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LETTER TO THE EDITORS

PEDF: A Pigment Epithelium-derived Factor with Potent Neuronal Differentiative Activity

It has long been recognized that the retinal pigmented epithelium (RPE) is crucial to the normal development and function of the neural retina. A variety of molecules, including a number of growth factors, are synthesized and secreted by RPE cells and may play a role in the organization, differentiation and normal functioning of the retina (Adler and Severin, 1981; Bryan and Campochiaro, 1986; Rosenbaum *et al.*, 1987; Schweigerer et al., 1987; 1987; Campochiaro et al., 1988; Connor et al., 1988; Wong et al., 1988; Elner et al., 1990; Hewitt et al., 1990). Recently, RPE cells and medium conditioned by RPE cell cultures have been shown to influence retinal cell development both in vivo and in vitro (Vollmer et al., 1984; Vollmer and Layer, 1986; Liu et al., 1988, 1990; Allen et al., 1988; Spoerri et al., 1988; Sheedlo and Li, 1990).

In a previous study, we used the human Y79 retinoblastoma cell line as a model to study the effects of RPE secretory products on cells of neural retinal origin. Retinoblastoma is an intraocular tumor thought to be derived from primitive, multipotential retinoblasts. Cultured retinoblastoma cells have been shown to retain the multipotential nature characteristic of their cells of origin (Kyritsis et al., 1987). Human fetal RPE cell-conditioned medium (RPE-CM) was shown to potentiate the differentiation of Y79 retinoblastoma cells into cells that exhibit both morphological and biochemical characteristics of neurons (Tombran-Tink and Johnson, 1989a). In an attempt to identify specific factors in RPE-CM responsible for this neurotrophic activity, RPE-secreted proteins were fractionated by SDS polyacrylamide gel electrophoresis, selected proteins were electroeluted from gel slices, and the eluted proteins were tested for effects on the differentiation of Y79 cells in vitro. A fraction containing a 50–55-kDa protein doublet (RPE-54) unique to human fetal RPE-CM when compared to control, non-conditioned medium, was found to mimic the neurotrophic effects exhibited by the complete human fetal RPE-CM (Tombran-Tink and Johnson, 1989b).

Recently, ion-exchange and size exclusion HPLC have been utilized in an improved purification regime to purify further this pigmented epithelium derived factor (PEDF) (manuscript in preparation). Following purification, PEDF appears as a single, closely spaced 50-kDa doublet in SDS-gels stained with Coomassie Blue (Fig. 1) or silver. Preliminary sequence analyses have confirmed the presence of a single protein species in the purified preparations. As described here, we have further characterized PEDF as a potent inducer of neuronal differentiation of Y79 retinoblastoma cells. The experimental procedure used to test neurotrophic effects of PEDF on Y79 cells were the same as those previously used to study the effects of complete human fetal RPE-conditioned medium and RPE-54 (Tombran-Tink and Johnson, 1989a, b). Briefly, Y79 cells were pre-treated (stimulated) in suspension culture for 7 days with 50–250 ng ml^{-1} PEDF in serum-free MEM supplemented with insulin, transferrin, selenium, sodium pyruvate, Hepes and minimum essential amino acids. Following stimulation with PEDF, the cells were seeded onto poly-D-lysine-coated glass coverslips and maintained for the duration of the

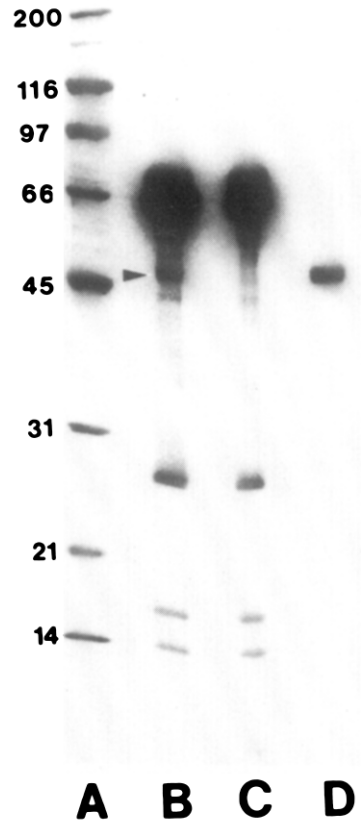

FIG. 1. Coomassie blue-stained SDS polyacrylamide gel showing the purification of PEDF from human fetal RPE-conditioned medium. Lane A, Molecular weight standards (labeled in kDa). Lane B, Human fetal RPE-conditioned medium; the PEDF doublet that is unique to RPE-CM (compare with Lane C) is indicated by the arrowhead. Lane C, Control non-conditioned medium. Lane D, PEDF as purified from RPE-CM by ion exchange and size exclusion HPLC.

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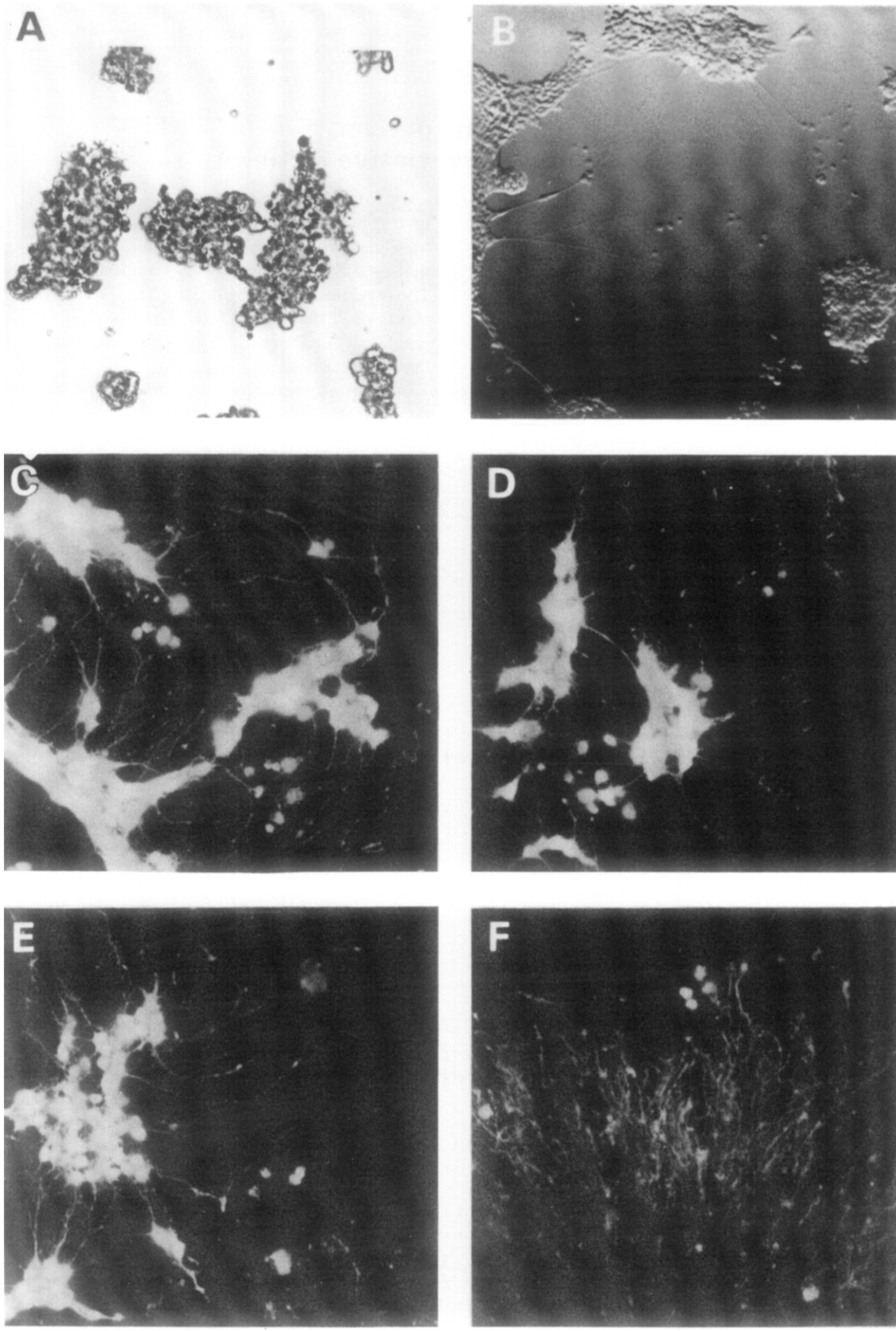
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experiment in serum-free MEM without PEDF. In comparison to untreated cells [Fig. 2(A)], the majority of PEDF-treated Y79 retinoblastoma cells extend long neurites within 3 days of attachment [Fig. 2(B)]. These can be seen to interact with neurites from adjacent cells, forming a complex meshwork of interlacing processes. Y79 cells induced to differentiate by PEDF also express the neuronal marker molecules, neuron-specific enolase [Fig. 2(C) and (D)] and the neurofilament proteins [Fig. 2(E) and (F)].

Both human fetal RPE-CM and the crude RPE-54 fraction produce similar effects in Y79 cells, but the effects are usually observed 8–10 days following attachment of stimulated cells. Moreover, this response usually requires the addition of laminin (a promoter of neurite outgrowth). However, purified PEDF stimulates elaborate neurite outgrowth at very low concentrations (as low as 50 ng ml^{-1}) in a shorter period of time (i.e. 3 days) and its effect is not dependent on the presence of laminin. In addition, the length of the stimulatory (suspension culture) period required for PEDF is shorter (3–5 days) than that for human fetal RPE-CM or the RPE-54 fraction (7–10 days). The relatively high potency of PEDF in comparison to complete RPE-CM may be due, at least in part, to inhibitors or degrading enzymes present in the complete medium that reduce the concentration of bioactive PEDF.

Thus, we have purified a 50-kDa protein, termed PEDF, that is secreted by human fetal RPE cells and is a potent inducer of neuronal differentiation of Y79 retinoblastoma cells. Sequence analysis of PEDF is under way and its possible therapeutic use in treatment of retinal degenerative and other retinal diseases is currently being examined.

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References

Adler, A. J. and Severin, K. M. (1981). Proteins of the bovine interphotoreceptor matrix: tissues of origin. *Exp. Eye Res.* **32**, 755–69.

Allen, C. B., Spoerri, P. E., Kelley, K. C. and Ulshafer, R. J. (1988). Differentiation of photoreceptor cells in cultures of embryonic chick retina supplemented with pigment epithelium conditioned medium. *Invest. Ophthalmol. Vis. Sci.* **29**, 242.

Bryan, J. A. and Campochiaro, P. A. (1986). A retinal pigment epithelial cell-derived growth factor(s). *Arch. Ophthalmol.* **104**, 422–5.

Campochiaro, P. A., Sugg, R., Grotendorst, G. and Jhelmeland, L. (1988). Retinal pigment epithelial cells produce PDGF-like proteins and secrete them into their media. *Invest. Ophthalmol Vis. Sci.* **29**, 305.

Connor, T., Roberts, A., Sporn, M., Davis, J. and Glasser, B. (1988). RPE cells synthesize and release transforming growth factor-beta, a modulator of endothelial cell growth and wound healing. *Invest. Ophthalmol Vis. Sci.* **29**, 307.

Elner, V. M., Strieter, R. M., Elner, S. G., Baggiolini, M., Lindley, I. and Kunkel, S. L. (1990). Neutrophil chemotactic factor (IL-8) gene expression by cytokine-treated retinal pigment epithelial cells. *Am J. Pathol.* **136**, 745–50.

Hewitt, A. T., Lindsey, J. D., Carbott, D. and Adler, R. (1990). Photoreceptor survival-promoting activity in interphotoreceptor matrix preparations: characterization and partial purification. *Exp. Eye Res.* **50**, 79–88.

Kyritsis, A. P., Tsokos, M. and Chader, G. J. (1987). Behavior of human retinoblastoma cells in tissue culture. *Prog. Retinal Res.* **6**, 245–4.

Liu, L., Chen, S.-H. G., Jiang, L.-Z., Hansmann, G. and Layer, P. G. (1988). The pigmented epithelium sustains cell growth and tissue differentiation of chicken retinal explants in vitro. *Exp. Eye Res.* **46**, 801–12.

Liu, Y., Gaur, V. and Turner, J. E. (1990). Photoreceptor cell survival and differentiation stimulated by rat retinal pigment epithelium conditioned medium in tissue culture. *Invest. Ophthalmol. Vis. Sci.* **31**, 75.

Rosenbaum, J. T., O'Rourke, L., Davies, G., Wenger, C., David, L. and Robertson, J. E. (1987). Retinal pigment epithelial cells secrete substances that are chemotactic for monocytes. *Curr. Eye Res.* **6**, 793–800.

Schweigerer, K., Malerstein, B., Neufeld, G. and Gospodarowicz, D. (1987). Basic fibroblast growth factor is synthesized in cultured retinal pigment epithelial cells. *Biochem. Biophys. Res. Commun.* **143**, 934–40.

Sheedlo, H. J. and Li, L. (1990). Effects of a putative RPE-cell trophic factor(s) on photoreceptor cell survival in RCS dystrophic rats. *Invest. Ophthalmol. Vis. Sci.* **31**, 595.

Spoerri, P. E., Ulshafer, R. J., Ludwigh, H. C., Allen, C. B. and

FIG. 2. A, Y79 cells (control) attached to poly-D-lysine for 3 days. × 94. B, Nomarski micrograph showing several long neurites extending between and making contacts with Y79-cell aggregates. Cells treated with 50 ng ml^{-1} PEDF for 7 days in suspension culture followed by 3 days without PEDF in attachment culture. × 94. C, A meshwork of processes seen between two aggregates of Y79 cells. Immunofluorescence for neurofilament protein 200 kDa in cells treated as above. × 94. D, Immunohistochemical staining of neurofilament protein 200 kDa in PEDF-differentiated Y79 cells. Cells treated as above. × 94. E and F. Differentiated Y79 cells (3 days attached) showing positive immunofluorescence for neuron-specific enolase. Note the elaborate neurite outgrowths (F) induced by PEDF (Y79 cell-aggregates were detached during immunohistochemical procedures). Cells treated with PEDF as above. × 94.

Kelly, K. C. (1988). Photoreceptor cell development in vitro: influence of pigment epithelium conditioned medium on outer segment differentiation. *Eur. J. Cell Biol.* **46**, 362–7.

Tombran-Tink, J. and Johnson, L. V. (1989a). Neuronal differentiation of retinoblastoma cells induced by medium conditioned by human RPE cells. *Invest. Ophthalmol. Vis. Sci.* **30**, 1700–7.

Tombran-Tink, J. and Johnson, L. V. (1989b). RPE-54 – a unique RPE product with neuronal differentiating activity. *Invest. Ophthalmol. Vis. Sci.* **29**, 414.

Vollmer, G. and Layer, P. G. (1986). An in vitro model of proliferation and differentiation of the chick retina: coaggregates of retinal and pigment epithelial cells. *J. Neurosci.* **6**, 1885–96.

Vollmer, G. S., Layer, P. G. and Giere, R. A. (1984). Reaggregation of embryonic chick retina cells: pigment epithelial cells induce a high order of stratification. *Neurosci. Lett.* **48**, 919–1984.

Wong, C., Boulton, M., McLeod, D., Bayly, M., Clark, G. and Marshall, J. (1988). Retinal pigment epithelial cells in culture produce retinal vascular mitogens. *Arch. Ophthalmol.* **106**, 1439–43.

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