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Introduction

Pigment Epithelium-Derived Factor (PEDF) is a potent antiangiogenic agent with neuroprotective activity. A major goal of our Phase II SBIR grant application is to produce large amounts of clinical grade protein which will be used to investigate the therapeutic benefit of PEDF in patients with visual disorders such as wet age-related macular degeneration (AMD) and diabetic retinopathy (DR). A number of purification methodologies have been tested and one identified to be translatable to large scale GMP manufacture. Production has been scaled up to 30 liters and a scheme able to handle larger scale purifications has been devised yielding high quality protein. It has also been shown that hPEDF can be injected intraocularly into the mouse eye with no adverse side effects, and is detectable out to 7 days post injection at a 9 µg dose. Currently, investigation of the hPEDF protein to enhance the longevity of its biological activity and its pharmacokinetic profile is underway.

Background

Currently AMD and DR are the leading causes of blindness within the United States. Wet AMD is estimated to afflict ~1.6 million individuals in the US. This number is expected to grow as more of our population enters their senior years. There is a significant need for new and better treatments for this blinding disease. Based on its multifunctional mechanisms of activity, its potent antiangiogenic properties¹, and its ability to prevent as well as lead to selective regression of abnormal vasculature, hPEDF could provide added benefit over current agents in development.

Diabetic retinopathy also afflicts a very large number of patients. Currently, there are approximately 5.3 million individuals with diabetic retinopathy in the US. The numbers are only predicted to increase with the rise of obesity in this nation. Pre-clinical studies performed with our collaborators using experimental ocular models simulating abnormal blood vessel growth in DR show that increased PEDF expression significantly inhibits retinal neovascularization. Therefore, PEDF is likely to have application for the treatment of DR as well.¹

Lastly, PEDF may have applications to neurodegenerative ocular disorders, since PEDF is also a neuroprotective agent and has demonstrated protective activity in several models of neurodegeneration.²⁻⁶ The implications of these studies suggest PEDF could be used in the treatment of glaucoma, dry AMD and retinal degenerative disorders.

PEDF: Pluripotent Protein with Multiple Mechanisms of Action

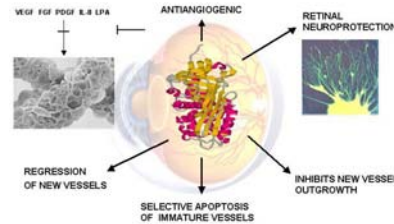


Figure 1: Schematic diagram of PEDF and its known biological activity.

Current Human PEDF (hPEDF) Purification Scheme

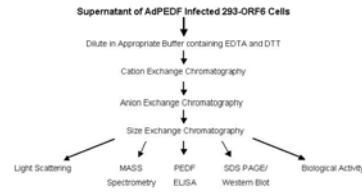


Figure 2: Process flow diagram for purification and biochemical characterization of hPEDF protein.

Purification of hPEDF Yields High Purity Product

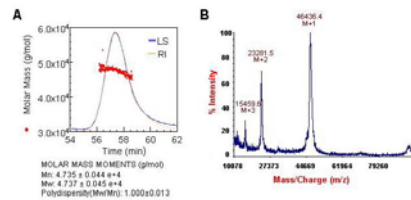


Figure 3: Characterization of purified hPEDF by size exclusion chromatography with multi-angle light scattering (SEC-MALS) and Mass Spectrometry. A. SEC-MALS analysis of purified hPEDF shows a single protein peak and a homogeneous molar mass distribution (red diamonds) indicating a monomeric form for the protein in solution. B. MALDI-TOF mass spectrometry of purified hPEDF shows the expected molecular weight for mature glycosylated hPEDF, M+1 = 46,500 Da. Multiple ionization forms of the protein, M+2 and M+3, are also observed. Also note the absence of contaminant proteins.

Purified hPEDF Is Biologically Active

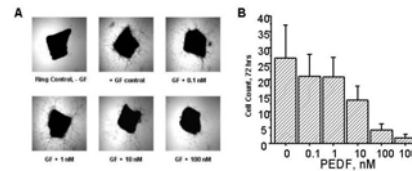


Figure 4: The anti-angiogenic activity of hPEDF was tested in a mouse aortic ring assay. In this assay, 0.5-0.8 mm rings from the aortas of C57BL/6 mice were placed on liquid MatriGel in 24 well plates, and the MatriGel was allowed to solidify at 37°C. Human basal endothelial cell media supplemented with 0.25% mouse serum and antibiotic/antimycotic was then added. The endothelial growth factors bFGF and VEGF were added at 2 ng/mL and 25 ng/mL, respectively, to elicit endothelial cell outgrowth. Various concentrations of purified hPEDF were also added to the media. A: Representative photomicrographs of the aortic rings after 120 hours of incubation with different concentrations of hPEDF; Ring control - GF contains no bFGF or VEGF or hPEDF; +GF control contains bFGF and VEGF but no hPEDF; GF + concentration contains bFGF and VEGF and the stated concentration of hPEDF. B: Quantization of endothelial cell outgrowth by cell count at 72 hours showing the potent anti-angiogenic activity of hPEDF.

hPEDF Protein Levels Rapidly Decline Following a Single Intravitreal Injection

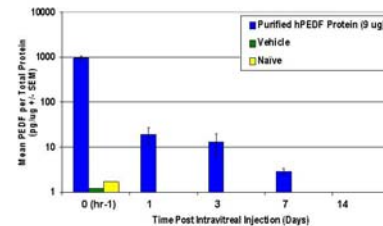


Figure 5: Purified recombinant human PEDF undergoes rapid elimination ($t_{1/2} \sim 3.5$ hours) following a single intravitreal injection (9 µg) in C57BL/6 mice. Approximately 70% of the delivered protein is cleared from the eye within 1 hour, and 99.5% is eliminated within 24 hours. In contrast to the hPEDF elimination profile, the PEDF gene delivered approach (1e9 pu) has an initial accumulation step for the first 24 hours followed by a slower elimination rate ($t_{1/2} \sim 41.0$ hours) over the next six days (data on file). For comparison purposes, a dose of 1e9 pu of AdPEDF corresponds to approximately 250 ng of total protein. hPEDF levels are undetectable at day 14 post-injection.

Next Steps

- Determine biological activity of hPEDF protein in an *in vivo* ocular experimental model
- Investigate variety of excipients to enhance protein stability
- Improve current pharmacokinetic profile of hPEDF
- Further optimize the purification process and assays to generate hPEDF for toxicological studies, and future eventual testing

Conclusions

- Purification method at the 12 liter scale yields pure, full-length hPEDF protein
- Purified hPEDF protein is biologically active as shown in the aortic ring assay and in other *in vitro* assays (data on file)
- Capability to generate up to 30 liters of crude material with high protein production levels

References

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