REVIEW

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Pigment epithelium-derived factor (PEDF): drug developmental challenges ahead for a budding anti-angiogenic

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Received September 19, 2007, accepted October 3, 2007

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Pharmazie 63: 4–6 (2008) *doi: 10.1691/ph.2008.7736*

One of the most potent anti-angiogenic biological discovered to date, pigment epithelium-derived factor (PEDF) is fast becoming an exciting and promising lead candidate. Recent studies have shown that even shortened versions of the protein can serve as therapeutic agents with similar activity as the parent molecule. The next obvious challenge is to find ways to deliver this molecule *in vivo* with enhanced pharmacodynamics and reduced toxicity. Several methods to achieve this are proposed.

1. Introduction

Pigment epithelium-derived factor (PEDF) was identified as an effective neurotrophic factor, with purified PEDF concentrations being able to convert active Y79 retinoblastoma cells into differentiated non-proliferating neurons (Tombran-Tink et al. 1991). Further studies have shown that in fact PEDF possesses multiple biological properties, not only neurotrophic, but also neuroprotective, anti-tumorigenic and potent anti-angiogenic activity (Ek et al. 2006a). PEDF has been demonstrated to be the most potent endogenous inhibitor of angiogenesis in various assays for identifying potential anti-angiogenic compounds (Phung and Dass 2006), being more than twice as potent as angiostatin, and more than seven times as potent as endostatin (Dawson et al. 1999).

Studies have already shown that decreased levels of PEDF in the eye is associated with a number of ocular neovascular and neurodegenerative diseases. Furthermore, low expression of PEDF has been correlated with the increased incidence of metastasis and poorer prognosis in prostate cancer, pancreatic cancer, neuroblastomas and gliomas (Ek et al. 2006b). In this review, we focus on the key issues surrounding the ability of PEDF to demonstrate therapeutic properties against various biomedical conditions via a reduction in angiogenesis.

2. Angiogenesis regulation

Much focus on PEDF as a promising therapeutic target in cancer has stemmed from its potent anti-angiogenic activity, which has shown to be more effective than any other known endogenous angiogenic inhibitor (Dawson et al. 1999). Moreover, PEDF inhibits endothelial cell migration even in the presence of pro-angiogenic factors such as VEGF, FGF-1, FGF-2 and interleukin-8 (Tombran-Tink and Barnstable 2003). PEDF plays a key role as a natural angiogenesis inhibitor, as PEDF-null mice phenotypically exhibit increased stromal microvessel density in several organs including the pancreas and prostate (Doll et al. 2003). PEDF's activity is selective in that it targets only new vessel growth and spares the pre-existing vasculature, which makes it an appealing candidate as an inhibitor of tumour angiogenesis (Bouck 2002). While no ADME/Tox study has yet been performed on the protein or its peptides, one could predict that treatment with PEDF should be readily harmless. Nevertheless, given the fine balance between VEGF and PEDF in maintaining the angiogenic status in the body, the effects of PEDF infusion need to be closely monitored especially for females in their menstrual cycle, and those who have wounds that require repair.

Although the mechanisms via which PEDF reduces neovascularization remain elusive, it involves endothelial cell apoptosis, through the activation of the Fas/FasL death pathway (Volpert et al. 2002) and also via a disruption in the critical balance between pro- and anti-angiogenic factors, in particular VEGF. PEDF has an inhibitory effect on VEGF-induced angiogenesis in bovine retinal microvascular endothelial cells via enhancing γ-secretase-dependent cleavage of the C-terminus of VEGFR-1 which consequently inhibits VEGFR-2 induced angiogenesis (Cai et al. 2006). PEDF has been demonstrated to inhibit VEGF binding to its receptor in retinal capillary endothelial cells (RCECs) and to be downregulated by VEGF (Zhang et al. 2006).

Moreover, in the human MG63 osteosarcoma cell line, PEDF down-regulates VEGF expression (Takenaka et al. 2005). We have corroborated this finding in our own two orthotopic clinically relevant models of osteosarcoma with both rPEDF (Ek et al. 2007a) and its shortened peptides (Ek et al. 2007b). Western blotting confirmed a dose-de-

pendent decrease in the level of VEGF with rPEDF or peptide. *In vivo*, a reduced microvessel density was noted with rPEDF treatment (Ek et al. 2007a) and with PEDF overexpression (Ek et al. 2007c).

The expression patterns of VEGF, a potent pro-angiogenic factor, and PEDF have been well characterised in the eye and it is the balance of these opposing stimuli that prevents the development of choroidal neovascularisation that is involved in diabetic proliferative retinopathy and macular degeneration (Ogata et al. 2001; Holekamp et al. 2005). PEDF and VEGF are both co-localised in the striatum of patients with Parkinson's disease, and this balance is believed to be critical for changes that the authors speculate to take place in the blood vessel walls in sufferers (Yasuda et al. 2007). We hypothesise that this factor may well be useful for therapy of multiple sclerosis in which patients suffer from breached blood vessel walls (Carvalho et al. 2007).

This inverse correlation also resides in bone epiphyseal growth plates where PEDF is highly expressed in the avascular resting and proliferative zones, and VEGF is mostly in the lowermost layers of the hypertrophic zone (Quan et al. 2003). Fine-tuning of this balance permits the growth plate to switch from an angiostatic to angiogenic state during endochondral ossification. PEDF has now directly been shown to inhibit osteosarcoma from penetrating the avascular resting zones of the growth plate (Dass et al. 2007; Ek et al. 2007a, b) in a clinically relevant model of the disease (Dass et al. 2006). Akin to osteosarcoma, low PEDF and high VEGF levels have been noted in lymphangioma and are believed to enhance pathogenesis in this disorder (Sidel et al. 2005).

Given that increased intratumoural mean vessel density has shown to be associated with a more aggressive and metastatic phenotype in the majority of cancers, reduction of tumour vascularity by PEDF may prove to be a promising candidate for targeted cancer therapy.

3. Delivery – the key to therapeutic success

Native PEDF is either purified from plasma (Sawant et al. 2004) or retinal cells (Steele et al. 1992) using classical liquid chromatography or in a recombinant form, nowadays mostly using a mammalian cell line such as HEK 293 cells (Simonovic et al. 2001). Commercial supplies are also available, but the cost of PEDF is high and is not feasible for *in vivo* studies in large animals where milligram quantities of the protein will be required. However, for *in vitro* and even *in vivo* small animal studies, commercial supplies of PEDF are economical since often in-house synthesis or hiring contractors may turn out to be more costly in the long run. For tumour therapy, the extra need for targeting PEDF to diseased site(s) presents an added challenge. In contrast, for ocular indications, delivery is usually local, precluding the need for bulk amounts of the protein. Furthermore, for ocular indications, it has been demonstrated recently that transscleral movement of PEDF is possible via subconjunctival administration (Amaral et al. 2005).

PEDF is a protein that is 418 amino acids in length, and it has already been elucidated that fragmented versions of the protein (34-mer and 44-mer peptides) possess activities per se (Filleur et al. 2005). In our lab, we have found that a couple of 25-mer peptides have potent activity against osteosarcoma, and we believe that they may have activity against other cancers as well. A major advantage of using shorter peptides is that in the event that the peptide is therapeutic, the cost of medical intervention to the patient is significantly reduced, even by a factor of 100-fold. Shorter peptides also broaden the scope for improving on biodistribution of the active agent in comparison to that of the parent protein. It may also lessen the possibility of immunological recognition of the protein causing adverse effects. Such improvements usually pave the way to enhanced efficacy via increasing the therapeutic window of the peptide. Looking at natural examples such as endostatin and canstatin, antiangiogenic and anticancer peptides derived from much larger proteins, this does not seem implausible. PEDF fragments will need empirical testing in each disease model, at least at the cell culture stage, to determine whether each has potential.

It has already been shown that systemic administration of recombinant PEDF causes tumour regression mediated by a selective effect of the protein on the tumour as well as its vasculature (Zhang et al. 2006), as does administration of a PEDF viral vector (Abe et al. 2004). Such positive findings now have to be verified in these same models as well as others, using drug administration regimens that closely mimic those used clinically. The feasibility of smart drug delivery systems (DDSs) such as nanoparticles need to be evaluated for this protein.

PEDF has both positive and negative charged domains, which could be used for encapsulation in formulations that rely on charge interaction such as chitosan nanoparticles (Dass et al. 2007) or cationic liposomes (Dass and Choong 2006a). Recently, a biocompatible poly(lactide-co-glycolide; PLGA) nanosphere-based delivery system for PEDF peptides has been trialed in the rat eye that showed sustained delivery of PEDF and beneficial results up to 7 days (Li et al. 2006). In addition, devices such as transdermal and invasive controlled release implants may be useful as they have been for a select number of other peptidic agents (Dass and Choong 2006b).

Therefore, for treatment of biomedical conditions with PEDF, smart solutions for restricting the delivery to affected sites are essential. In any case, systemic administration of recombinant PEDF causes tumour regression mediated by a selective effect of the protein on the tumour as well as its vasculature (Abramson et al. 2003). Thus, this sets a very important and promising precedence for PEDF usage.

In concluding there are some promising studies reported highlighting the therapeutic potential of PEDF, whether given as a protein or as shortened peptides based on the full length protein. While a majority of diseases amenable to PEDF therapy will also suffer from an increased vascular pattern in the lesion, this in fact may well serve as the Achille's heel of the pathologies. For cancer, most tumours have well-established vasculatures, for instance osteosarcoma.

Better benefits of PEDF-mediated therapy will eventuate when appropriate drug delivery systems (DDSs) are discovered, optimised and implemented. So far, mostly naked (free, unmodified) protein has been tested in vivo, and it is believed that with such DDSs, enhanced drug activity will be realised.

4. Perspectives

PEDF, whether full length protein or shortened versions of it, can serve as therapeutic agents against a variety of ailments having a perturbed angiogenesis as a driving factor for pathogenesis. A majority of studies have demonstrated that this factor indeed changes the cause of these disor-

ders, which include cancer and ocular lesions. The key to success with this promising new protein lies in the implementation of better means to target treatment to the diseased site.

Acknowledgements: The authors acknowledge the support of the Australian Orthopaedic Association, the Victorian Orthopaedic Research Trust Grant and the Cancer Council of Victoria.

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