Published in final edited form as:

Growth Factors. 2010 August ; 28(4): 280–285. doi:10.3109/08977191003604513.

The applied biochemistry of PEDF and implications for tissue homeostasis

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Abstract

Pigment epithelium-derived factor (PEDF) is an endogenously produced glycoprotein with a spectrum of biological roles across diverse pathologies. Recent research has focused on the biochemical properties of PEDF and its associated receptors. This review discusses the recent developments in PEDF biochemistry and how this new knowledge will help progress our understanding of PEDF as a molecular mediator for anti-angiogenesis and -tumorigenesis. Additionally, pathophysiological roles for PEDF in healing and tissue homeostasis are being revealed and our enhanced understanding of the interactions between PEDF and its receptors may yet prove useful in propelling PEDF towards clinical application.

Keywords

Pigment epithelium-derived factor; structure; receptor; biochemistry; healing; repair

Pigment epithelium-derived factor—A “biologically-enriched” protein

Pigment epithelium-derived factor (PEDF) was first discovered in 1991 as a factor secreted by the pigment epithelium of the human foetal eye (Becerra 2006). Retinal pigment epithelium produces a number of trophic factors that aid development and promote differentiation of the neural retina. Among these, PEDF was shown to be capable of inducing differentiation of retinoblastoma cells (Tombran-Tink and Johnson 1989). Following this, focus then shifted to the potent anti-angiogenic properties of PEDF. It is the most potent anti-angiogenic agent currently known, twice as potent as angiostatin and seven

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

times more potent than endostatin (Dawson et al. 1999). PEDF suppresses migration and proliferation and induces apoptosis of endothelial cells (Ek et al. 2006).

Angiogenesis is an essential process in the development and spread of all tumour types and PEDF has great tumour specificity, not only by targeting neovasculature but also by inducing differentiation and inhibiting proliferation of tumour cells (Ek et al. 2006). Its growth-inhibitory ability involves interaction with apoptotic and cell cycle control mechanisms. PEDF has been shown to be capable of stimulating apoptosis in malignancies ranging from prostatic (Doll et al. 2003; Halin et al. 2004; Filleur et al. 2005; Guan et al. 2007), ovarian (Phillips et al. 1996; Cheung et al. 2006) and pancreatic carcinomas (Doll et al. 2003; Halin et al. 2004), melanoma (Abe et al. 2004; Garcia et al. 2004; Yang et al. 2006), glioma (Guan et al. 2003, 2004; Zhang et al. 2007) and osteosarcoma (Quan and Choong 2006; Ek et al. 2007a, b, c). For osteosarcoma, our laboratory has progressed the evaluation of PEDF from *in vitro* studies to preclinical studies in orthotopic models of spontaneously metastasizing disease. Nevertheless, before PEDF can be taken beyond the laboratory, a greater understanding of its molecular mechanisms is essential.

Biochemistry—A tool to understanding underlying molecular mechanisms of PEDF function

An endogenously produced 50-kDa glycoprotein (Becerra 2006), PEDF is encoded by the SERPINF1 gene on chromosome 17p13. It is a 418-amino acid polypeptide (Stratikos et al. 1996), expressed by a number of foetal and adult tissues, including brain, spinal cord, eye, plasma, bone, prostate, pancreas, heart and lung (Ek et al. 2006).

PEDF belongs to the serine protease inhibitor (serpin) family of proteins as demonstrated by both amino acid and DNA sequence data. Most of its amino acids form secondary structures with the exception of the first 35 residues at the N-terminus (residues 1–35). It possesses a similar tertiary structure to other members of the serpin family, with three β -sheets and 10 α -helices (Simonovic et al. 2001; Filleur et al. 2009). It also possesses the typical reactive centre loop (RCL; residues 373–380) near the C-terminus (Steele et al. 1993; Dass et al. 2008). For inhibitory serpins, the RCL determines specificity for binding to target proteases (Filleur et al. 2009).

Despite being structurally homologous with other serpins, PEDF is in fact a non-inhibitory serpin, and subtle differences in the RCL may explain this inability to inhibit serine proteases. The RCL is protease sensitive; however there is no conformational change (the stressed (S) $\rightarrow$ relaxed (R) transition) when the loop is cleaved as is typical for inhibitory serpins. This lack of conformational change has also been demonstrated for ovalbumin, another non-inhibitory serpin (Becerra et al. 1995).

PEDF associates with a number of extracellular matrix components, including glycosaminoglycans (GAGs; heparin, heparin sulphate and hyaluronan) and collagens (Alberdi et al. 1998; Kozaki et al. 1998; Meyer et al. 2002; Yasui et al. 2003; Becerra et al. 2008). Using protein chemistry and genetic engineering, collagen-, heparin- and hyaluronan-binding sites on the human PEDF protein have been mapped (Figure 1). The acidic amino acid residues Asp²⁵⁵, Asp²⁵⁷ and Asp²⁹⁹ are critical to collagen-I-binding, three clustered basic amino acid residues Arg¹⁴⁵, Lys¹⁴⁶ and Arg¹⁴⁸ are necessary for heparin binding, and Lys¹⁸⁹, Lys¹⁹¹, Arg¹⁹⁴ and Lys¹⁹⁷ form a motif that is critical for hyaluronan binding (Becerra et al. 2008). These findings are important because mutational analysis supports the involvement of the collagen-I-binding site in the anti-angiogenic activity of PEDF and the involvement of heparin/heparan sulphate in efficient cell surface receptor binding of PEDF (Alberdi et al. 2003). For instance, collagen-I is the most abundant protein in bone, and

PEDF has distinct activity against osteosarcoma cells, in both culture and *in vivo* (Ek et al. 2007b, c). This activity is linked to collagen-I, though the exact mechanisms have yet to be teased out. For other tumour types, other GAGs or even certain collagens may be important to PEDF function. Heparin binding induces a conformational change in PEDF that would allow for better interactions with its receptor (Valnickova et al. 2007). In addition to contributing to deposition in hyaluronan-rich extracellular matrixes, such as the interphotoreceptor matrix, the hyaluronan-binding activity of PEDF may contribute to its reported antitumour/antimetastatic effects (Becerra et al. 2008).

More interesting are the studies on binding partners for PEDF that led to the identification of a plasma membrane-linked phospholipase protein as a PEDF receptor (PEDF-R; Notari et al. 2006). PEDF-R is a transmembrane protein present on the surface of cells. It specifically binds PEDF with high affinity and exhibits phospholipase A2 (PLA2) activity. PEDF stimulates the PLA2 activity of PEDF-R causing the release of fatty acids and lysophosphatidic acid from phospholipids. It has been demonstrated that PEDF increases the release of a fatty acid derivative, neuroprotectin D1, from retinal pigment epithelial cells (Mukherjee et al. 2007). It is speculated that upon binding PEDF-R, PEDF stimulates the neuroprotectin D1 signalling to mediate the neurotrophic and anti-inflammatory activities of PEDF. Depending on the composition of the plasma membrane phospholipids, PEDF can stimulate the PEDF-R-mediated release of bioactive lipids for its different functions. These findings provide insight into how a PEDF ligand/receptor interaction on the cell surface could generate a signalling pathway capable of attenuating cell biology.

Recently, (Bernard et al. 2009) also identified a laminin receptor (LR) for PEDF by a yeast two-hybrid screening. PEDF was confirmed as ligand in this setting by co-immunoprecipitation, his-tag pulldown and surface plasmon resonance assays. Furthermore, the binding of PEDF to LR was attributed to the 34-mer peptide region with amino acids 44–77 on PEDF and amino acids 120–210 on LR. The PEDF-derived P46 (amino acids 46–70) was shown to interact with LR and inhibit angiogenesis both *in vitro* using human bone marrow endothelial cells and *in vivo* using a corneal angiogenesis model in C57/Bl6 mice.

PEDF as the healer—maintaining tissue homeostasis

Models of PEDF knockdown have recently advanced our understanding of PEDF's role in maintaining tissue homeostasis. Doll et al. (2003) were the first to perform a PEDF knockdown study, examining PEDF's role in regulating the vasculature of the prostate. The anti-angiogenic ability of PEDF was confirmed with increased stromal vasculature evident in PEDF-deficient mice, along with striking epithelial hyperplasia. More recently however, Huang et al. (2008) used PEDF-deficient mice to examine the role of PEDF in the vascularisation of the retina during the normal post-natal period and in response to oxygen-induced ischaemic retinopathy. By removing the anti-angiogenic influence of PEDF, retinal vascularisation was found to proceed at an increased rate. Hyperoxia-mediated vessel obliteration was also more problematic. Finally, Chung et al. (2008) have used PEDF-deficient mice to reveal a novel role for PEDF in lipid metabolism. PEDF-deficient mice exhibited striking hepatic steatosis and increased levels of hepatic triglycerides. These effects were decreased by treatment with rPEDF, highlighting a potential therapeutic role for PEDF in lipid homeostasis.

The clinical use of an anti-angiogenic such as PEDF may potentially be limited by the disruption of physiological processes that rely on neovascularisation. For this reason, we must consider the particular implications for tissue repair, in response to ischaemic, traumatic and metabolic damage. The neovascularisation of chronically ischaemic tissue is critical for re-establishing oxygenation, nutritional supply lines, as well as a means for waste

removal. Traumatic wound healing relies on an acute inflammatory response and the arrival of fibroblasts for collagen deposition. Reversal of the cellular damage induced by deranged metabolic processes or toxic agents may depend on various antioxidants. All these processes are partially reliant on vascularisation.

Rychli et al. (2009a, b) recently examined PEDF as a therapeutic target in cardiovascular disease. PEDF expression is downregulated by low oxygen levels in human hearts, and levels of this factor are significantly lower in explanted human ischaemic hearts as compared to their healthy counterparts. Furthermore, human cardiac myocytes and fibroblasts downregulate PEDF expression not only in response to hypoxia, but also when exposed to the toxic agents such as cobalt chloride, 2,2'-dipyridyl and dimethoxally glycine (Rychli et al. 2009a). In contrast, the anti-oxidant, -inflammatory and -thrombotic properties of PEDF are protective in the setting of atherosclerosis. Advanced glycation end products and platelet CD40L are known to progress atherosclerotic disease under hyperglycaemic conditions. Yamagishi et al. (2009a, b, c) showed that not only did AGE injection into non-diabetic rats cause a 1.3-fold increase in platelet CD40L expression, but also that these effects could be completely abrogated by PEDF. Furthermore, PEDF could partially block the 2-fold increase in platelet CD40L in diabetic rats. Intraplatelet PEDF levels were significantly lower in diabetic vs. control rats.

The healing process in response to traumatic tissue injury is a finely orchestrated event with respect to neovascularisation. Leukocytes and fibroblasts localise to the injured microenvironment; however, over-vascularisation may pose a problem in the guise of excessive granulation tissue. To this end, the temporal expression of PEDF was examined by Ipina et al. (2009) using a model of dermal wound healing in horses. PEDF expression was evaluated in normal skin and biopsies were taken from the edge of experimentally induced wounds. RT-PCR demonstrated that wounding caused a temporal increase in PEDF mRNA, with results suggesting that PEDF may function in effecting a superior form of wound healing, one in which exuberant granulation tissue is avoided.

PEDF's role as an anti-angiogenic agent in wound healing is by no means a new concept and its ability to reign in excessive neovascularisation is absolutely critical to healing in the eye. Here, optical clarity and performance depend on avascularity. Using a rat model of penetrating ocular injury, a 30% decrease in local neovascularisation of retinas was observed. PEDF appeared to be the main effector, with injury causing a 4.1-fold increase in retinal PEDF mRNA and a 1.5-fold increase in PEDF protein. Furthermore, there was a 3.4-fold increase in PEDF protein in the vitreous humour of injured eyes and PEDF mRNA increased primarily around the puncture site (Penn et al. 2006). These findings have also been extended to the healing of the cornea by Azar et al. (2006). Recently, it has been demonstrated that subconjunctival injections of PEDF protein at doses as low as 0.1 pmol protein/day result in decrease in injured choroidal neovascularisation (Amaral in press).

With mounting evidence in support of PEDF's role in wound healing, particularly in the eye, it was thought that PEDF might in fact be a key mediator for a number of established treatments. For example, retinal neovascularisation is inhibited by laser therapy while preserving visual function. Using a transgenic rat model of neovascularisation, Yu et al. (2009) demonstrated that low power laser therapy achieved this with an inversely proportional relationship between PEDF and neovascularisation. Similarly, intravitreal bevacizumab was tested for macular oedema secondary to branch retinal vein occlusion. Here, the ratio of vascular endothelium growth factor (VEGF) to PEDF was reduced in responders to bevacizumab injection, suggesting that a shift in the balance towards PEDF may in part account for the response (Matsuyama et al. 2009; Park and Ahn 2009).

Interestingly, a similar effect on the VEGF to PEDF ratio was seen when diabetic rats were treated with perindopril (Zheng et al. 2009).

This apparent role of PEDF in the pathophysiology of wound healing may hinge on its ability as a chemotactic agent rather than as an anti-angiogenic. Among factors secreted from multipotent murine mesenchymal stromal cells, PEDF is the predominant fibroblast chemoattractant (Sarojini et al. 2008). This stimulatory effect of PEDF on fibroblast chemotaxis is in contrast to the PEDF-mediated inhibition of endothelial cell migration. Differential effects of PEDF towards fibroblasts and endothelial cells may serve to programme an ordered temporal sequence of scaffold building followed by angiogenesis during physiological wound healing. In addition, it again confirms that PEDF may have several receptors for its separate and sometimes contradicting functions.

Drug-induced renal toxicity, such as that caused by doxorubicin, is a real dose-limiting phenomenon faced by clinicians. In a recent rat study (Fujimura et al. 2009), when animals were administered doxorubicin, levels of PEDF in isolated glomeruli were significantly reduced. In addition, marked proteinuria and increased urinary excretion of nephrin, an index of podocyte (cells of the visceral epithelium in the kidneys which form a crucial component of the glomerular filtration barrier, contributing size selectivity and maintaining a large surface area for filtration) damage, were noted. When rats were administered PEDF for 14 days, these changes were reduced sufficiently such that renal damage was kept to a minimum. However, the protective effects of PEDF, if any, on other organs were not observed. For certain pathologies such as osteosarcoma, doxorubicin is in fact a frontline therapeutic that does have side effects in patients. Since PEDF is very effective against osteosarcoma, the combined effects of doxorubicin and PEDF therapy, found to be promising (Fujimura et al. 2009), need further evaluation.

Summary

PEDF influences cell proliferation via both the apoptotic and cell cycle regulatory pathways. Recent biochemical studies have provided some insight into PEDF structure, function and receptors. These have to be exploited now to reveal novel functions of PEDF, and hence research such that the true therapeutic potential of PEDF is realised. Such biochemical analyses may also facilitate better ways for healing damaged tissues with PEDF via its abilities of an anti-angiogenic, antioxidant and chemotactic agent.

Acknowledgments

Dr M. L. Broadhead is supported by a Postgraduate Scholarship awarded by the National Health and Medical Research Council (NHMRC). The authors would also like to acknowledge the continuing support of the Royal Australasian College of Surgeons, the Australian Orthopaedic Association and the St Vincent's Research Endowment Fund.

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Figure 1.

Illustration of the 3D structure of PEDF showing structural features associated to function. The backbone tracing of the PEDF polypeptide is shown in grey. The 34-mer (green) and 44-mer (orange) peptide regions are shown in tube representations. The required acidic residues (red) for collagen binding, the required basic residues (blue) for heparin binding, the phosphorylation sites Ser114 and Ser227 (green and orange, respectively) and glycosylation site Asn285 with sugar attachment (fuchsia) are all shown in ball-and-stick representations. From left to right, each view is rotated (vertical axis) about 90° from the previous one, and has the RCL oriented to the top of the figure.