Binding of Pigment Epithelium-derived Factor (PEDF) to Retinoblastoma Cells and Cerebellar Granule Neurons

EVIDENCE FOR A PEDF RECEPTOR*†‡§

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Pigment epithelium-derived factor (PEDF) has neuronal differentiation and survival activity on retinoblastoma and cerebellar granule (CG) cells. Here, we investigated the presence of PEDF receptors on retinoblastoma Y-79 and CG cells. PEDF radiolabeled with 125I remained biologically active and was used for radioligand binding analysis. The binding was saturable and specific to a single class of receptors on both cells and with similar affinities (Kd = 5 ± 0.5–2.7 × 10⁸ M⁻¹), Bmax = 1.1 ± 10⁸ sites/Y-79 cell; and Kd = 3.2 nm, Bmax = 3.1 ± 10⁸ sites/CN cell). A polyclonal antiserum to PEDF, previously shown to block the PEDF neurotrophic activity, prevented the 125I-PEDF binding. We designed two peptides from a region previously shown to confer the neurotrophic property to human PEDF, synthetic peptides 55-mer (positions 44–77) and 44-mer (positions 78–121). Only peptide 44-mer competed for the binding to Y-79 cell receptors (EC₅₀ = 5 nm) and exhibited neuronal differentiating activity. PEDF affinity column chromatography of membrane proteins from both cell types revealed a PEDF-binding protein of ~80 kDa. These results are the first demonstration of a PEDF-binding protein with characteristics of a PEDF receptor and suggest that the region comprising amino acid positions 78–121 of PEDF might be involved in ligand-receptor interactions.

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§ The abbreviations used are: PEDF, pigment epithelium-derived factor; Ab-rPEDF, polyclonal antiserum to PEDF; CHAPS, 3-(3-cholamidopropyl)dimethylammonio)-1-propanesulfonate; CG, cerebellar granule; BHK, baby hamster kidney; PAGE, polyacrylamide gel electrophoresis; DIV, day(s) in vitro; PBS, phosphate-buffered saline; BSA, bovine serum albumin.

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parameters of such binding were established. We have used cultures of human retinoblastoma cells and rat CG cells because they respond to PEDF stimuli, and we used a biologically active form of 125I-PEDF to define the basic physicochemical parameters of such binding. We have used PEDF purified from bovine eyes (12, 13), recombinant human PEDF (26), synthetic peptides derived from BA, and a polyclonal antiserum to PEDF, Ab-rPEDF (12), to further investigate the specificity of the binding. Finally, PEDF affinity column chromatography was used to isolate a PEDF-binding protein from retinoblastoma and CG cell membranes. We describe here that PEDF exhibits a saturable and specific binding to target cells for neurotrophic activity and demonstrate for the first time a PEDF-binding protein with characteristics of a PEDF receptor.

**EXPERIMENTAL PROCEDURES**

**Materials**—Minimum Eagle’s medium (without l-glutamine), Dulbecco’s modified Eagle’s medium, Dulbecco’s modified Eagle’s medium/ Ham’s F-12 medium, L-glutamine, sodium pyruvate, nonessential amino acids, HEPES, fetal bovine serum, and phosphate-buffered saline (PBS) (0.144 4-mer were designed from amino acid positions 44–77 (DPFFKFPVKNAALASNVGFYLDVSSMPT) and 78–121 (VLLPSLVSATALSLSAQGRTESIHRYLDDISPDHTG) of the human PEDF sequence (GenBank™ accession number U29953), respectively, and prepared by Biosynthesis, Inc., followed by high pressure liquid chromatography purification (>90% purity) and amino-terminal sequence determination. The resulting peptides were soluble in aqueous solutions. rPEDF-BH is the recombinant human PEDF protein obtained from 25 10^6) were collected by centrifugation, washed twice, resuspended in 10 ml of homogenization buffer (20 mM HEPES, pH 7.0, 100 mM KCl containing 1 mM EDTA, 5 μg/ml aprotinin, 1 mM AEBSF, 1 μg/ml pepstatin, and 0.5 μg/ml leupeptin). After incubation on ice for 15 min, cells were disrupted by sonication with two bursts, in 5 s pulses at 4°C. The suspension was fractionated by centrifugation at 1,000 × g for 10 min at 4°C to remove nuclei and unbroken cells. The plasma membranes in the supernatant were separated by centrifugation at 83,000 × g for 30 min at 4°C. The particulate extracts were resuspended in 0.1 ml of solubilization buffer (20 mM sodium phosphate buffer, pH 6.5, 10% glycerol, 1 mM CaCl_2, and 0.5% CHAPS) at 4°C. The suspension was mixed with gentle pipetting, and the soluble proteins were fractionated by centrifugation at 83,000 × g for 40 min at 4°C. The supernatant was resuspended in 0.5 ml of ice-cold PBS containing 0.1% BSA, and the pellet was extracted a second time with solubilization buffer by the same procedure. The first and second extracts were combined and constituted the detergent-soluble membrane fraction. About 50 μg of protein was obtained from 25 × 10^6 Y-79 cells.

Membranes from rat CG cells were prepared as above and with the following modifications. The homogenization buffer was 72 ml of 0.32 M sucrose/0.05 M NaCl containing protease inhibitor cocktail and 0.1 mg 10^6 cells) were incubated at 4°C for 24 h. Briefly, 2 ml of Y-79 cell culture (1.25 × 10^6 cells/ml) in serum-free medium as above plus 0.1% ITS were treated with 25 μl of a solution containing PEDF protein in phosphate-buffered saline with 1% BSA. After 7 days of treatment, the cells were attached to poly-n-lysine coated plates. The differentiation state of the cells was monitored by light microscopy at intervals after attachment.

**Preparation of Detergent-soluble Membrane Extracts**—Human retinoblastoma Y-79 cells (25 × 10^6) were collected by centrifugation, washed twice with PBS, and resuspended in 100 ml of homogenization buffer (20 mM HEPES, pH 7.0, 100 mM KCl containing 1 mM EDTA, 5 μg/ml aprotinin, 1 mM AEBSF, 1 μg/ml pepstatin, and 0.5 μg/ml leupeptin). After incubation on ice for 15 min, cells were disrupted by sonication with two bursts, in 5 s pulses at 4°C. The suspension was fractionated by centrifugation at 1,000 × g for 10 min at 4°C to remove nuclei and unbroken cells. The plasma membranes in the supernatant were separated by centrifugation at 83,000 × g for 30 min at 4°C. The particulate extracts were resuspended in 0.1 ml of solubilization buffer (20 mM sodium phosphate buffer, pH 6.5, 10% glycerol, 1 mM CaCl_2, and 0.5% CHAPS) at 4°C. The suspension was mixed with gentle pipetting, and the soluble proteins were fractionated by centrifugation at 83,000 × g for 40 min at 4°C. The supernatant was resuspended in 0.5 ml of ice-cold PBS containing 0.1% BSA, and the pellet was extracted a second time with solubilization buffer by the same procedure. The first and second extracts were combined and constituted the detergent-soluble membrane fraction. About 50 μg of protein was obtained from 25 × 10^6 Y-79 cells.

Membranes from rat CG cells were prepared as above and with the following modifications. The homogenization buffer was 72 ml of 0.32 M sucrose/0.05 M NaCl containing protease inhibitor cocktail and 0.1 mg
then centrifuged at 1200 × g for 10 min at 25 °C, and the amount of uncoated protein was determined from the supernatant. The amount of uncoated protein ranged between 0.4 and 6.5% of the total protein added to the mixture. To block the remaining active groups in the coupled beads, 10 column volumes of 3 M of ethanolamine, pH 9, were added, and the mixture was rotated for 3 h at 25 °C. The quenching solution was removed after centrifugation and the beads were washed with 15 ml of PBS, followed with 5 ml of 1× NaCl. Finally, the beads were washed with 30 ml of PBS and stored in 0.05% sodium azide/PBS at 4 °C. The PEDF affinity resin contained about 1–6 mg of PEDF per ml of resin.

**PEDF Affinity Column Chromatography—** PEDF affinity and control resins were packed in Polyprop chromatography columns (Bio-Rad) to yield a 0.25-ml settled bed volume and equilibrated with binding buffer (20 mM sodium phosphate buffer, pH 6.5, 150 mM NaCl, 10% glycerol, 1 mM CaCl₂, and 0.5% CHAPS). Detergent-soluble membrane proteins from 25 × 10⁷ Y-79 cells were applied to the control column to absorb Ultralink™ binding proteins. The flow-through and the first 7-column volume wash with the binding buffer were pooled and mixed with PEDF affinity beads (1.8 mg of PEDF/ml of beads) at a volume ratio of 7:1, and the mixture was rocked at 4 °C for 16 h. The resin was transferred into a column and washed with 12 column volumes of binding buffer, and the bound proteins were eluted with 14 column volumes of low pH buffer (10 mM glycine, pH 2.0, 150 mM NaCl, 10% glycerol, 1 mM CaCl₂, and 0.5% CHAPS) followed by 14 column volumes of 1× NaCl in low pH buffer, 14 column volumes in high pH buffer (100 mM glycine, pH 11.0, 150 mM NaCl, 10% glycerol, 1 mM CaCl₂, and 0.25% CHAPS), and finally with 1× NaCl in high pH buffer. Proteins from each elution were precipitated with trichloroacetic acid.

Detergent-soluble membrane extract from 360 × 10⁶ CG cells was applied to a control resin column (0.5 ml) prequadrilled with 100 mM Tris-acetate acid, pH 8.1, 1 mM EDTA, and 0.5% CHAPS. The flow-through was mixed with 1 ml of PEDF beads (6 mg of PEDF/ml of Ultralink™) and incubated with gentle rotation at 4 °C for 16 h. The PEDF affinity resin was transferred into a column and washed with 20 column volumes of 150 mM NaCl in binding buffer, and the proteins were eluted with 6 column volumes of low pH buffer (100 mM glycine, pH 2.5, 150 mM NaCl, 10% glycerol, 1 mM CaCl₂, and 0.5% CHAPS) followed by 6 column volumes of the same buffer at pH 11. Eluted proteins were concentrated by ultrafiltration with Centricon-30 (Amicon), following the manufacturer’s protocol.

**RESULTS**

**125I-PEDF Induces Morphological Differentiation on Y-79 Cells**—Before we could investigate the binding characteristics of a ligand to target cells, it was necessary to analyze its structural and biological properties. PEDF was chemically modified by iodination with 125I and resolved by SDS-PAGE. A photograph of the autoradiogram is shown to the left. Migration positions of SDS-PAGE standards (MW) and volume of the radiolabeled protein applied to each lane are indicated. Neurite outgrowth assay was performed with radiolabeled PEDF on retinoblastoma Y-79 cells, as described under “Experimental Procedures.” Morphological characteristics of 7-day postattachment cultures treated with (top) and without (bottom) 125I-PEDF (0.2 nM) are shown as indicated.

The free and bound radioligand did not undergo major degradation and had an identical migration pattern by SDS-PAGE, even after incubation for 16 h, but the binding signal decreased in the presence of an excess of unlabeled PEDF, confirming the specificity of 125I-PEDF binding to Y-79 cells (Fig. 2B). The specific binding augmented linearly with increasing concentrations of 125I-PEDF in the range of 0–1 nM, representing 4% of the total radioactivity added to the cells (Fig. 2C). The amount of nonspecific binding was similar in the presence of an excess of unlabeled PEDF or without cells.

We then analyzed the PEDF binding profile to Y-79 cells to determine its physicochemical parameters. Binding was performed with a given amount of radioligand and increasing concentrations of unlabeled PEDF. Fig. 2D shows that Y-79 cells exhibited a saturable and specific binding of PEDF. Scatchard analysis of the binding data revealed a single class of binding sites (Bmax = 271,200 sites per Y-79 cell) with an apparent dissociation constant (Kd) of 3.55 nM. A second and third experiment performed with different batches of Y-79 cells revealed similar kinetics, with Kd = 1.7–3 nM and Bmax = 45,500–97,000 sites per Y-79 cell (data not shown).

**Effect of Antiserum to PEDF and Ovalbumin on the 125I-PEDF Binding**—We have previously shown that a rabbit polyclonal antiserum developed against recombinant human PEDF, Ab-rPEDF, reacts in a specific, sensitive, and linear fashion with bovine PEDF, and when preincubated with PEDF, it blocks its inducing-neurotrophic activity on human retinoblastoma Y-79 cells (12). To evaluate the effect of Ab-rPEDF on the 125I-PEDF binding to receptors on Y-79 cells, the radioligand was preincubated with an excess of Ab-rPEDF before adding it to the cells binding reaction mixtures. We found that...
in the presence of Ab-rPEDF the $^{125}$I-PEDF binding decreased to nonspecific binding levels (Fig. 3A), suggesting that Ab-rPEDF blocked the PEDF neurotrophic activity by preventing the binding of PEDF to receptors on Y-79 cells.

To determine whether serpins can compete for the $^{125}$I-PEDF binding to Y-79 cells, we chose ovalbumin because it does not have inhibitory activity against proteases, which could interfere in the assay. In addition, it shares the lack of S to R conformational change upon cleavage of the serpin-exposed loop with PEDF (25, 28) but failed to induce neurite outgrowth in assays with Y-79 cells (data not shown). We found that in the presence of excess ovalbumin the binding of $^{125}$I-PEDF to Y-79 cells was partially inhibited (Fig. 3B). Similar results were obtained when bound $^{125}$I-PEDF was separated from free by centrifugation, and its radioactivity was determined after SDS-PAGE (data not shown).

**Competition of $^{125}$I-PEDF Binding with Synthetic Peptides 34- and 44-Mer**—We have previously shown that recombinant PEDF polypeptide fragments with truncations from the carboxy terminus retain neurotrophic activity (25). These results suggest that the smallest biologically active fragment, BA (positions 44–121 of human PEDF) contains the site that binds to cell surface receptor(s) on Y-79 cells. We have prepared two peptides from BA, peptides 34-mer (amino acid positions 44–77) and 44-mer (amino acid positions 78–121) to use as competitors of the $^{125}$I-PEDF binding. As shown in Fig. 4, increasing concentrations of peptide 44-mer inhibited the $^{125}$I-PEDF binding in a concentration-dependent manner, with an EC$_{50}$ = 5 nM. It is worth noting that the EC$_{50}$ of bovine PEDF was 3 nM, as calculated from data in Fig. 2D. Peptide 34-mer had an insignificant effect. Peptides 34-mer and 44-mer were used for neurite outgrowth assays with Y-79 cells, to evaluate their biological activity. After 22 days postattachment, only cells that had been treated with 44-mer showed elongation of processes similar to those for rPEDF-BH (24), the bacterially derived recombinant human PEDF (positions 44–418) (Fig. 5). These results demonstrate that peptide 44-mer competed efficiently for the binding of PEDF to receptors on Y-79 and suggest that it contains the structural determinants for binding and neurotrophic activity of PEDF.

**Cell Type Specificity of Cell Surface $^{125}$I-PEDF Binding**—We examined whether the binding was confined to Y-79 cells. Two human retinoblastoma cell lines (Y-79 and Weri), primary cultures of rat CG cells, mouse NIH3T3, hamster BHK, and monkey COS-7 cells were used in $^{125}$I-PEDF binding reactions. The neuronal survival and differentiating effects of PEDF on the CG and retinoblastoma cells have been previously demonstrated (2–6), whereas the response of the other cells to PEDF stimuli is still unknown. Fig. 6 shows that the specific $^{125}$I-PEDF binding for Y-79 and Weri cells reached 12 and 1.7%, respectively, whereas for the other cells, it was below 1%. The binding to NIH3T3 was the highest of the others, reaching 0.8% compared with CG and BHK cells with about 0.1% and no detection of binding to COS-7. These results suggest that the
Retinoblastoma cells have a higher number of PEDF-binding sites on their cell surface and/or binding sites with a higher affinity for PEDF than CG cells.

Binding Analysis of 125I-PEDF to Cerebellar Granule Cell Neurons—CG cells, the most abundant neuronal subtype cells in the mammalian brain, undergo a maturation process during the course of culture, i.e., they can be distinguished between immature (DIV 0–3) and mature (DIV 5+) cells. We have observed that when added at specific DIVs, PEDF has a variety of rescuing effects on CG neurons, e.g., neuronal survival effect is observed after PEDF addition at DIV 1 (4), protection against glutamate neurotoxicity at DIV 8 (5) and antiapoptotic effect at DIV 2 but not at DIV 5 (6). We have determined the physico-chemical characteristics of PEDF binding by CG cells cultured at different DIV. Fig. 7 shows that 125I-PEDF bound to CG cells in a specific and saturable fashion, with binding parameters that were similar and independent of the age of the cultures. The ligand bound as a 50-kDa protein to about 1000 sites per CG cell, with a $K_d$ of 3 nM. The $K_d$ value is in agreement with the half-maximum dose for the PEDF biological activity on CG cells, 0.5–3 nM (4, 5), and is similar to that of Y-79 cells (see Fig. 2D). However, the number of PEDF-binding sites were 100-fold lower than those for Y-79 cells (see Fig. 2D). These results suggest that PEDF binds to a similar cell surface protein on immature and mature CG cells and on retinoblastoma cells.

PEDF Affinity Column Chromatography of Y-79 Retinoblastoma and CG Cells Membrane Proteins—To isolate the cell surface protein(s) with binding affinity for PEDF, plasma membrane proteins of Y-79 retinoblastoma and CG cells were extracted and solubilized with detergents, CHAPS, or Triton X-114. Preliminary experiments showed that the detergent-soluble membrane extracts contained the PEDF binding activity, e.g., 0.5–7.5 fmol of 125I-PEDF specifically bound to detergent-soluble membrane proteins of 0.2–2 x 10^6 Y-79 cells (radioligand at 2.4 nM). Membrane proteins with affinity for PEDF were fractionated by PEDF affinity column chromatography (Fig. 8). Most of the solubilized membrane proteins from Y-79 cells did not bind to PEDF; however, a main protein with an apparent molecular weight of 50,000 bound and eluted from the column with buffers of elevated pH (Fig. 8A). Another band of less intensity and migrating as a molecular weight 60,000 protein was also detected in the eluates. Biotinylation of Y-79 cell surface proteins followed by PEDF affinity chromatography showed that a PEDF-binding protein of 80 kDa is a cell surface protein (data not shown). Similarly, a CG cell mem-
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Fig. 7. PEDF binding to cell surface receptors on CG cells. Radioligand was prepared from recombinant human PEDF. A, cultures of CG cells at DIV 9 were incubated with 2.9 nM 125I-PEDF at 4 °C for 16 h. Medium was removed, and the cells were washed with binding buffer and lysed with 1% SDS. The proteins were precipitated with trichloroacetic acid before resolving by SDS-PAGE, followed by autoradiography. Samples were applied to a 10–20% polyacrylamide gel as follows: lane 1, flow-through; lane 2, CG cells. Binding assays were performed two times, and a photograph of a representative x-ray film is shown. Migration of molecular weight standards is indicated to the right. B and D, photographs of CG cells at DIV 2 and 7, respectively, visualized under light microscopy. C and E, saturation and Scatchard analysis of PEDF binding by CG cells at DIV 2 and 7, respectively. Binding was performed with 0.8 nM 125I-PEDF and increasing concentrations of unlabeled human PEDF. Data was analyzed by linear regression with Microsoft Excel 97 to obtain Scatchard binding plots. The following values were obtained: for CG cells at DIV 2 (C), Kd = 4.1 nM and Bmax = 1187 sites/cell; for CG cells at DIV 7 (E), Kd = 2.25 nM and Bmax = 1000 sites/cell. Similar results were obtained by nonlinear regression, and Scatchard analyses were obtained using GraphPad Prism software. Experimental points are given as the average of duplicates for DIV 2 and triplicates for DIV 7.

DISCUSSION

Several lines of evidence implicate inhibition of proteases in serpin biological activities. However, PEDF has no known protease target for inhibition, and furthermore, its neurotrophic activity is determined by sequences away from its serpin-exposed loop, the structural determinant of serpin protease inhibition (25). The present study indicates that PEDF can interact with cells derived from the retina and the central nervous system. The data suggest that PEDF has a receptor on the surface of cells that respond to its stimuli. This conclusion is supported by several lines of evidence: 1) biologically active 125I-PEDF binds directly to both human retinoblastoma and rat CG cells; 2) 125I-PEDF binding is competed by native and recombinant PEDF and the biologically active peptide 44-mer; 3) 125I-PEDF binding is inhibited by Ab-rPEDF previously shown to block the PEDF biological activity; 4) PEDF binding affinity to receptors on retinoblastoma cells is similar to that of CG cells; and 5) the biochemical characteristics of a PEDF-binding protein from membranes of retinoblastoma are similar to those of CG cells. It is clear that further information is required to confirm that this binding activity and PEDF-binding protein is a PEDF receptor that, upon its interaction with its ligand, triggers the necessary signal transduction events for neurotrophic activity. However, the present study indicates that the first step in the biological events of PEDF is established as binding to receptors on the surface of target cells.

The data reported here also provide pharmacological and biochemical evidence that PEDF and peptide 44-mer bind to the same site, called PEDF receptor, on Y-79 cells: the specific and saturable PEDF binding to cells is blocked by peptide 44-mer in a concentration-dependent fashion. In addition, this peptide is biologically active, e.g. exhibits neuronal differentiating activity on human retinoblastoma cells (Fig. 4) and is protective for motor neurons against chronic glutamate toxicity in organotypic spinal cord cultures (8). These observations suggest that the region spanning positions 78–121 is the site in PEDF that binds to receptors on Y-79 cells. The region of 44-mer is conserved among the human, bovine, and mouse PEDF sequences. Sequence comparison and alignment using a BLAST program (29) of the 44-mer and GenBank™ database revealed that the highest similarity is found for regions toward the amino-terminal end of other serpins (35 ± 10% identity), including ovalbumin and protease nexin-1. Sequence similarity could account for the partial inhibition of PEDF binding by ovalbumin. In a previous study, we analyzed the heparin-binding site of PEDF and proposed a spatial structural model for PEDF (16). In this model, the 44-mer region, the receptor-
binding site, is located in a distinct area and away from the heparin-binding site. It will be necessary, however, to isolate cDNA clones for PEDF receptor and express PEDF receptor in a heterologous cell to provide evidence that peptide 44-mer and PEDF bind to it.

Previous reports have demonstrated two different receptors for serpins, the low density lipoprotein receptor-related protein and the serpin-enzyme complex receptor. The low density lipoprotein receptor-related protein, a multifunctional cell surface receptor that binds and endocytoses several distinct ligands including serpin-enzyme complexes, is the hepatic receptor responsible for the clearance of serpin-enzyme complexes (30–32). Interestingly, its gene is abundantly expressed in most neurons of the central nervous system, particularly in the cerebellum (33). In addition, the low density lipoprotein receptor-related protein can mediate the neurite outgrowth activity of apolipoprotein E in a central nervous system-derived neuronal cell line (34, 35). These observations suggest that the low density lipoprotein receptor-related protein contains the necessary biochemical components for signaling the transduction of neurite outgrowth upon binding to a ligand and becomes a candidate receptor for PEDF. However, the low density lipoprotein receptor-related protein is unable to bind to immobilized native or proteolytically modified forms of serpins antithrombin III, heparin cofactor II and 1-antitrypsin, suggesting that it would be unable to bind PEDF (30). In addition, retinoblastoma cells do not have the ligand-binding subunit of the low density lipoprotein receptor-related protein (585-kDa). These observations exclude the possibility that the PEDF binding activity described here is directed toward the low density lipoprotein receptor-related protein.

On the other hand, the serpin-enzyme complex receptor has a ligand-binding subunit of about 78 kDa, binds several serpin-enzyme complexes and soluble amyloid-β peptide, and is also present in neuronal cells (36–38). Several observations suggest that the serpin-enzyme complex receptor might not be related to the PEDF receptor: 1) proteolytically modified α-antitrypsin competes for binding of serpin-enzyme complexes to the serpin-enzyme complex receptor and vice versa, whereas the purified 51-kDa amino-terminal fragment of α-antitrypsin does not compete for binding of serpin-enzyme complex (39); and 2) on the other hand, an amino-terminal fragment of PEDF, peptide 44-mer, competes for the binding of 125I-PEDF to Y-79 cells (Fig. 4A). It has been shown that the serpin-enzyme complex receptor recognizes a pentapeptide domain in the carboxyl-terminal fragment of α-antitrypsin-protease complex (FVFLM, amino acid positions 370–374) and in the amyloid-β peptide (IILGMM, positions 31–35) (38, 40). We have compared these sequences to the 44-mer and have not been able to identify significant similarity. Thus, the present studies cannot reveal the identity of the PEDF receptor, but they provide evidence that PEDF acts to promote neuronal differentiation and survival on retinoblastoma and CG cells via a novel receptor-mediated mechanism, which may indicate a novel role for serpins in neurotrophic activity.

The results of this study may also have important implications for neurodegenerative diseases. In the vertebrate retina, the PEDF gene is expressed by retinal pigment epithelial cells (14, 18), and the protein product is secreted from these cells and localized in the interphotoreceptor matrix (12, 15). The PEDF gene maps next to loci for several inherited retinal dystrophies, such as retinitis pigmentosa, progressive cone dystrophy, and central areolar choroidal dystrophy (41–44). We have recently shown that intravitreal injections of PEDF in animal models for retinitis pigmentos resulted in a delay of photoreceptor cell degeneration, implying a survival effect on photoreceptor cells in vivo (10). It is not known yet whether PEDF acts directly on receptors on the photoreceptor cells or via receptors on other retinal cells, which will eventually have an effect on the photoreceptor cells. The reported neurotrophic activities on CG cells in vitro also point out important implications for cerebellar neurodegenerative diseases, e.g. xeroderma pigmentosum and Cockayne syndrome, hereditary disorders characterized by impaired DNA repair and neurodegeneration, in particular apoptosis of CG neurons (45). Although the protein is detected in cerebrospinal fluid (8), we have not detected it in the CG cell culture media, suggesting that PEDF could be a paracrine neurotrophic factor for cerebellar neurons. Taken together with these data, the present study reveals the binding of PEDF to its cell surface receptor and constitutes the first step in understanding its mechanism of action as a potential neurotrophic factor for the retina and central nervous system.

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