To examine the molecular mechanisms that regulate changes in EPC-1 gene expression in WI-38 cells, we isolated and characterized the human EPC-1 gene and determined the mRNA cap site. Transcriptional assays showed no change in the transcription rates of EPC-1 between young proliferating, quiescent, and senescent WI-38 cells. These results suggest posttranscriptional regulation of the EPC-1 gene. Reverse transcriptase polymerase chain reaction measurements (of hnRNA) indicate regulation at the hnRNA level. The regulation of the EPC-1 gene at the level of hnRNA can explain the observed slow increase in the steady-state EPC-1 mRNA levels when cells become quiescent. The reduction of EPC-1 mRNA levels that occurs when cells exit G(0) and

are induced to proliferate can be accounted for by a reduction of the EPC-1 mRNA stability in stimulated cells as compared to quiescent cells.

Science. 1999 Jul 9;285(5425):245-8.

Pigment epithelium-derived factor: a potent inhibitor of angiogenesis.

Dawson DW, Volpert OV, Gillis P, Crawford SE, Xu H, Benedict W, Bouck NP.

In the absence of disease, the vasculature of the mammalian eye is quiescent, in part because of the action of angiogenic inhibitors that prevent vessels from invading the cornea and vitreous. Here, an inhibitor responsible for the avascularity of these ocular compartments is identified as pigment epithelium-derived factor (**PEDF**), a protein previously shown to have neurotrophic activity. The amount of inhibitory PEDF produced by retinal cells was positively correlated with oxygen concentrations, suggesting that its loss plays a permissive role in ischemia-driven retinal neovascularization. These results suggest that PEDF may be of therapeutic use, especially in retinopathies where pathological neovascularization compromises vision and leads to blindness.