Looking into anti-angiogenic gene therapies for disorders of the eye

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Age-related macular degeneration (AMD) and proliferative diabetic retinopathy (DR) are the most common causes of visual impairment in the developed world. Because the key factor in AMD and DR is aberrant neovascularization in the retina (DR) or in the choroid (AMD), strategies to inhibit abnormal neovascularization represent a compelling therapeutic approach. Here we review various anti-angiogenic strategies for the treatment of ocular neovascular diseases with special emphasis on gene transfer as a way of achieving high, sustained concentrations of anti-angiogenic proteins in the back of the eye without concomitant systemic toxicity.

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▼ Anti-angiogenic gene therapy is a potential breakthrough therapy for ocular neovascular diseases, including age-related macular degeneration (AMD), proliferative diabetic retinopathy (pDR), ocular histoplasmosis and myopic retinopathy. The former two (AMD and pDR) are the most common causes of blindness in the developed world[1–2]. There are ~1.7 million people in the USA alone, who have impaired vision because of AMD, a number that is expected to triple by 2030 as the population ages[1]. Every year, >165,000 people develop the neovascular (also known as 'wet') form of AMD and ~16,000 individuals go blind because of it[2]. DR also represents a considerable problem, with ~50% of patients who have had insulin-dependent diabetes for >20 years developing pDR (Ref. 3). Without treatment, patients with pDR have at least a 50% chance of becoming blind within five years[4]; however, appropriate treatment strategies can reduce this risk to ~5%[5]. Nonetheless, there is still significant room for treatment improvement, considering that ~25,000 patients with DR go blind every year in the USA[1].

The major factor in AMD, as well as in pDR, is aberrant neovascularization in the retina (pDR) or in the choroid (AMD). In the neovascular form of AMD, abnormal new blood vessels invade the macula, which is the region that provides sharp resolution and central vision[6]. Leakage from these vessels, and associated manifestations such as detachment and tears of the retinal pigment epithelium, fibrovascular disciform scarring and vitreous hemorrhage result in severe visual loss (20/200 or worse in either eye) in the majority of patients with the neovascular form[7–8].

In pDR, the diabetic microangiopathy causes capillary closure resulting in retinal ischemia, which in turn provides a potent stimulus for angiogenic stimulation, in particular, via vascular endothelial growth factor (VEGF)[9]. This leads to the ingrowth of new vessels from the retina and the optic nerve. Bleeding and leakage with subsequent scarring, as well as retinal detachment because of vitrioretinal traction, often leads to loss of vision, as does the development of macular edema[10].

Anti-angiogenic therapies

Recognizing that the key pathogenic factor in AMD and pDR is aberrant neovascularization, strategies to curb neovascularization by administration of anti-angiogenic agents represent a compelling approach, and one that is currently being evaluated in numerous clinical trials. Many endogenous, as well as synthetic factors, have been shown to possess potent anti-angiogenic activity, including endostatin, angiostatin and soluble VEGF-receptor inhibitors[11–13]. Several of these are currently in clinical trials, mostly for cancer[14], although a few have also been tested for ocular neovascular disorders[15–16].

However, ocular application of anti-angiogenics might not be straightforward, as

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illustrated by the first few randomized trials, which evaluated the activity of anti-angiogenic agents in the treatment of AMD or pDR and failed to demonstrate any benefit[15–16]. Indeed, there was some indication that interferon (IFN) -α2a was harmful in AMD compared with placebo[15].

AMD and pDR are chronic diseases and, consequently, inhibition of the neovascular stimuli for prolonged periods of time is likely to be required. Oral administration of angiogenesis inhibitors[15–16] carry with them the risk of systemic toxicity and chronic oral administration could potentially interfere with normal physiological functions that require angiogenesis, such as wound healing, collateral formation and menstruation. The two anti-angiogenic drugs tested in randomized clinical trials for AMD, the matrix metalloproteinase (MMP) inhibitor Prinomastat™ and IFN-α2a, both demonstrated non-trivial systemic toxicities[15–16]. Furthermore, because of the blood–retina barrier, it is often difficult with many different therapeutics (including most proteins) to achieve adequate therapeutic levels in the posterior part of the eye[17]. One way to circumvent this is by direct intravitreal injection, which was first introduced therapeutically with ganciclovir for the treatment of HIV-induced cytomegalovirus (CMV) infection[18]. Various anti-angiogenic proteins have been administered this way, including EYE001, which is an injectible anti-VEGF aptamer (an oligonucleotide)[19] and humanized anti-VEGF antibodies[20]. Both approaches have shown substantial activity in various models of ocular neovascularization and are now in clinical trials. However, the clinical applicability of both these, and other proteins, are likely to be impaired by their relatively short half-life, which could require frequently repeated intra-vitreous injections to maintain elevated levels of inhibitor in the posterior part of the eye.

Gene transfer

Gene transfer could represent a potential solution for delivery of therapeutic proteins to the posterior part of the eye. This technology involves the introduction of normal or modified genes into the somatic cells of a target organ to modify cell functions as a means of treating or preventing pathological processes. *In vivo* gene transfer relies on a vector to introduce a therapeutic gene into the cell. Delivery systems currently in use include plasmids, adeno- and retroviruses as well as adeno-associated viruses[21]. For ocular use, adenovirus (Ad)[22], adeno-associated virus (AAV)[23] and lentivirus (HIV)[24] have shown promise in preclinical models. So far, only one clinical trial using gene therapy is underway in ophthalmology (Baylor University Medical Centre, Houston, TX, USA). This involves administration of an adenoviral vector containing the herpex simplex virus (HSV) thymidine kinase gene followed by treatment with the prodrug ganciclovir for the treatment of retinoblastoma (protocol on file with the Recombinant DNA Advisory Committee (RAC) to the NIH Director). However, this is likely to change as the field of ocular gene therapy continues to be refined with the development of increasingly sophisticated vector systems. Studies like the one recently published by Acland *et al.*[25], which demonstrates restoration of vision in a dog model of Leber's Congenital Amaurosis (LCA) is likely to further stimulate the field.

The eye has several inherent properties that make gene transfer potentially appealing. First, the relative immune-privileged status of the eye is likely to limit the extent of the host immune response to the vector, which in other tissues has been a limiting factor for the clinical applicability of gene transfer[26–27]. Second, the eye is a relatively secluded organ, which makes local treatment approaches like gene transfer appealing, because the treatment is unlikely to spread outside the eye thereby minimizing non-ocular toxicity. Third, the symmetry and bilaterality of the eyes provide a superb internal control for clinical trials in which one eye is treated and the contra-lateral eye used as a control. Finally, the eye is easily inspectable and the effects of various treatment modalities as well as their toxicity can be readily monitored.

Anti-angiogenic gene therapy

The limitations of using proteins for anti-angiogenic therapy has led to an increased interest in anti-angiogenic gene therapy. Preclinical proof-of-principle studies, using either recombinant adenovectors to carry the genes encoding pigment epithelium-derived factor (PEDF)[28] and endostatin[29], or recombinant adeno-associated viruses carrying the transgene encoding for angiostatin[30], have recently been published and demonstrated that significant inhibition of neovascularization in various models indicative of AMD or DR is feasible. Recently, Mori *et al.* tested intravenous administration of an adenoviral construct carrying the murine endostatin gene driven by a CMV promoter in a murine model of choroidal neovascularization (CNV) and found almost complete inhibition of CNV (Ref. 29). The authors also found a strong inverse correlation between endostatin serum levels and area of CNV (Ref. 29). Similarly, subcutaneous injection of an AAV virus carrying a truncated angiostatin gene resulted in significant inhibition of retinal neovascularization in a model of retinal neovascularization[30]. These studies provide compelling preclinical evidence that systemic administration of potent endogenous angiogenesis inhibitors, such as endostatin and angiostatin can prevent or reduce retinal and/or CNV.

These encouraging results with endostatin and angiostatin suggest a potential role of anti-angiogenesis in ocular disease.

More recently, there has been great interest and excitement in a promising potent, natural inhibitor of angiogenesis, PEDF (Ref. 31), and the remainder of this review will focus on this molecule.

PEDF

PEDF was first described in 1989 by Tombran-Tink in conditioned medium from cultured, fetal retinal-pigment-epithelial (RPE) cells as a potent neurotrophic factor[32]. In a test system using cultured, primitive retinoblastoma cells, extensive neuronal-like processes were induced after exposure to the RPE-conditioned medium. Subsequently, PEDF has now been purified[33] and cloned both from humans[34] and mice[35]. The gene is expressed as early as 17 weeks in human fetal RPE cells, suggesting that PEDF is intimately involved in early neuronal development[36]. The neurotrophic activity of PEDF was further substantiated by Tanawaki *et al.*[37], who demonstrated that PEDF had a marked effect on cerebellar granule-cell survival in primary cell cultures. These effects of PEDF are not limited to brain neurons, as demonstrated by Araki *et al.*[38] who discovered a similar effect on developing spinal neurons.

PEDF attracted even more attention when Dawson *et al.* demonstrated that, in addition to the previously described neurotrophic activity, PEDF also demonstrated potent anti-angiogenic effects in a rat corneal assay[39]. *In vitro*, PEDF inhibited endothelial-cell migration in a dose-dependent manner with a median effective dose of 0.4 nM, placing PEDF among the most potent natural inhibitors of angiogenesis. Furthermore, the same paper demonstrated that PEDF inhibited endothelial cell migration towards a variety of different angiogenic factors, including VEGF, fibroblast growth factor (FGF) and interleukin-8 (IL-8)[39]. 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[39].

The notion that PEDF plays an important role in regulating vascularity in the posterior part of the eye was further supported by the findings of Gao *et al.*[40], who demonstrated a correlation between changes in VEGF:PEDF ratio and the degree of retinal neovascularization in a rat model of retinal neovascularization. In this model, retinas with neovascularization showed a fivefold increase in retinal VEGF levels and a twofold decrease in retinal PEDF levels compared with age-matched control rats with normal eyes, which resulted in a substantial increase in VEGF:PEDF ratio[40].

The proposed important physiological role that PEDF has in inhibiting ocular neovascularization is further supported by Ogata *et al.*[41], who measured PEDF levels in vitreous fluid in patients who underwent vitrectomy for the treatment of diabetic retinopathy, rhegmatogenous retinal detachment and idiopathic macular hole[41]. They also reported a significant decrease in PEDF levels in patients with active pDR compared with inactive pDR, which further suggests that PEDF is a basal inhibitor of ocular neovascularization[41]. This could have potentially important therapeutic implications for the treatment of ocular neovascular disorders.

The hypothesis that pharmacologically induced elevations in ocular PEDF levels could reduce or prevent ocular neovascularization was tested by Stellmach *et al.*[42]. Recombinant PEDF was intra-peritoneally administered daily from days 12 to 16 in a retinopathy-of-prematurity (ROP) model, in which neonatal mice were exposed to hyperoxide conditions for five days (from day 7 to day 12) while their retinal vasculature is developing. When the mice are returned to the lower oxygen level of air on day 12, the retina becomes ischemic and, in response, excessive neovascularization occurs. By day 17, vessels have proliferated into the retina and the vitreous, creating a retinopathy similar to that seen in retinopathy of prematurity and sharing many of the features of diabetic retinopathy[42]. PEDF administration resulted in substantial dose-dependent inhibition of neovascularization, with doses as low as 2.2 mg kg^{-1} being effective in preventing neovascularization. However, because the half-life of PEDF is short, strategies to provide sustained levels of PEDF over longer periods of time are needed to treat chronic diseases like AMD and/or pDR. Furthermore, systemic administration of a potent angiogenesis inhibitor like PEDF might theoretically interfere with normal repair functions in the body that are dependent on angiogenesis, thereby increasing the risk of systemic toxicity; a strategy for local delivery would, therefore, be important. A gene therapy approach could appease these concerns and might represent the most attractive way of ensuring high, sustained levels of PEDF intra-ocularly.

Gene therapy with PEDF (AdPEDF)

AdGVPEDF.11 (AdPEDF) is a second-generation E1, E4- and partial E3-deleted replication deficient adenovirus vector, designed to deliver the human PEDF gene, controlled by a CMV promoter. Expression of PEDF was measured by RT-PCR for PEDF mRNA and immunohistochemical staining for PEDF protein throughout the retina. Intravitreous injection of AdPEDF resulted in increased expression of PEDF mRNA in the eye compared with eyes injected with AdNull

(the vector without the transgene) or uninjected controls, where no PEDF mRNA was present[43]. PEDF staining was present not only in the retina but in other parts of the eye, including the iris, the lens and the corneal epithelium[43–44]. After subretinal administration of AdPEDF, much stronger staining for PEDF was detected in the RPE cells compared with other ocular structures[43–44]. Gene expression was shown to be sustained for at least one month, using a luciferase marker gene[45].

AdPEDF has been tested in three different models of ocular neovascularization[43–44]. In mice with laser-induced rupture of Bruch's membrane causing CNV, a model indicative of the pathology in AMD, a single injection of AdPEDF administered intravitreously [1×10^9 particle units (pu)] or subretinally (1×10^8 pu) resulted in significant inhibition of CNV (Ref. 43) compared with controls[43]. In a transgenic model, rhodopsin–VEGF transgenic mice have increased expression of VEGF in photoreceptors beginning at day seven, and by day 21 have extensive subretinal neovascularization, sharing several characteristics with the pathology in pDR (Ref. 46). A single intravitreal injection of AdPEDF (1×10^9 pu) resulted in almost complete inhibition of subretinal neovascularization as compared to AdNull and the control group[44]. The third commonly used and widely accepted model for retinal neovascularization is the ROP model described previously. The effect of AdPEDF in this model was similar to the other two models, that is, highly significant inhibition of neovascularization. The percentage inhibition of neovascularization, compared with untreated controls was typically 45–90%[44].

Conclusion

Ocular neovascularization is a key factor of the most common causes of blindness in humans in the developed world: age-related macular degeneration and proliferative diabetic retinopathy. Prevention of ocular neovascularization by deployment of anti-angiogenic drugs represents a rational and appealing therapeutic approach. However, because these are chronic diseases characterized by ongoing new vessel formation, long-term inhibition of the angiogenic stimuli is likely to be needed. However, associated with the long-term suppression of angiogenesis is the risk of systemic side effects, considering that angiogenesis is needed for several physiological functions including wound healing, collateral formation and menstruation. A local treatment modality, resulting in high levels of an angiogenesis inhibitor in the eye (or preferably in the back of the eye) with no systemic exposure would be ideal. However, delivery is complicated because most known anti-angiogenic proteins have short half-lives. A gene therapy approach, using a replication-deficient viral vector to carry the gene encoding the anti-angiogenic substance represents one potential solution to this problem. AdPEDF represents such an approach. Preclinical studies in models of retinal neovascularization, as well as CNV, have demonstrated that AdPEDF, administered by intravitreal or subretinal injection is highly effective in preventing neovascularization. Studies in larger animals are under way to explore the safety and toxicology of this approach. Assuming that toxicology studies identify a well-tolerated dose range, AdPEDF should be tested in clinical trials in patients with ocular neovascular disorders.

References

1 Varmus, H. (1997) Age-related macular degeneration. Status of research. *National Eye Institute Annual Report 1997*, Department of Health and Human Services

2 Seddon, J.M. (2001) Epidemiology of age-related macular degeneration. In *Retina* (Vol. 2, 3rd edn) (Ryan, S.J., ed.), pp. 1039–1050, Mosby

3 Wood, A.J. (1999) Treatment of diabetic retinopathy. *New Engl. J. Med.* 341, 667–678

4 Caird, F.I. *et al.* (1968) Diabetic retinopathy: a further study of prognosis for vision. *Diabetes* 17, 121–123

5 Ferris, F.L., III (1993) How effective are treatments for diabetic retinopathy? *J. Am. Med. Assoc.* 269, 1290–1291

6 Bressler, N.M. *et al.* (2001) Neovascular (exudative) age-related macular degeneration. In *Retina* (Vol. 2, 3rd edn) (Ryan, S.J. ed.) pp. 1100–1136, Mosby

7 Bressler, N.M. *et al.* (1988) Age-related macular degeneration. *Surv. Ophthalmol.* 32, 375–413

8 Ferris, F.L., III *et al.* (1984) Age-related macular degeneration and blindness due to neovascular maculopathy. *Arch. Ophthalmol.* 102, 1640–1642

9 Miller, J.M. (1997) Vascular endothelial growth factor and ocular neovascularization. *Am. J. Physiol.* 151, 13–23

10 Ferris, F.L., III *et al.* (1999) Treatment of diabetic retinopathy. *New Engl. J. Med.* 341, 667–678

11 O'Reilly, M.S. *et al.* (1997) Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. *Cell* 88, 1–20

12 O'Reilly, M.S. *et al.* (1994) Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis Lung carcinoma. *Cell* 79, 315–328

13 Kendall, R.L. and Thomas, K.A. (1993) Inhibition of vascular endothelial growth factor activity by an endogenously endcoded soluble receptor. *Proc. Natl. Acad. Sci. U. S. A.* 90, 10705–10709

14 Zetter, B.R. (1998) Anti-angiogenesis and tumor metastasis. *Annu. Rev. Med.* 49, 407–424

15 Pharmacological Therapy for Macular Degeneration Study Group (1997) Interferon α-2a is ineffective for patients with CNV secondary to age-related macular degeneration: results of a prospective, randomized, placebo-controlled clinical trial. *Arch. Ophthalmol.* 115, 865–882

16 Blodi, B.A. *et al.* (2000) The angiogenesis inhibitor, Prinomastat (AG3340) in the treatment of age-related macular degeneration: study design and baseline characteristics. *Invest. Ophthalmol. Vis. Sci.*, 41, S641

17 Magone, M.T. and Whitcup, S.M. (1999) Mechanisms of intraocular inflammation. In *Immune Response and The Eye* (Vol. 73), (Steilein, J.W., ed.) pp. 90–119, Karger

18 Heineman, M.H. (1989) Long-term intravitreal ganciclovir therapy for cytomegalovirus retinopathy. *Arch. Ophthalmol.* 107, 984–994

19 Guyer, D.R. *et al.* (2001) Anti-VEGF therapy in patients with exudative age-related macular degeneration. *Invest. Ophthalmol. Vis. Sci.* 42, S522

20 Aiello, P.L. *et al.* (1995) Suppression of retinal neovascularization *in vivo* by inhibition of vascular endothelial growth factor (VEGF) using soluble VEGF-receptor chimeric proteins. *Proc. Natl. Acad. Sci. U. S. A.* 92, 10457–10461

21 Murata, T. *et al.* (1997) Ocular gene therapy: experimental studies and clinical possibilities. *Ophthalmic Res.* 29, 242–251

22 Bennett, J. *et al.* (1994) Adenovirus vector mediated *in vivo* gene transfer into adult murine retina. *Invest. Ophthalmol. Vis. Sci.* 35, 2535–2542

23 Grant, C. *et al.* (1997) Evaluation of recombinant adeno-associated virus as gene transfer vector for the retina. *Curr. Eye Res.* 16, 949–956

24 Miyoshi, H. *et al.* (1997) Stable and efficient gene transfer into the retina using an HIV-based lentiviral vector. *Proc. Natl. Acad. Sci. U. S. A.* 94, 10319–10323

25 Acland, G.M. *et al.* (2001) Gene therapy restores vision in a canine model of childhood blindness. *Nat. Genet.* 28, 92–95

26 Bennett, J. and Maguire, A.M. (2000) Gene therapy for ocular disease. *Mol. Ther.* 1, 501–505

27 Sterilein, J. (1995) Enhanced perspective: unraveling immune privilege. *Science* 270, 1158

28 Mori, K. *et al.* (2001) Pigment epithelium-derived factor inhibits retinal and choroidal neovascularization. *J. Cell. Physiol.* 188, 253–263

29 Mori, K. *et al.* (2001) Inhibition of choroidal neovascularization by intravenous injection of adenoviral vectors expression secretable endostatin. *Am. J. Pathol.* 159, 313–320

30 Meneses, P.I. *et al.* (2001) Recombinant angiostatin prevents retinal neovascularization in a murine proliferative retinopathy model. *Gene Ther.* 8, 646–648

31 Chader, G. (2001) Commentary. PEDF: raising both hopes and questions in controlling angiogenesis. *Proc. Natl. Acad. Sci. U. S. A.* 98, 2122–2124

32 Tombran-Tink, J. and Johnson, L. (1989) Neuronal differentiation of retinoblastoma cells induced by medium conditioned by human RPE cells. *Invest. Ophthalmol. Vis. Sci.* 30, 1700–1709

33 Tombran-Tink, J. *et al.* (1991) PEDF: A pigment epithelium-derived factor with potent neuronal differentiative activity. *Exp. Eye Res.* 53, 411–414

34 Steele, F. *et al.* (1992) Pigment epithelium-derived factor: neurotrophic activity and identification as a member of the serine-protease inhibitor gene-family. *Proc. Natl. Acad. Sci. U. S. A.* 90, 1526–1530

35 Singh, V. *et al.* (1998) Structural and comparative analysis of the mouse gene for pigment epithelium-derived factor (PEDF). *Mol. Vis.* 4, 7–12

36 Tombran-Tink, J. *et al.* (1995) Expression, secretion, and age-related downregulation of pigment epithelium-derived factor, a serpin with neurothropic activity. *J. Neurosci.* 15, 4992–5003

37 Taniwaki, T. *et al.* (1997) Pigment epithelium-derived factor protects cultured cerebellar granule cells against glutamate-induced neurotoxicity. *J. Neurochem.* 68, 26–32

38 Araki, T. *et al.* (1998) Pigment epithelium-derived factor (PEDF) differentially protects immature but not mature cerebellar granule cells against apoptotic cell death. *J. Neurosci. Res.* 53, 7–15

39 Dawson, D.W. *et al.* (1999) Pigment epithelium-derived factor: a potent inhibitor of angiogenesis. *Science* 285, 245–248

40 Gao, G. *et al.*(2001) Unbalanced expression of VEGF and PEDF in ischemia-induced retinal neovascularization. *FEBS Lett.* 489, 270–276

41 Ogata, N. *et al.*(2001) Pigment epithelium-derived factor in the vitreous is low in diabetic retinopathy and high in rhegmatogenous retinal detachment. *Am. J. Ophthalmol.* 132, 378–382

42 Stellmach, V. *et al.* (2001) Prevention of ischemia-induced retinopathy by the natural ocular anti-angiogenic agent pigment epithelium-derived factor. *Proc. Natl. Acad. Sci. U. S. A.* 98, 2593–2597

43 Duh, E.J. *et al.* (2001) *In vivo* expression of pigment epithelium-derived factor by adenovirus-mediated gene therapy. *Invest. Ophthalmol. Vis. Sci.* 42, S506

44 Mori, K. *et al.* (2001) Pigment epithelium-derived factor inhibits retinal and choroidal neovascularization. *J. Cell. Physiol.* 188, 253–263

45 Wei, L.L. *et al.* (2001) Adenovirus gene delivery for the treatment of ocular diseases. *Invest. Ophthalmol. Vis. Sci.* 42, S343

46 Okamoto, N. *et al.* (1997) Transgenic mice with increased expression of vascular endothelial growth factor in the retina. *Am. J. Pathol.* 151, 281–291