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THE NEUROPROTECTIVE AND ANGIOGENESIS INHIBITORY SERPIN, PEDF: NEW INSIGHTS INTO PHYLOGENY, FUNCTION, AND SIGNALING

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1. ABSTRACT

Pigment Epithelial-Derived Factor (PEDF) is a non inhibitory serpin with neuroprotective and antiangiogenic actions. It is a potent and broadly acting neurotrophic factor that protects neurons from many regions of the CNS against a wide range of neurodegenerative insults including glutamate toxicity and oxidative stress. PEDF also functions as a natural inhibitor of angiogenesis, targeting the growth of only new vessels. The 50 kD protein is encoded by a single gene that shows strong conservation across phyla from fish to mammals. Two specific domains on the PEDF protein interact with extracellular matrix components and may mediate some of the biological actions of this protein. The transducers through which PEDF signals neurons and endothelial cells are defined and involves major pathways including Akt/NFkB , MAPK, and the caspases. PEDF is widely expressed in the nervous system and in most tissues of the body. A significant amount of the protein is found in the cerebral spinal fluid and circulating plasma as well. Therapeutic administration of the soluble protein or viral-mediated transfer of the gene in experimental in vivo models suggests that PEDF is an excellent pharmacological tool for slowing the progression of a range of neurodegenerative diseases and those pathologies associated with abnormal vessel growth in the eye and metastatic cancers of various tissues.

2. INTRODUCTION

Serpins are of biological importance because they are modulators of proteolytic networks essential to a wide range of physiological processes. Blood clotting defects, inflammation, cirrhosis, emphysema, and dementia are just a few of the disorders associated with defective serpins (1,2) . Some serpins modulate immune function by protecting cells of the immune system from cytotoxic proteases. Others are implicated in molecular signals controlling apoptosis (3,4). Serpins are of structural

Table 1. cross-species conservation of the introns and exons in PEDF

	5'-ncr	exon 1	intron 1	exon 2	intron 2	exon 3	intron 3	exon 4	intron 4	exon 5	intron 5	exon 6	intron 6	exon 7	intron 7	exon 8	3' ncr	bp SAM
Human	100	128	4789	102	2743	199	772	156	567	204	2868	143	1119	211	308	379	100	14888
Mouse	100	108	4449	88	1430	199	449	156	400	204	821	143	2812	211	440	362	100	12472
Chick		54	2542	88	415	199	79	156	778	204	803	143	378	211	361	379		6790
Fugu		46	683	61	77	196	297	156	110	198	81	140	165	211	162	239	100	2922
Xenopus		65		83		178		156		204		143		193		443		
Zebrafish		42		68		190		153		207		140		211		269		

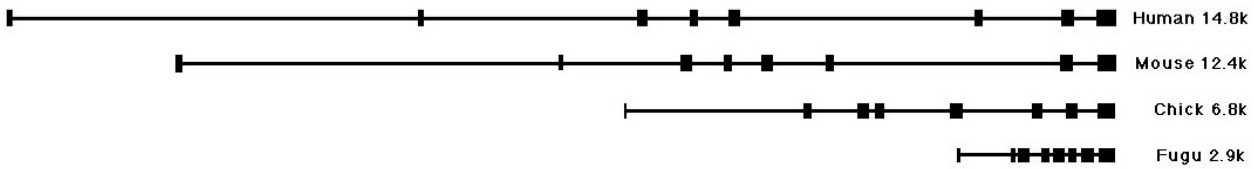

Figure 1. PEDF gene structure is conserved in phylogeny. Exon-intron arrangements of the PEDF gene in human, mouse, chick and fugu. The PEDF gene in each species consists of 8 exons and 7 introns. The considerable overall size difference of the PEDF gene among the species is almost entirely due to the variation in intron size. Black box shows exons and line shows introns.

interest because of their remarkable ligand-binding features and exquisite kinetically controlled folding properties that are critical for inhibition of target proteinases (5,6). Strategically placed hinges, gates, helices, and strands within the molecule allow inhibitory members of the serpin family to adopt a structurally unique conformation that is flexible and which permits finely tuned interactions with target proteases or allows for self-association and polymerization. A subfamily of serpins, however, does not undergo the same conformational transitions as the classical serine protease inhibitors. These include many secreted, cytoplasmic, and nuclear proteins whose structural mechanisms of action are less well understood.

Pigment Epithelium Derived Factor (PEDF) is a member of the second group of serpins. It is not known to target a specific protease but is associated with three important cellular processes: neuroprotection, cell differentiation, and angiogenesis. The 50 kDa protein was first identified as a secretory product of the pigmented cells of the neural retina because of its neurotrophic actions on a human retinoblastoma cell line (7,8) . Like many serpins, PEDF is synthesized by most mammalian tissues, including the brain and spinal cord but thus far, knowledge of much of its function has been limited to the ophthalmic field (9). Characterization of the gene for PEDF has promoted several important studies including structural analysis of the PEDF gene in phylogeny, expression and regulation studies, gene therapy, derivation the PEDF crystal structure, chromosomal localization, and structural analysis with other members of the serpin gene family (9,10).

3. PEDF Structure-Function relationships

3.1. Gene Structure

Recently we performed a comprehensive search of all available genomic databases and have identified the PEDFs from an additional 9 species, bringing the total to 14 including those that have been previously cloned and sequenced from human, mouse, bovine, *Xenopus tropicalis*, and zebra fish (11). The computer-assembled sequences are from chimpanzee, dog, pig, rat, chicken, *Xenopus Laevis*, trout, medaka, and *fugu*. Alignment of the PEDF gene of those species for which genomic data are available shows structural conservation of the 8 exons and 7 introns gene structure, a characteristic feature of the genes for many serpins (Figure 1). While there is strong conservation of exon sizes, there is significant variation in intron sizes of the PEDFs (Table 1). The overall gene size for PEDF in phylogeny varies from 14.8 kb in human to 2.9 kb in *Fugu*. The PEDF promoter regions are less homologous in phylogeny with only the proximal 200 bp showing extensive similarity between species. The promoter region and introns of mammalian PEDF contain a large number of repetitive DNA elements, a putative CAAT box, and several transcription binding elements including a retinoic acid receptor motif (RARE) [9]. In promoter mapping studies, we show that this RARE may be functional since RPE and glioma cells transfected with the PEDF promoter region containing this element respond to all trans retinoic acid treatment by increasing luciferase activity (12).

3.2. Chromosomal localization

We used FISH and linkage analysis to map the PEDF gene to human chromosome 17p13.3 and to mouse chromosome 11B4 and showed that the fine structure of these regions is conserved in other species including chick and *Fugu* (11,13,14). Flanking the gene in both humans and mouse is Serpin F2 (α 2-antiplasmin), an inhibitory serpin, and Smyd4, a gene of unknown function (Figure 2). Interestingly, serpin F2, an inhibitory serpin, is the gene that is most closely related to PEDF in phylogeny suggesting that both genes could have evolved from the duplication of a single ancestral serpin gene. Since inhibitory serpins are found much earlier in evolution, it is likely that serpin F2 retained its functional and structural similarities to the ancestral gene while PEDF diverged from adopting the serpin-like conformational transitions essential for the actions of many inhibitory serpins.

3.3. Phylogenetic analysis

Serpins are organized into 16 clades based on the sequence similarities of the proteins and exon-intron

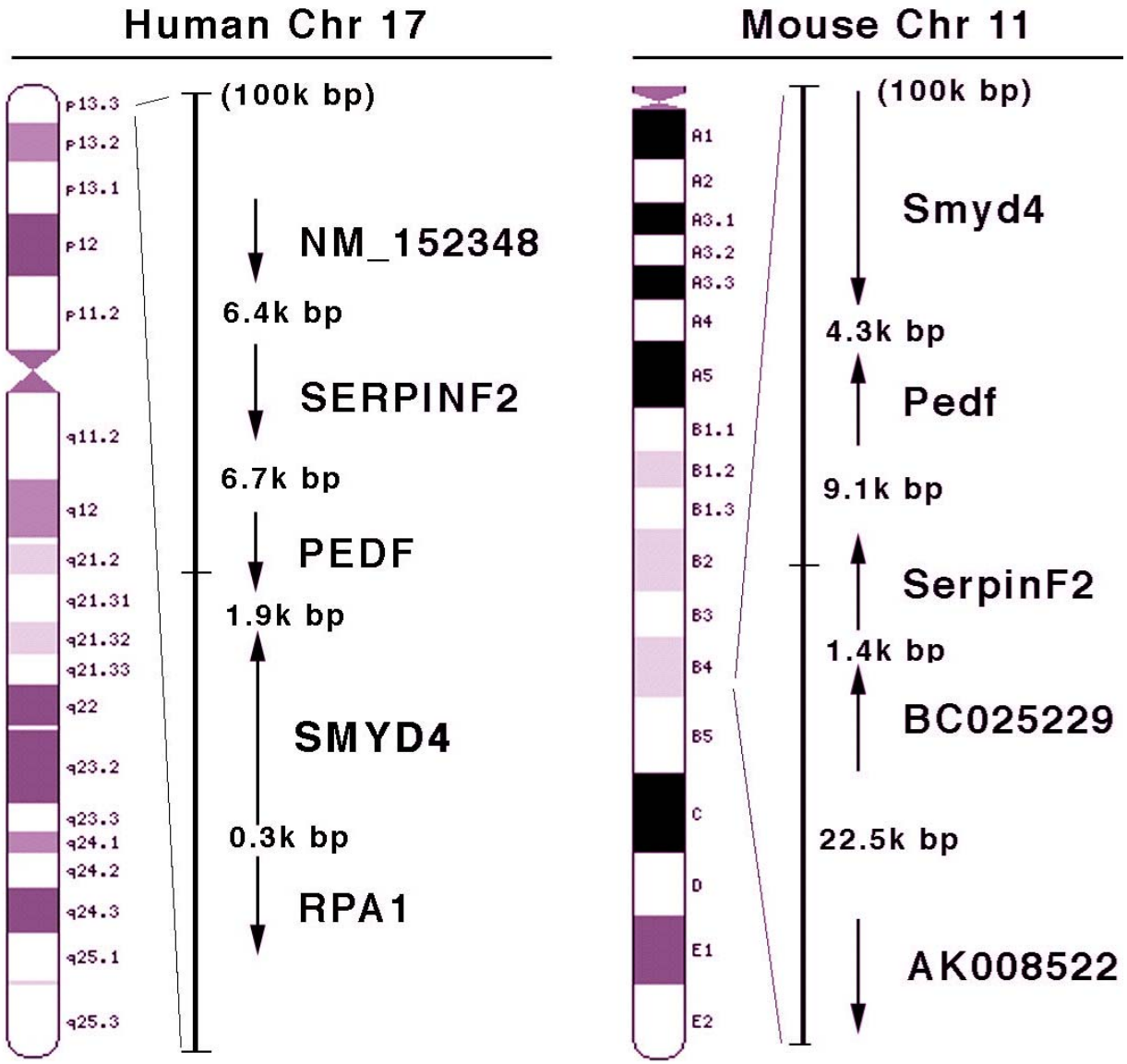

Figure 2. Map location of human and mouse PEDF. In the expanded views, a region of 100 kb of human chromosome 17p13.3 and mouse 11B4 are shown. The genes, their direction, and the intergenic distances are indicated on the maps. Chr 17, Chromosome 17; Chr 11, Chromosome 11.

organization of the genes (15,16). PEDF was placed in the serpin F1 clade based on the few mammalian sequences available. We used computational tools to extend our previous work on the evolutionary conservation and genomic structure of human PEDF to identify PEDF sequences in other species and to show their relatedness to mammalian PEDF as well as to other serpins (Figure 3) (9). Phylogenetic clustering of PEDF with other serpins indicates that there is a close relationship between PEDF and the protease inhibitors serpin F2/α2-antiplasmin and serpin G1/C1 inhibitor. Alpha 2-antiplasmin and C1 inhibitors are both inhibitory serpins involved in maintaining vascular integrity and function. Deficiency of C1 inhibitor leads to increased vascular permeability and angioedema (17). Deficiency of alpha 2-antiplasmin leads to uncontrollable bleeding (18). These three serpins, PEDF, alpha-2-antiplasmin, and C1 inhibitor, are not only phylogenetically close relatives but are secreted in the blood and are associated with various aspects of blood vessel function. Because functional constraints tend to conserve essential biological sequences between distantly related organisms, the molecular phylogeny of PEDF will undoubtedly provide insights into its regulation and structure-function relationships.

3.4. Protein Structure and homologies with other serpins

A number of features characterize the PEDF subgroup of clade f serpins. A leader domain is present in all the PEDF sequences we have examined suggesting that

Table 2. Cross Species alignment and Homology of the PEDF Leader Sequence

		Hs	Chimp	Bt	Pig	Mm	Rt	Chick	Xt	Xl	Fugu	Trout	Zebra
	** ***** * ****												
Hs	MQALVLLLCIGALLGHSSC	100											
Chimp	MQALVLLLCIGALLGHSSC	100	100										
Bt	MQALVLLLWTGALLGFGRC	75	75	100									
Pig	MQALVLLLWTGALLGSGSC	80	80	90	100								
Mm	MQALVLLLWTGALLGHGSS	80	80	85	90	100							
Rt	MQTLVLLLWTGALLGHGSS	75	75	80	85	95	100						
Chick	MQIPAVLLLLGLLTIPSKS	40	40	35	35	40	40	100					
Xt	MKIYLALLFTGSFLSYTSA	35	35	35	40	40	40	30	100				
Xl	MKIYLALLFTGTFLSSTSA	35	35	35	45	40	40	30	90	100			
Fugu	MKGTTFLLVIGVILRFCQA	35	35	35	30	30	30	25	40	40	100		
Trout	MMRTTLLLCLGFLLSLSYA	50	50	40	40	40	40	40	40	40	45	100	
Zebra	MKKIVLLVGLWSLLSLSHA	40	40	35	35	35	35	30	40	35	30	55	100
	* *												

An asterisk above the sequence indicates absolute conservation among mammals. An asterisk below the sequence indicates absolute conservation across all species

the biological activity of PEDF is, in part, tightly linked to its actions as a secreted protein (Table 2). Like other inhibitory and non-inhibitory serpins, PEDF has a Reactive Center Loop (RCL) region. The PEDF RCL is conserved in evolution and has certain features unique to this serpin. PEDF falls within the size range of most serpins. The protein has a molecular weight of 50 kD and contains an open reading frame of 418 amino acids which is organized into beta sheets A, B, and C and 9 alpha helices as is seen in most other inhibitory and non-inhibitory serpins. From cross species alignment of the 14 PEDF sequences, we identified conserved structural domains in the gene in addition to the leader sequence and RCL. A single C-terminal glycosylation site, collagen binding residues, and four specific PEDF peptides are conserved among the species. The C-terminus containing residues 384-415 and an N-terminal region containing residues 78-95, are highly homologous with 97 and 39 inhibitory serpins, respectively. These regions are believed to be structurally important to serpins. There is also strong conservation of 39 of the 51 consensus key residues involved in serpin structure and function. Much less homology was found between PEDF and known non-inhibitory serpins. Two peptide regions containing residues 40-67 and 277-301, are unique to PEDF and are highly conserved across species. These domains may be important in mediating the specific actions of PEDF. Conserved residues at the N-terminus, helix D, and helix A of PEDF form a structure similar to the heparin-binding groove of other serpins (19).

3.4.1. PEDF reactive center loop (RCL)

Serpins use finely tuned conformational transitions, unique to their structure, to inhibit target proteases (20-23). The reactive center loop of the few inhibitory serpins that have been studied so far, is shown to be a flexible structure that mediates protease inhibitory function. Like other serpins, PEDF has an RCL comprising 17 amino acids and an absolutely conserved glycine residue at the P15 position. However, PEDF is a non-loop insertable, non-inhibitory serpin because its RCL lacks the tetrad of alanine residues between P12 and P9, which predicts alpha helix structure in the N-terminal of the reactive center region of inhibitory serpins (23). It is hypothesized that the P12-P9 alanine residues in the RCL may be linked to the inhibitory properties of the serpins rather than an alpha helix structural requirement of the RCL for serpins to adopt a metastable conformation (23). In phylogeny, the sequence of the PEDF RCL shows some variation but in all cases it appears to be a prominent canonical structure that extends from the molecule (24). The three proline residues found in the PEDF RCL suggests that it is unlikely to interact with target proteases in the same way that classical serpins have been shown to behave (25). Although its function is not yet elucidated, the PEDF RCL is a prominently exposed target for interactions with diffusible factors and matrix molecules that could augment PEDF angioinhibitory and neuroprotective functions.

3.4.2. Leader Sequence

PEDF is secreted as a 50 kDa protein in vitro and in vivo and its transcripts are widely distributed in almost all human tissues (7,9). Alignment of the PEDF sequences across phyla, which represent approximately 300 million years of evolution, shows that PEDF has a hydrophobic signal sequence that is highly conserved among mammals with a number of conservative substitutions in species of other phyla (Table 2). The biological activity of PEDF is probably tightly linked to its secretion, although functions within the cell cannot be excluded. It is estimated that approximately 20% of all serpins are secreted in the blood. Both intracellular and extracelluar branches of the serpin family regulate a wide range of blood diseases and control vascular integrity. Although the secreted PEDF plays an important role in neovascular diseases, there is new evidence presented in this review suggesting that PEDF may also act intracellularly.

3.4.3. PEDF extracellular matrix binding domains

There are several exposed domains on the PEDF protein, based on the crystal structure of the 418 amino acid human PEDF which was resolved at 2.85Å (24). There are two sites on PEDF for interactions with extracellular matrix molecules that may contribute to both the neurotrophic and antiangiogenic actions of PEDF (24). There is a concentration of aspartic and glutamic acid side chains in the N-terminal portion of the protein, which promote binding with high affinity to type I collagen and with lower affinity to type III collagen (26-28). When measured by surface plasmon resonance in 10 mM salt, the dissociation

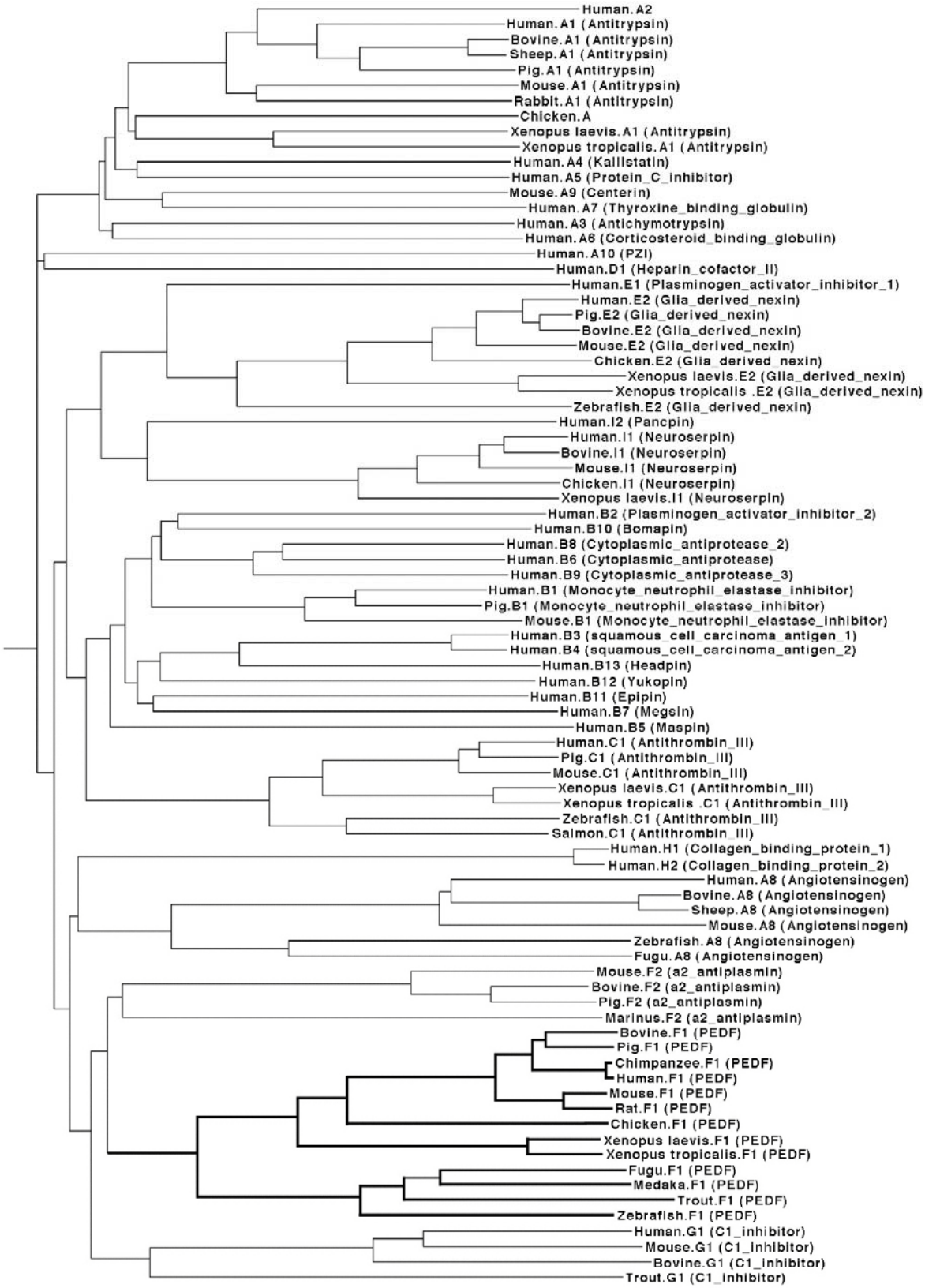

Figure 3. Phylogenetic relationship of PEDF to other serpins. A total of 87 serpins with 13 PEDF sequences were used for this analysis. Related serpins are organized in the various clades (A-I) and are listed on the right of the dendrogram.

	Species	PEDF Residues
		134 151
	Hs	KNLKS ASRIVFEKKL RIK
	Chimp	KNLKS ASRIVFEKKL RIK
	Bt	KNLKS ASRIIFERKL RIK
	Pig	KNLKS ASRIIFEKKL RIK
	Mm	KNLKR ASRIVFERKL RVK
	Rn	KNFKS ASRIVFERKL RVK
	Chick	KSLKS ASRIIVEKRL RVK
	Xt	SGLKS TWRIMLERRL RLR
	Xl	SGLKS TWRIMLERKL RLR
	Fugu	KGLST AARLYLSRRL RLK
	Medaka	KGLST AARLYLARRL RPK
	Trout	KGLSI AARVYLARRL RLK
	Zebra	KGFKS AERILLARKL RLR
A	*Clustal Consensus*	..:. : *: . ::* * :
	Human PEDF	KNLKSASRIVFEKKLRIK
	Monopartite	
	HSFA1	KHLLKSISRRKPAHG
	HSFA2	KHLLKTIKRRRNVGQ
	Bipartite	
	LANA2	RRHERPTTRRIRHRKLRS
	Human pRb	KRSAEGSNPPKPL-KKLR
	Mouse RB	KRSAEGPNPPKPLKNVR
	Xenopus RB	KRSADTGTTPKLPKKLR
	Human IL5	KKYIDGQKKKCGEERRR
	Human p53	KRALPNNTSSSPQPKKKP
	Nucleoplasmin	KRPAATKKAGQA–KKKK
	ICP22 NLS1	RRPALRSPPLGT–RKRK
	N1N2	RKKRKTEEESPLKDKAKKSK
	SWI5	KKYENVVIKRSPRKRGRPRK
	Human IL-5	KKYIDGQKKKCGEERRRVNQ
	MAPKAP Kinase 2	KKIEDASNPLLL–KRRKK
B	P55-C-fos proto-oncogene protein	RRERNKMAAAKSRNRR

Figure 4. A putative NLS motif in PEDF. **A**. cross-species alignment of PEDF NLS. Residue 134- 151 is based on the precursor human PEDF protein. Clustal consensus sequences are labeled '*' indicating positions which have a single, fully conserved residue; ':' shows one of the 'strong' groups is fully conserved; and '.' indicates that one of the 'weaker' groups is fully conserved. **B**. Nuclear localization sequences in other proteins. Bold letter shows key residues in the NLS.

constant for PEDF binding to type I collagen was found to be 8 nM (28). PEDF binds collagen in physiological salt solution, implying that this interaction is likely to take place in vivo as well (27). Low affinity interactions between PEDF and heparan and other glycosaminoglycans have also been found. These are probably mediated by a large surface rich in the basic amino acids**,** lysine and arginine (24, 29). Binding to heparan, collagen or both is critical for the activation and function of several serpins including the well studied CI-inhibitor and antithrombin (30,25). Interaction with other extracellular matrix components is important as well to the function of factors, such as the FGF gene family.

As with other serpins, the heparan-binding domain of PEDF could be important to its antiangiogenic activity. Actively proliferating endothelial cells are shown to secrete extracellular matrix in culture, which contains heparan sulfate as the predominant glycosaminoglycans (GAGs) as compared to the extracellular matrix of confluent and post confluent endothelial cells, which contains dermatan sulfate as the major component. In these cultures, the activity of the endothelial cell mitogen FGF, whose binding to its cognate receptor on endothelial cells is strongly mediated by heparan sulfate, is impaired on post confluent endothelial cell cultures but not on mitotic cultures (31.32). Heparan sulfate promotes ligand-receptor interactions between other angiogenesis factors and their receptors on endothelial cells as well. For example, heparan sulfate tethers angiopoietin 3 to the cell surface where it can interact with its receptor Tie 2 to transmit mitogenic signals to the endothelial cells (33). A significant role for heparan sulfate in angiogenesis is also seen in studies that show FGF-2-induced corneal neovascularization is inhibited in percalan heparan sulfate deficient mice (34). The local concentration of heparan sulfate and its interaction with promoters and inhibitors of angiogenesis is thus an important one in modulating the angiogenesis process. Similarly, it is likely that heparan sulfate could tether and localize PEDF to newly sprouting vessels, where a critical concentration of PEDF is essential for angioinhibitory activities. Whether interactions with extracellular matrix components modulate the activity of PEDF or provide a local reservoir of functional PEDF that could be released under specific normal or pathological conditions is currently an area of research focus. Another possible mechanism of regulating PEDF activity is suggested by a recent report that extracellular phosphorylation of PEDF plays a key role in controlling both the anti-angiogenic and the neuroprotective activities of PEDF (35). The extent of phosphorylation of the serine residues in vivo is not clear since a complete map of all posttranslational modifications of serum PEDF does not indicate the presence of any phosphate groups (36). If phosphorylation of PEDF occurs, it still has to be shown that it is a regulated process and responsive to pathogenic stimuli.

3.4.4. Putative PEDF Nuclear Localization Signal

We recently identified a motif in PEDF, KKLR, at residues 146-149, in the loop region between sheet 2A and helix E, that is homologous to the nuclear localization signals of several other proteins that function in the nucleus (Figure 4) (19). Yasui et al, however, have performed site mutagenesis studies, which showed that these residues (Arg^{145}, Lys^{146} and Arg^{148} in recombinant mouse PEDF) contribute to the interaction between PEDF and heparin (28). It is possible that the heparin binding domain of PEDF also function as a nuclear localization signal as is seen for other proteins such as superoxide dismutase or nuclear localization is mediated by heparin sulfate proteoglycans as is shown for fibroblast growth factor (39,107). Immunocytochemistry and western blot analysis of nuclear and cytoplasmic fractions of cells indicate that there is a bitopographical localization of PEDF in cells (Figure 5). The KKLR motif is present in other proteins that function in the nucleus including the retinoblastoma protein, a major regulator of cell proliferation, and the

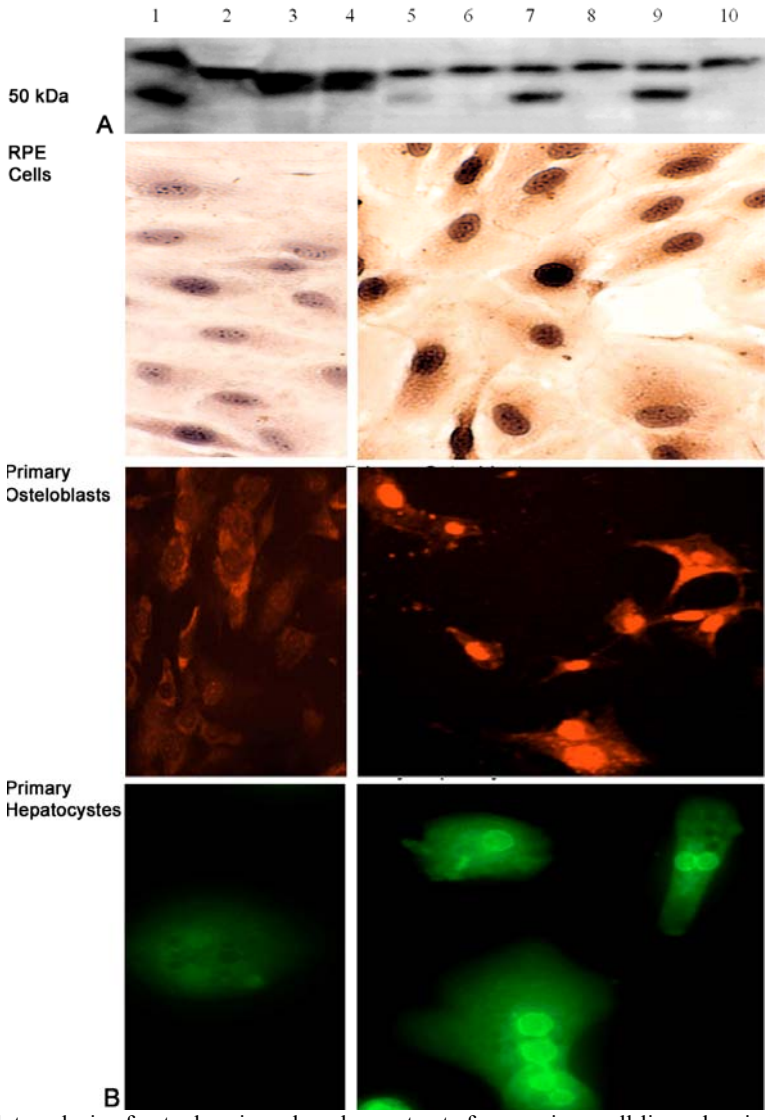

Figure 5 A: Western blot analysis of cytoplasmic and nuclear extracts from various cell lines showing levels of PEDF in each cellular compartment. The fractions were extracted using Pierce nuclear and cytoplasmic extraction reagents. 10 microgram of each sample was loaded onto a 10% SDS gel and electrophoresed by conventional methods. The proteins were transferred to nitrocellulose membranes and signal detected with a PEDF polyclonal antibody using western blotting reagents from Biorad. 1; MWS, 2. positive control, RPE conditioned-medium, 3,4 cytoplasmic and nuclear extract of human A-RPE-19 cells, respectively. 5,6. cytoplasmic and nuclear extract of human Y79 retinoblastoma cells, respectively. 7,8. cytoplasmic and nuclear extract of a human neuroblastoma cell line, respectively. 9,10. cytoplasmic and nuclear extract of human HepG2 cells (hepatocellular carcinoma cell line). The data show that a strong 50 kDa band is present in all cytoplasmic and nuclear samples. An extra band at approximately 36 kDa is observed in cytoplasmic preparations but not in the nuclear fractions suggesting that the preparations are not contaminated with each other. The lower band most likely represents non-specific binding of PEDF and has been seen in several studies using different PEDF antibodies including monoclonal antibodies. Preliminary mass spectrometry data indicate that this is not a PEDF fragment. B: Immunolocalization of PEDF in the cytoplasm and nucleus of human RPE cells. All cultures were maintained in DMEM + 10% FBS. Prior to immunolabeling, the cells were fixed with 4% paraformaldehyde for 10 min, washed, and non-specific binding sites blocked with normal goat serum. The samples were incubated in the primary antibody or goat serum (control) for 1 hr, washed, and subsequently incubated with the secondary antibody (goat-anti-rabbit IgG conjugated to either Texas red (primary osteoblasts) or FITC (primary hepatocytes). Streptavidin conjugated horseradish peroxidase was used for immunohistochemistry (RPE cells). Left panels: goat serum control for A-RPE-19 (top), primary osteoblasts (middle), primary hepatocytes (bottom). Right panels: Cells labeled by a PEDF polyclonal antibody with the same top to bottom labeling as for the controls. The data show that PEDF is distributed in both the cytoplasmic and nuclear compartments of the cells with some nuclei more densely stained that others possibly reflecting the cell cycle activity state of the cells.

serpin MENT, which is found in the nucleus of several avian blood cell types and which inhibits the nuclear cystein proteinases, cathepsin L and V. In MENT, this motif is present in the "M loop" (positions 60 – 91) which extends out from the molecule and which is not found in other serpins (37). Ectopic expression of MENT leads to its sequestration in the nucleus, a process which can be blocked by mutation or deletion of the basic residues in the M-loop (38). MENT induces chromatin condensation by a process thought to involve both the M-loop and RCL of MENT. Although it is unclear why PEDF accumulates in the nucleus, there is strong evidence that the protein promotes cell differentiation and regulates specific cell cycle processes such as the cells transition from G1 to G0 stage, which may, in part, explain its presence in the nucleus (40).

3.5. Polymorphisms in the PEDF Gene

The region of chromosome 17p to which PEDF maps is a hot spot for several neurodegenerative diseases including at least 5 other retinal degenerative diseases. The DNA of patients with Leber's congenital amaurosis (LCA) (17p13.1) and Retinitis Pigmentosa (RP) (17p13.3), two ocular diseases resulting in severe visual impairment, were genotyped using markers flanking the PEDF gene, PCR-SSCP analysis, and direct sequencing. Although PEDF was excluded as a common cause of either of the disease, several intragenic single nucleotide polymorphisms in the gene have been identified. These include a Met72Thr polymorphism in exon 3, Thr130Thr in exon 4, a G to A transition in intron 5, and a Tyr321Tyr polymorphism in exon 7 in the PEDF gene of LCA patients (41). A T to C and a G to C substitution were also indentified in exon 4, codon 130 and 132 of the PEDF gene, resulting in a neutral and missense mutation respectively (14). Although PEDF is excluded from these diseases, the polymorphisms are informative and are useful in facilitating gene linkage studies for other candidate diseases.

4. PEDF EXPRESSION

PEDF was first identified as a neurotrophic factor in conditioned medium obtained from fetal human RPE cell cultures. At concentrations as low as 1 nM, the purified 50kDa protein effectively switched Y79 retinoblastoma cells from an actively growing suspension cell line to non-proliferating cells that attached to a substrate, extended neurites, and increased expression of molecules associated with differentiated neurons (7,8).

The interphotoreceptor matrix, a matrix bathing the apical projections of the RPE cells and adjacent photoreceptors, is a major reservoir for the PEDF secreted by RPE cells in vivo (42). The vitreous contains a significant amount of PEDF as well, possibly from sources such as the retinal ganglion cells, several cell types in the cornea, and the non-pigmented ciliary epithelial cells, which have all been shown to synthesize the protein (43-45, 78). Detection of PEDF in cultured Muller cells suggests that other types of retinal cells can also be induced to express this protein (12)

PEDF transcripts are found in many other regions of the central nervous system, the brain and spinal cord (). and the protein detected in cerebral spinal fluid (CSF). Ependymal cells are responsible for some of the PEDF detected in the CSF, but a variety of neurons also express this protein. In the spinal cord, the protein is localized to motor neurons of the ventral horn and some neurons in the dorsal horn. In the brain, PEDF mRNA is transcribed in almost all regions. Thus, like the retina, much of the brain and spinal cord are bathed in this neurotrophic factor. Several non-neural tissues including skeletal muscle, bone, heart, placenta, testis, ovaries, and liver also synthesize PEDF but its function in these organs is not yet elucidated (9,46,47).

5. BIOLOGICAL ACTIVITY OF PEDF

Serpins have a wide range of physiological functions in the body. Some serpins function in the nervous system and are involved in pathogenesis of diseases such as Parkinsons and Alzheimers disease. Others function in the circulatory and in the immune systems. PEDF has been identified in almost all human tissues and in most extracellular fluids. Three important biological activities have been attributed to PEDF: angiogenesis, cell differentiation, and neuroprotection, indicating that this serpin functions in more that one target region of the body.

5.1. PEDF and Angiogenesis

5.1.1. PEDF inhibits ocular angiogenesis

The vasculature of most tissues is held in a state of quiescence through a finely tuned balance between pro- and anti-angiogenic factors. Normal tissue repair, reproductive cycles, and wound healing represent a few adult processes that require neovascularization and which stimulate physiological angiogenesis (48,49). Abnormal blood vessels can be generated in response to numerous pathological stimuli as well. These are often leaky and underlie the progression of most tumors, many inflammatory conditions, and a wide range of human disorders (50).

Pathological angiogenesis is by far the most common aspect of eye diseases. Corneal lesions and inflammatory ocular diseases have a strong angiogenesis component and are the most common causes of visits to ophthalmologists. Proliferative diabetic retinopathy (PDR) and age-related macular degeneration (AMD), two blinding eye disease with a neovascular phenotype, affect over 7 million people in the USA alone. Pathological angiogenesis is an important component of many other diseases of the eye as well.

Dozens of pro- and anti-angiogenic factors that are essential to maintain vascular quiescence in the adults have been identified. Gene knockout and transgenic animal studies implicate vascular endothelial growth factor (VEGF) as one of the major initiators of both normal and pathological angiogenesis (51,52). There is growing evidence that one of the key endogeneous factors that antagonize VEGF proangiogenic activity is Pigment Epithelium Derived Factor (PEDF).

An antiangiogenic role for PEDF in the retina emerged when Dawson et al. (53) showed that PEDF inhibited angiogenic processes and was more effective than the well studied angiogenesis inhibitor angiostatin. In those studies, PEDF prevented endothelial cell migration alone or in the presence of the potent proangiogenic factors, FGF1, FGF2, VEGF, interleukin-8, and lysophosphatic acid.

In gene therapeutic strategies, PEDF reduces blood vessel growth in the eye using viral-mediated gene transfer approaches (54). Ocular injection of an adenoviral construct containing the PEDF gene inhibits the formation of both retinal and choroidal neovascularization in mouse models of ocular angiogenesis. Even more importantly, PEDF causes regression of neovascularization already underway (55,56). In other studies where mice are placed in hyperoxic conditions, intraocular application of an AAV-PEDF vector results in high levels of PEDF expression in the eye over extended periods and a significant correlation with reduced development of ocular vessels was evident (57,58) . Choroidal neovascularization arising from laser-induced damage to Bruch's membrane can also be inhibited by both intravitreous and subretinal injections of AAV-PEDF (59). These studies are convincing and indicate that PEDF establishes specific mechanisms of interference that mitigate vascular growth propelling signals.

In addition to the growth of new vessels, many ocular problems arise because of increased vessel permeability. One of the earliest activities observed for VEGF was increased permeability of blood vessels (60). Recently, it has been shown that PEDF cancels VEGF-induced increases in vascular permeability (61). Co-injection of PEDF with VEGF into mouse eyes results in much lower fluorescein leakage than in eyes injected with VEGF alone. Over 95% of the VEGF induced permeability can be abolished by PEDF as determined by quantitative assays using Evans Blue. We have recently shown that PEDF increases the expression of VEGF-C and its cognate receptor VEGF R3, both of which are implicated in the control of vascular leakage in physiological conditions (62,63). This additional activity of PEDF strengthens the argument for its use as a major component of any ocular neovascular disease therapy.

Controlling the growth of blood vessels, therefore, offers a unique opportunity to impact a wide spectrum of physiological and pathological functions.

5.1.2. PEDF inhibits tumor angiogenesis and promotes cell differentiation

The antiangiogenic activities of PEDF are not limited to neovascular eye diseases. There is evidence that the action of PEDF on tumor regression is two fold: partly due to its antiangiogenic properties and partly due to cell differentiation effects. The original identification of PEDF was directly related to a measure of its differentiation and antiproliferative actions on human retinoblastoma cells (7,8). This differentiation activity is also noticed in primitive neuroblastoma which, when treated with the protein, is converted into the less malignant ganglionic or other cell types that**,** in turn**,** produces more PEDF (64). In support of this biological activity, it is shown that PEDF expression is lost in metastatic subclones of some tumors, and that there is allelic loss of the PEDF gene in others (65-67). In addition, there is evidence that mouse lung cancer cells infected with a PEDF adenovirus construct have less tumor burden, and that the proliferation rates decrease in melanoma cells transfected to express PEDF (68,69). In human melanoma xenografts in mice, PEDF inhibits both liver and lung migration as well subcutaneous survival of the tumor cells thereby reducing the melanoma tumor burden in these animal (70).

In many of the tumor studies, it is difficult to separate direct anti-tumor effects of PEDF from its powerful antiangiogenic activity. In numerous models including lung carcinoma, hepatocellular carcinoma, melanoma, elevating the levels of PEDF reduces the growth of blood vessels into the tumor thereby reducing the tumor mass (69-72). Conversely, a decrease in the levels of PEDF can result in tumor formation. This has been shown in studies where transformation of VEGF deficient fibroblasts with either ras or neu oncogenes decreased synthesis of the antiangiogenic molecules, PEDF and TSP1, resulting in highly tumorigenic and angiogenic fibrosarcomas (73). These experimental studies are supported by clinical observations that patients whose tumors have higher levels of PEDF expression show fewer metastases and have a better prognosis (74).

These actions of PEDF on cell proliferation and blood vessel growth are further confirmed in a PEDF knockout mouse strain (75) where a lack of PEDF expression results in hyperplasia of organs such as the prostate and increased microvasculature in several tissues including the retina. By shifting the balance between proliferation and differentiation, PEDF slows the expansion of tumor cells (64,71,76). PEDF may alter this balance in tumor cells by mediating the movement of cells into growth arrest stage as seen in cultured fibroblasts where PEDF promotes exit from the cell cycle and movement into a G0 phase (40). PEDF also inhibits the growth of endothelial cells that form new blood vessels in cancers and in some neovascular diseases, particularly in the eye (53,69,76-77). Its secretion in the hypertrophic layer of epiphyseal cartilage is shown to form a barrier to osteosarcoma invasion into the matrix (79). The multiple biological roles of this serpin are confirmed in PEDF null mice. These mice show epithelial hyperplasia, increased endothelial cell proliferation, and increased microvessel density in several organs (75).

Based on these findings, it could be argued that PEDF contributes to vascular quiescence, in part, by maintaining the differentiated state of endothelial cells and by inhibiting growth promoting signals that lead to the aberrant proliferation of these cells in neovascular diseases

5.1.3. A Role for PEDF in sculpting the vasculature during normal embryonic development

PEDF is detected early in human and mouse development (42,78). The embryonic expression of this serpin suggests that it may play a role in early vasculogenesis as well, although there is currently little information on the ways in which anti-angiogenic factors, such as PEDF, can regulate this process. As a factor that is shown

to bind to specific extracellular matrix components, PEDF could promote the spatial definition of developing vessel pathways. Such a role has been postulated for PEDF in the hypophyseal plate of bone and the uterine endometrium (79,80). This could also be true in the eye where the concentration of PEDF in the limbal region of the cornea may be part of the barrier that keeps the cornea avascular.

5.2. PEDF and Neuroprotection

While the aberrant growth of blood vessels is a major factor contributing to the progression of neovascular eye disease, it is the damage that it does to surrounding tissues that ultimately leads to visual loss or progression of the disease. In the retina, invading leaky choroidal vessels contributes to the degeneration of the RPE and photoreceptor cells as seen in the wet form of age related macular degeneration. In this condition, controlling neovascularization alone does not eliminate the degeneration already in progress unless there is very early diagnosis. Thus, it is important that neuroprotective factors are an essential component of therapeutic strategies for these pathologies.

In addition to its antiangiogenic properties, PEDF is an effective neuroprotective factor in many parts of the nervous system. It interferes with progression of neurodegenerative cascades that are promoted by axotomy, glutamate excitotoxicity, and oxidative stress in many types of neurons ,damage induced by removal of trophic support or exposure to chronic bright light (81-83).

In the eye, PEDF reduces apoptosis induced by H_2O_2 or light damage in rat photoreceptors, preserves the spatial organization, morphology, and function of photoreceptors and Muller cells after RPE detachment in a *Xenopus* model of retinal degeneration, and protects retinal neurons from injuries caused by increased intraocular pressure from transient ischemia and reperfusion (82,84-86). In cells of other parts of the nervous system, such as cerebellar granule cells, hippocampal neurons and spinal cord motor neurons, nanogram amounts of PEDF provide protection from the damaging effects of glutamate toxicity (87-89). These protective effects add to the value of PEDF as a therapeutic factor since neurodegeneration is a common result of neovascular disease.

Summarizing the data presented above, we can conclude that PEDF facilitates cell movement into a quiescent phase of the cell cycle, aids in differentiation, protects neurons from damage, and blocks angiogenesis. It is possible that these actions reflect activation of a few key signaling molecules that receive input from a wide spectrum of normal and pathological stimuli.

6. PEDF MECHANISM OF ACTION

6.1. PEDF and VEGF strike a balance in angiogenesis

As mentioned earlier, there are many pro- and anti-angiogenic factors capable of modulating vessel growth, though not all play an equal role in this process. There is increasing evidence that, at least in the eye, the balance between the pro-angiogenic factor, VEGF, and the anti-angiogenic factor, PEDF, appears to determine the formation of new vessel (90). Both factors are expressed early in embryological development. For example, in the highly vascularized liver, PEDF and VEGF are coexpressed, although there is differential expression of the VEGF isoforms during development [47]. In the adult, VEGF levels increase and PEDF levels decrease in several angiogenic diseases of the eye. For example, an inverse relationship is noted between these two factors in the vitreous of patients with PDR and AMD, suggesting an underlying cooperative relationship between these proteins in maintaining vascular quiescence. Analogous observations that PEDF levels are lower in the vitreous of active compared with inactive forms of diabetic retinopathy and that PEDF and VEGF are detected in choroidal neovascular membranes and polypoidal choroidal vasculopathy, with decreased expression of both in new vessels where fibrosis is present further support their relationship in the angiogenesis process (91-96).

While it is attractive to envisage a direct and unique relationship between PEDF and VEGF, there is currently no evidence to support the direct regulation of one molecule by the other at the molecular level. We have shown that PEDF does not alter VEGF transcription in either basal or hypoxia-stimulated conditions, and that VEGF does not alter the level of PEDF transcripts in vitro (62). However, PEDF reduces the transcription of the Flk-1 (VEGF-R2) receptor in the retina, a vascularized tissue, as well as in a cell line derived from the retinal retinal pigment epithelium (Figure 6A), suggesting that one way in which PEDF might antagonize the mitogenic activity of VEGF is by reducing the availability of the key angiogenesis promoting Flk-1 receptor (62).

6.2. PEDF signaling

The evidence that PEDF exerts neuroprotective effects in the nervous system and apoptosis in endothelial cells appears contradictory. How does PEDF intercept growth-promoting signals, accelerate cell death cascades, and prolong cellular life span? Are the different activities of PEDF based on different receptors? Or do separate fragments of PEDF contain different biological activities? Could cellular diversity or modulations in the cellular environment account for the variations in response to PEDF?

Unfortunately, a receptor for PEDF has not yet been identified to allow us to clearly address these questions. An 80 kDa PEDF binding protein has been detected on Y79, cerebellar granule cells, and the retina but whether this is a receptor for PEDF or an associated regulatory protein is still not clear(97). The activities of many proteins including serpins are not receptor mediated and often occur because of interactions with other ligands. There are data, which show that PEDF, whether through binding to a specific receptor or other proteins, activates specific signaling molecules in both neurons and endothelial cells and that PEDF uses specific signaling modules to transmit its biological actions.

6.2.1. Angiogenic signaling

In addition to its transcriptional regulation of the VEGFR2/KDR/Flk-1 receptor, PEDF modulates activation

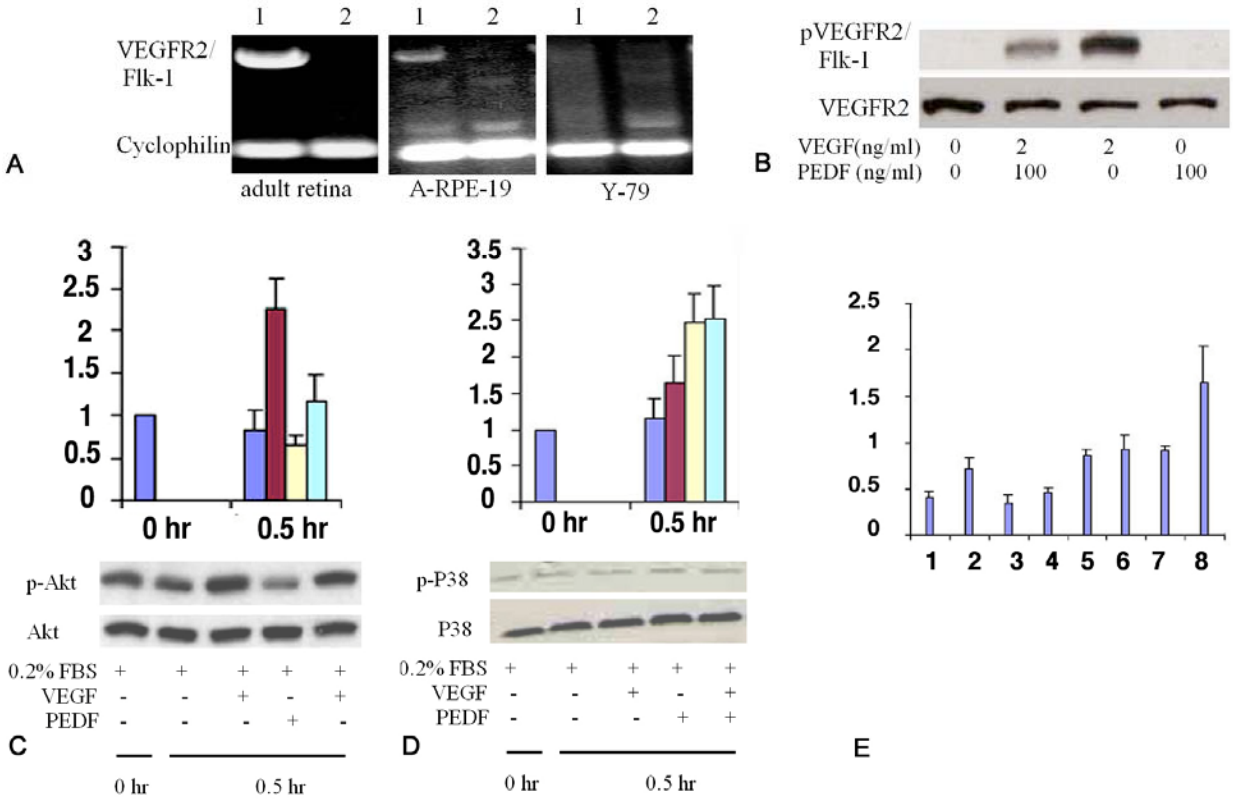

Figure 6. PEDF modulates phosphorylation of VEGFR2, Akt, and P38 signaling molecules. **A.** PEDF downregulates transcription of VEGFR2/Flk-1 receptor in the retina. The figure shows expression and regulation of VEGFR2 transcripts in adult monkey retina, human A-RPE-19 and Y79 retinoblastoma cells lines after treatment with: 1. serum free medium, 2. 100 ng/ml PEDF for 48 hrs in normoxic conditions. RNA samples were used in PCR reactions with Flk-1 primers. Cyclophilin primers were included in each tube to ensure equal concentrations of cDNA in the PCR reactions and loading of amplification products. The strong Flk-1 band in monkey retina, and the weaker band in ARPE-19 are significantly down regulated after treatment with PEDF. B. PEDF inhibits phosphorylation of VEGFR2/Flk-1 receptor in HUVECs in the presence of its ligand, VEGF. The Western blot shows Flk-1 phosphorylation after treatment of HUVEC cells with VEGF and PEDF. HUVECs were treated with PEDF (100ng/ml), or with 2ng/ml VEGF for 10 min. Cell lysates were resolved by gel electrophoresis, transferred to nylon membranes and probed with specific antibodies to measure phosphorylation of Flk-1 (p-VEGFR2/Flk-1) and expression of total receptor (VEGFR2) in the samples. Phosphorylation of the VEGFR2 receptor was not detected in the control or PEDF treated samples. Strong phosphorylation of VEGFR2 is seen after treatment with VEGF and this is substantially reduced in the presence of PEDF. C. PEDF inhibits VEGF-induced activation of Akt in HUVECs. Serum starved HUVEC cells were treated with PBS, VEGF (2ng/ml), PEDF(100ng/ml) or VEGF + PEDF. After 30 min of treatment, the cells were lysed and proteins analysed by western blot for phosphorylation of Akt (p-Akt) and the expression of total Akt (Akt) in the samples. The gel, and more clearly the histogram, show that PEDF reduces VEGF stimulated phosphorylation of Akt. The histogram represents the mean ± SD for three separate experiments. The abcissa shows phosphorylation relative to the control sample. D. PEDF stimulates phosphorylation of P38 in HUVECs. Cells were treated as in Fig 6C and anlysed by western blot for phosphorylation of P38 (lanes p-P38) and the expression of total P38 in the samples (lanes P38). VEGF caused a modest increase in the phosphorylation of the P38 signaling molecule but PEDF induced a much stronger phosphorylation of P38. There was no additive effect in the presence of both VEGF and PEDF. The histogram represents the mean ± SD for three separate experiments. The abcissa shows phosphorylation relative to the control sample. E. PEDF inhibits VEGF-induced survival of HUVEC cells by a caspase dependent mechanism. PEDF blocks the VEGF induced increase in HUVEC cell survival in serum-deprived conditions but this effect is cancelled out by the broad spectrum caspase inhibitor Z-VAD-FMK. Healthy monolayers of HUVECs were placed in serum free medium for the duration of the experiment. After 6 hrs of serum deprivation the cultures were treated as follows: 1: 0% FBS; 2: VEGF (2ng/ml); 3: PEDF (100 ng/ml); 4: VEGF + PEDF; 5: PEDF + Z-VAD-FMK (100μM) 6: VEGF + PEDF + Z-VAD-FMK; 7: Z-VAD-FMK; 8: 20% FBS. After 24 hrs of treatment with these agents, cell survival was estimated by fluorescence from viable cells following uptake of Calcein AM. Numbers on the abcissa represent fluorescence units as a measure of cell survival. The histogram represents the mean ± SD for six replicate experiments. The results show that serum deprivation induces >60% cell death in HUVECs (1) and that this can be reduced by VEGF (2). PEDF enhanced the cell loss induced by serum starvation in the absence (3) or presence (4) of VEGF. The general caspase inhibitor blocks cell death induced by either serum deprivation or PEDF (5,7) with no additional affects in the presence of VEGF (6). The observation suggests that the apoptotic effects of PEDF are dependent on the active presence of caspases in HUVECs.

of Flk-1 on endothelial cells in culture. It reduces VEGF growth-promoting signals by decreasing VEGF-induced phosphorylation of Flk-1 in human umbilical vein endothelial cells (HUVECs) (Figure 6B). Flk-1, possibly the most important VEGF angiogenesis receptor, transmits mitogenic signals to endothelial cells by interacting with its ligand, VEGF-A. Inactivation of Flk-1, therefore, may be a second way in which PEDF interrupts VEGF growth promoting actions on new vessel formation.

There is also evidence that PEDF can block VEGF mitogenic effects on endothelial cells through the modulation of three major pathways: the Akt, MAP kinase, and the Fas/FasL pathways. The survival of HUVEC cells is thought to be contingent on the activity of one or more specific signaling molecules including Akt, Erk1/2, and P38.

In a recent study, we showed that when HUVEC cells were treated with VEGF, there was an increase in the amount of phosphorylated Akt present in cell lysates within 30 min of the treatment (Figure 6C). PEDF had little effect on the basal level of Akt phosphorylation but almost completely blocked the increase induced by VEGF. The signal molecule Akt plays an active role in the NFkB transduction cassette and is widely known to signal survival/neuroprotective activities in many cell types. In addition to modulating signaling by Flk-1 and Akt, we found that PEDF also increases the phosphorylation of P38 over the modest increase induced by VEGF (Figure 6D). P38 is a proapoptotic molecule and its activation may be a third mechanism by which PEDF controls the growth and viability states of HUVEC cells.

A fourth way by which PEDF could block endothelial cell growth and reduce angiogenesis is by regulating the MAPK signaling module. In bovine retinal endothelial cells this could occur in two ways: by decreasing the transcription of MAP kinase kinase 1(MAPKK1) and by altering VEGF-induced phosphorylation of ERK1/2 (98,106). The regulation of ERK1/2 phosphorylation by PEDF however, varies according to the growth conditions under which endothelial cells are exposed (98). When cells are adapted to growth in VEGF, treatment with PEDF causes little change in the phosphorylation state of Erk1/Erk2. whereas it can prevent VEGF-induced phosphorylation of Erk1/Erk2 in bovine endothelial cells that are not previously adapted to VEGF mitogenic stimulation.

In another model of endothelial cell growth, we observed as similar relationship between PEDF and VEGF. In serum-free conditions, VEGF promotes survival of endothelial cells, which would otherwise undergo apoptosis induced by serum starvation. VEGF-induced survival in serum-deprived HUVECs, however, can be blocked by the actions of PEDF in a caspase dependent manner. This was evident in cultures that were incubated with the general caspase inhibitor, Z- VAD-FMK in the presence of PEDF, VEGF, or both (FIGURE 6E). Although PEDF alone can enhance apoptosis in HUVEC in serum deprived conditions, it was unable to do so in the presence of the broad spectrum caspase inhibitor Z-VAD-FMK, suggesting that the executioner activity of caspases are linked to PEDF signaling in HUVECs.

Others have shown that antiangiogenic signals are generated by PEDF through activation of the Fas/FasL death cascade in endothelial cells (99). However, since PEDF inhibits ocular angiogenesis in mice deficient in Fas or FasL as well, it presumably has additional inhibitory actions independent of the Fas/FasL cascade on endothelial cells (100). In support of a PEDF induced Fas/FasL mediated transmission of apoptic signals to endothelial cells are studies which show that PEDF actions can be inhibited by interference with the activation of capase 8 and 3, two essential transducers of the Fas/FasL cascade. The control of apoptotic signals in these cells by PEDF may be linked to its regulation of Flip 1, an inhibitor of caspase 8 and a key mediator of cell death. Flip 1 is expressed above physiological levels when VEGF activates NFkB in endothelial cells (101). PEDF restores physiological levels of Flip1 in the presence of VEGF in endothelial cells and thus may restore activity of the caspase 8 executioner pathway. Similarly, recent studies suggest that VEGF induced activation of the transcription factor NFAT leads to increased Flip1 expression, and that this too can be blocked by PEDF (102).

Overlaid on this dynamic equilibrium of PEDF and VEGF levels is the interesting finding that there is an age-related decrease in PEDF expression in a number of cell types, and that this decline can be reset in cloned animals (103). Perhaps many age-related neovascular diseases occur because the amount or activity of angiogenic inhibitors, such as PEDF, has become attenuated.

6.2.2. Neuroprotective signaling

The NFkB pathway has been implicated in the transmission of neuroprotective signals. In cerebellar granule cells (CGCs), this pathways is also shown to be regulated by PEDF. Treatment of CGCs with PEDF stimulates phosphorylation of IkB, leading to activation and translocation of NFkB to the nucleus. This results in a chain of sequential events that link the NFkB pathway to a defined extracellular signal and transcription of antiapoptotic and neuroprotective genes (104). Whether the same pathways, or combination of pathways, are responsible for both the antiangiogenic and neuroprotective actions of PEDF has yet to be established.

It is worth noting that NFkB can also promote apoptosis by inducing FasL through binding regulatory motifs on FasL promoter and that VEGF can activate NFkB to promote cell survival by reducing caspase 8 death promoting signals. The available data suggest that NFkB is a primary intracellular junction molecule used by PEDF. However, cellular diversity and other extracellular signals may allow signal cross talk between the NFkB cascade and other transduction pathways, or for cell-type specific gene activation responses.

6.3. Cross talk in PEDF signaling

Although we are far from a comprehensive picture of how PEDF controls neuroprotective,

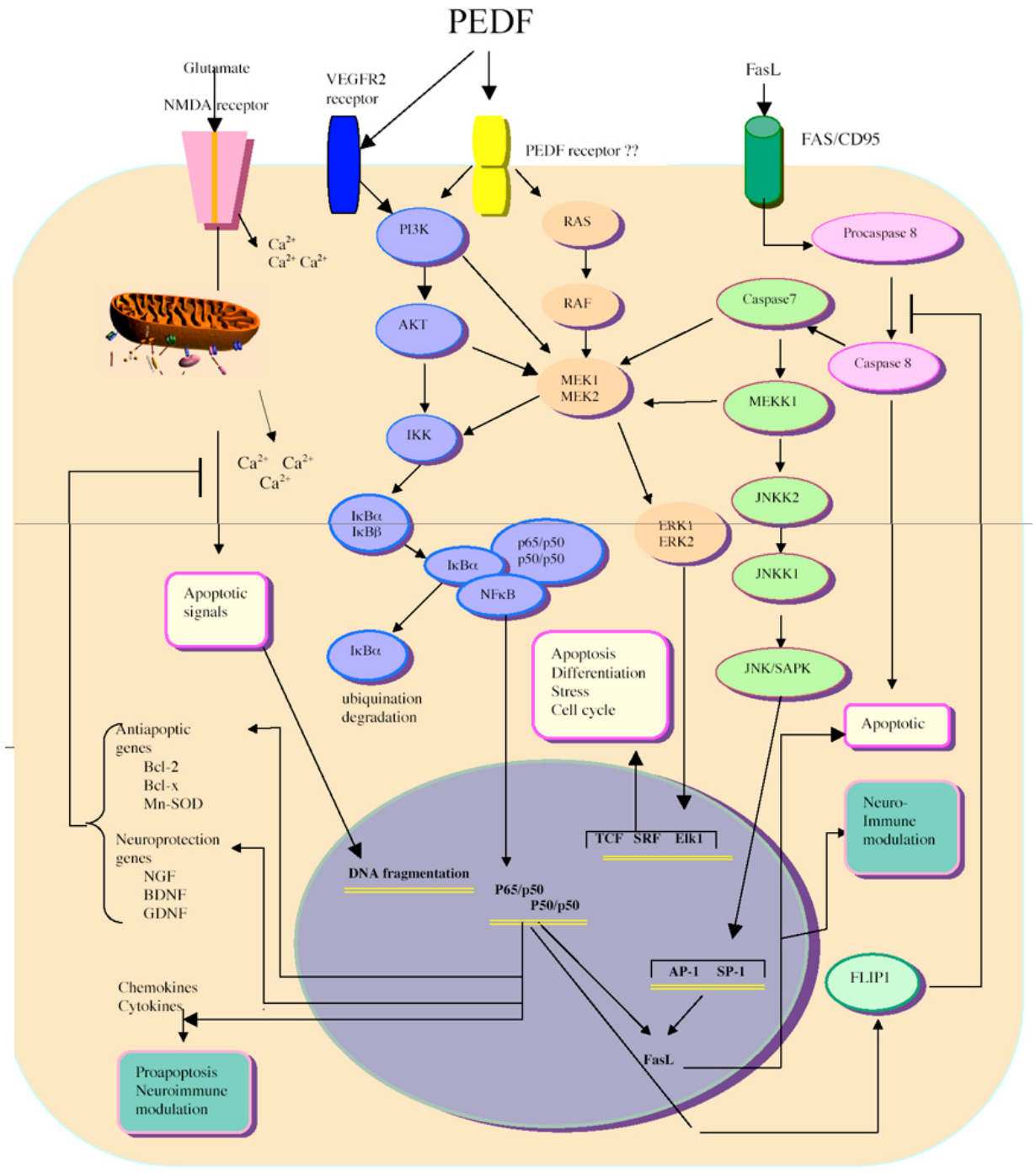

Figure 7. Schematic of signaling pathways modulated by PEDF. The diagram shows that PEDF can regulate several major transduction modules, which communicate with each other to control key events of cell growth, neuroprotection, and cell death. Modulation of these cascades by PEDF is dependent on the cell type and environmental conditions.

differentiation, and cell death signals, examination of the data presented so far suggests that PEDF activates three important signaling modules: the NFkB module, which is linked to neuroprotective signaling; the MAP kinase module, which is associated with cell differentiation and proliferation; and the caspase executioner module associated with cell death signaling (81,105) (Figure 7).

There is the potential for cross-talk and intersection between parallel transduction pathways that PEDF interacts with. These pathways link molecules that mediate key events that regulate mitogenesis, cell survival, and cell death with multiple intrinsic opportunities for communication between them. Such interactions could allow a single factor, like PEDF, to tweak multiple

molecular events in specific cell types in a given environment, or enhance or override the effects of another factor. The mechanisms by which PEDF signals information to multiple cell types will lead to a better understanding of the multifaceted role of this serpin in processes controlling angiogenesis and neuroprotection.

The hierarchy of PEDF's action and signaling diversity is most likely influenced by several factors including cellular environment, lateral complementation through cooperative signaling ligands, receptors, and specific codes that cells use to generate appropriate signals. Different cell types may also use various combinations of these mechanisms. In addition, actively proliferating cells may respond to PEDF in ways that are not similar to quiescent or senescent cells. Understanding how PEDF inhibits angiogenesis will potentially be rewarding since it could uncover other clinically relevant biological targets for angiogenesis.

7. THERAPEUTIC POTENTIAL OF PEDF IN NEOVASCULAR AND NEURODEGENERATIVE DISEASES

Antiangiogenic therapies could only be clinically effective if they selectively target new vessels and not preexisting ones so as to minimize undesirable side effects such as the collapse of normal vasculature, hemorrhaging, and inappropriate degradation of tissue extracellular matrix. PEDF meets these criteria as it targets only the growth of new vessels, has no known deleterious effects on mature vessels, and controls vascular leakage.

One advantage to using endogenous antiangiogenic molecules, such as PEDF, to target abnormally growing blood vessels is that these molecules would not be expected to activate drug resistant genes and, thus, may offer some of the most promising breakthroughs for effective long-term angiogenesis therapy. A further advantage of endogenous antiangiogenic molecules is that they are tolerated in the body and are unlikely to elicit an immunological response or produce the toxic side effects of synthetic inhibitors.

PEDF has the additional advantage of preserving neurons that are often damaged in vascular diseases of the nervous system. Its ability to promote differentiation of cancer cells as well as block angiogenesis is of additional therapeutic benefit in the treatment of a wide range of malignancies.

8. PERSPECTIVE

Serpins play a pivotal role in combating a wide range of human diseases. We know the structural mechanism of action of some of the classical inhibitory serpins in exquisite detail. The rapidly increasing knowledge of PEDF actions in molecular detail gives us a similar view of one member of the important class of non-inhibitory serpins. Comparison of the molecular actions of the two groups of serpins will undoubtedly lead to new insights into the function of both classes and to the evolution of this vitally important gene family.

The properties of the non-inhibitory serpin, PEDF, make it a strong candidate gene to be tested in ocular diseases associated with neovascularization and cell death as well as in neurodegenerative diseases promoted by aging, oxidative stress, and glutamate excitoxicty. The widespread tissue distribution of PEDF suggests that it should also be tested against a wider range of angiogenic diseases, including tumors. To maximize the therapeutic potential of this protein, it is essential that we develop a good working knowledge of the importance of PEDF-ligand interactions, the biochemical pathways it regulates, and other structure- function associations that mediate the actions of PEDF in physiological and pathological processes.

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