Possible Role of Pigment-epithelium-derived Factor in Corneal Angiogenesis

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Abstract

The detection of pigment-epithelium-derived factor (PEDF) in corneal tissue has allowed greater understanding of the avascularity of corneal tissue. The ability of the cornea to maintain the avascular nature of this tissue, also referred to as the angiogenic privilege of the cornea, could be partly attributed to the presence of this factor. This privilege is severely impaired by various diseases of the ocular surface associated with inflammation and infection that are often followed by neovascularisation, which compromises the transparency of the cornea and results in visual impairment. The rapidly increasing insights into the basic mechanisms controlling neovascularisation, i.e. balance of growth factor activation and enzymatic activity, has most recently led to the development of large-scale use of specific antiangiogenic agents in the treatment of neovascular age-related macular degeneration (AMD). Focusing on the effects of vascular endothelial growth factor (VEGF), the use of such agents, including bevacizumab (Avastin®), a humanised anti-VEGF monoclonal antibody originally used in the treatment of metastatic colorectal cancer, has been investigated in corneal angiogenesis. PEDF is only one of the many factors involved in ocular angiogenesis. However, although it is only a small protein, it has strong antiangiogenic actions that are expressed in the retinal pigment epithelial (RPE) layer, as well as in other parts of the eye. There are specific characteristics that could designate a special role for PEDF in the regulation of avascularity in the eye. In this article, we focus on corneal angiogenesis and highlight the special features of this somewhat unexplored cytokine, outlining the current knowledge and possible role of PEDF in corneal neovascularisation.

Keywords

Angiogenesis, corneal neovascularisation, pigment-epithelium-derived factor (PEDF), growth factors

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Pigment-epithelial-derived factor (PEDF) is an extracellular 50kDa secreted glycoprotein of the serpin family. In contrast to most other serpins, which are protease inhibitors, PEDF does not seem to exert inhibitory actions against any known proteases.[1,2] Only very few other serpins are considered to be non-inhibitory, including ovalalbumin and angiotensinogen. Interestingly, one product of angiotensinogen, angiotensin II, cross-talks with the endothelial growth factor receptors (EGFRs), consequently shedding new light on tumour cell proliferation.[3]

Furthermore, PEDF has antitumour actions as well,[4] but the detailed mechanisms of these actions are not yet clarified. However, PEDF most likely binds to receptors on the cell surface, leading to intracellular activity and altered transcription. In the search for PEDF receptors, the data suggested that PEDF has a 44-mer peptide cell-surface receptor responsible for its binding to retinoblastoma Y-79 cells.[5] The crystal structure of PEDF has been determined, showing a striking asymmetrical molecular charge distribution,[6] and two binding sites for PEDF to extracellular matrix components have been described: a high-affinity binding area to collagen type I and a low-affinity binding site to heparin, with evidence for a collagen-1 binding site on the negatively charged part of PEDF.[7] Different binding sites on PEDF for collagen and heparin were then located.[8] Other data indicate that some RPE cells may produce heparin with binding affinity to PEDF, which could be of importance in receptor binding on the cell surface.[9] Using an *in vitro* model, data showed that PEDF inhibits angiogenesis in microvascular endothelial cells via regulated translocation of vascular EGFR (VEGFR)-1 and phosphorylation of VEGFR-1; this in turn inhibits VEGF-2-induced angiogenesis.[10] A protein with phospholipase A_2 activity, PEDF receptor (PEDF-R), with high affinity for PEDF was detected in the human retinal pigment epithelia (RPE), suggesting a pathway for cell signalling at the cell surface.[11] PEDF further inhibited VEGF expression in the oxygen-induced hypoxic retina of mice, and also inhibited the binding of VEGF to the VEGFR-2, the main VEGF receptor for vascular permeability and angiogenesis.[12]

The effect of the observed structural change of PEDF following exposure to heparin[13] is not known, although an alteration of receptor kinetics should not be excluded. PEDF was first purified from human foetal (RPE) cells, where it promoted neuronal differentiation of cultured human Y-79 retinoblastoma cells.[14] PEDF is present in the retina and its RPE cells, but also in other parts of the eye, including ganglion cells and the ciliar epithelium,[15,16] as well as in tissues of the human body that are rich in collagen, such as bone and cartilage. The gene for PEDF has been localised to

chromosome 17p13,[17] the same chromosome responsible for autosomal retinitis pigmentosa[18] and Leber congenital amaurosis.[19]

Pigment-epithelium-derived Factor as a Neuroprotective Factor

Corneal innervation and reinnervation has been shown to be of major importance in corneal wound healing. In this context, the ability of PEDF to protect spinal motor neurons using a culture model based on a specific defect in glutamate transport in amyotrophic lateral sclerosis (ALS) was shown.[20] Additionally, PEDF treatment significantly increases the survival of embryonic chock spinal motor neurons in culture in a dose-dependent way, promoting neurite outgrowth of cultured motor neurons and also preventing death of axotomised motor neurons *in vivo*.[21]

The known neurotoxicity of glutamate is considered to be an important mechanism in programmed cell death, and is therefore related to different neurodegenerative disorders. PEDF inhibited glutamate-induced apoptosis in cerebellar granulae cells.[22,23] This has been shown to be mediated by activation of the transcription factor NF-kappa B (NF-κB); however, PEDF did not regulate the antiapoptotic genes *Bcl-2*, *Bcl-x* and *Mn-SOD*.[24] The earlier detected 44-mer peptide 78-121 out of 418 suggested cell-surface receptors binding to retinoblastoma cells[20] was identified as the PEDF region responsible for its neuroprotective actions.[25] It is not known whether PEDF has similar morphogenic effects within corneal development to those observed in the development of retinal photoreceptors.[26]

Pigment-epithelium-derived Factor Antiangiogenic Actions in the Eye

As outlined above, there has been significant progress in our understanding of how PEDF may exert its effects. However, the underlying mechanisms concerning the regulation and balance of PEDF and other growth factors involved in angiogenesis of the eye are still unclear.

The first presented antiangiogenic actions of PEDF in the mammalian eye showed that PEDF angiostatin inhibited neovascularisation in the retina more efficiently than the earlier well-studied antiangiogenic factors and, furthermore, that under hypoxia VEGF secretion was high and PEDF expression low.[27] Intravitreal injection of PEDF protected retinal photoreceptors from morphological and functional deterioration in light-exposed rats.[28] In another model with ischaemic-induced retinopathy in rats, VEGF levels were found to increase to a greater extent than PEDF levels, with a VEGF–PEDF ratio correlating to the observed retinal neovascularisation, suggesting an impaired balance between stimulators and inhibitors of angiogenesis that may contribute to retinal neovascularisation.[29] PEDF prevented retinal ischaemia-induced neovascularisation as it appeared from apoptosis of endothelial cells in a murine model,[30] and PEDF expression increased in retinal epithelial cells and the retina following laser photocoagulation, suggesting PEDF could play a role in inhibiting neovascularisation.[31] PEDF is further upgraded in differentiated but not in non-differentiated RPE cells.[32]

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The clinical importance of PEDF was emphasised by a study in which intraocular PEDF levels were found to be low in human eyes with retinal ischaemia, including proliferative diabetic retinopathy (DR), compared with controls, suggesting that the loss of antiangiogenic inhibition is of importance in mediating neovascularisation.[33] In a prospective case-control study, PEDF concentrations were low in the vitreous of patients with choroidal neovascularisation (CNV) and age-related macular degeneration (AMD) compared with age-matched control patients.[34] Experimental studies with intravitreal injections in a mouse model of oxygen-induced ischaemic retinopathy prevented retinal neovascularisation and suppressed VEGF-induced retinal microvascular endothelial cell proliferation.[35] PEDF was found in different tissues of the rat eye and expressed in areas of CNV following laser coagulation, suggesting that PEDF may modulate the process of CNV.[36] Lower levels of PEDF and high expression of VEGF were found in the vitreous of patients with DR compared with high levels of PEDF and undetectable levels of VEGF in vitreous samples from patients with idiopathic macular hole, suggesting that an inbalance of PEDF and VEGF may be related to angiogenesis in DR, which in turn can lead to active proliferative DR.[37] PEDF was also seen in the subretinal fluid of retinal detachment, which indicates a role in preventing subretinal neovascularisation.[38] The concentration of PEDF in tissues may be of crucial importance. Low PEDF concentrations in choroids may play a role in the development of choroidal neovascularisation, as PEDF levels were significantly lower in aged choroids with AMD compared with choroids of age-matched control patients, whereas levels of VEGF were similar in both groups.[39]

Vascular angiogenesis is possibly linked to lymphangiogenesis and should be considered an advanced multistep process defined as growth of new vessels from pre-existing ones[40] triggered by hypoxia and inflammation. In corneal angiogenesis, the limbal region is the site of origin and neovacularisation can be seen at the surface as well as in the stromal layers, depending on the underlying cause.[41] An imbalance between proangiogenic and antiangiogenic molecules seems to be of great importance,[42] as are other factors, including matrix metalloproteinases. Among the current experimental study designs, the corneal pocket assay is a widely used method in

angiogenesis research. Although originally developed for use in the eyes of rabbits,[43] later models often use mice instead,[44] where angiogenic-stimulating factors are implanted into the cornea and, as the healthy cornea is avascular, all emerging vessels are considered new vessels.[45]

The counteractive factors of PEDF-regulated vessel-inhibitory activity include VEGF, which is the pre-eminent growth factor promoting pathological angiogenesis. VEGF expression in the cornea was first described in the basal layer of the epithelium[46] and its receptors were expressed with increased intensity in inflamed and vascularised human corneal buttons compared with normal corneas, which suggests that VEGF may be involved in corneal vascularisation.[47] VEGF, transforming growth factor (TGF)-α and TGF-β expression were localised in human corneas with neovascularisation.[48] In corneas of Pax6± mice, bound VEGF-A and expression of soluble VEGFR-1 (also known as sFlt-1) were found.[49]

Based on this knowledge, current antiangiogenic strategies in the cornea focus on the use of modified antibodies against VEGF. Promising results have been achieved by subconjunctival injections with bevacizumab (Avastin®) in patients with superficial and deep corneal neovascularisation; neovascularisation was reduced but no significant change of the centricity of vessels was seen.[50] Corneal angiogenesis in animal rabbit models could be inhibited by using subconjunctival injections of bevacizumab.[51,52] However, there was no short-term regression of corneal vessels in recurrent pterygium disease after subconjunctival bevacizumab injection.[53] More similar studies[54–57] will further outline the effects of anti-VEGF treatment in the anterior segment.

Pigment-epithelium-derived Factor in the Cornea and Tear Fluid

Only a few studies have been conducted so far regarding PEDF in the anterior segment of the eye. PEDF was detected in the tear fluid of some healthy volunteers, suggesting that PEDF may play a role in the regulation of neovascularisation at the ocular surface.[58]

In normal samples, high levels of PEDF were seen in human corneas with less expression in the conjunctiva, but in patients with pterygia, only very few samples showed faint PEDF staining, and in the remaining samples PEDF was not detectable at all.[59] In the same samples, highly elevated levels of VEGF were seen in the pterygial samples and detectable levels were seen in the conjunctiva, but not in the cornea.

From clinical experience, it is well-known that with the use of amniotic membranes on the ocular surface, less corneal neovascularisation is seen. By investigating human amniotic membranes, PEDF expression was detected, suggesting that PEDF may contribute to the suppression of corneal angiogenesis.[60]

Matrix Metalloproteinases

The processing and downregulation of PEDF most likely involve major enzymatic factors of wound-healing events, such as matrix metalloproteinases (MMP). MMPs are of importance in angiogenesis as they dissolve basement membranes and extracellular matrix components, which in turn allow endothelial cells to proliferate, emphasising their possible role in pterygial disease. Pterygia is characterised by a proliferative and inflammatory growth of limbal cell origin that invades the cornea, causing dissolution of the Bowman's layer and leading to the loss of the natural collagenous barrier separating the epithelium from the stroma. The altered MMP expression in altered basal limbal epithelial cells compared with normal tissue suggests these cells play an important role in the formation and migration of a pterygium.[61] The expression of MMPs and their inhibitors at the pterygial head has also been identified.[62]

The positive feedback between MMP-9 and VEGF in human hypoxic RPE cells emphasises the importance of the enzymatic system in the regulation of VEGF. Raised levels of VEGF increased MMP-9 and exogenous administration of MMP-9 increased VEGF levels.[63] Increased expression of MMP-2, messenger RNA (mRNA) and VEGF was first presented in rat corneas following retinal photocoagulation,[64] and PEDF levels decreased in hypoxia in a mouse ROP model, most likely as an effect of increased proteolytic actions by MMP-2 and MMP-9.[65]

Conclusion

The underlying mechanisms of corneal angiogenesis are complex and include different growth factors. PEDF is not as well investigated in neovascularisation in the anterior segment of the eye as in the retina, and current studies so far mostly address the potential role of VEGF and the effects of anti-VEGF therapies. Furthermore, many studies in this area use *in vitro* or animal models with limitations. However, the balance between expression of PEDF and VEGF observed here, as well as in other parts of the eye, and the possible link via mediators such as MMPs described above suggest that PEDF may play an important role in corneal angiogenesis and wound healing. The tear fluid plays an important role in maintaining corneal homeostasis, and the fact that PEDF was found in detectable amounts in human tears in some samples may be of importance in the regulation of neovascularisation at the ocular surface, but the origin and mechanism of PEDF here are still unclear. Further research in the cornea and tear fluid is required to establish its role and the pathways in which it exerts its actions. Most interestingly, however, PEDF, as an antiangiogenic protein, has different biochemical characteristics from other vascular-inhibitory substances. Better understanding of this system may open the door to new therapeutic strategies in order to maintain or re-establish corneal transparency and vision. ■

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