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Research Report

# Microscopic characterization of rat retinal progenitor cells

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ABSTRACT

A progenitor cell line was developed from a postnatal day 2 (P2) rat retina to study the effects of secreted proteins of the retinal pigment epithelium (RPE) on isolated retinal progenitor cells and markers for immature and differentiated retinal cells. Progenitor cells were cloned from a P2 explant grown in secreted proteins of cultured RPE cells. A cell line was cloned from a single progenitor cell. During the period of RPE-secreted protein stimulation the cells were transformed with the psi AE1A virus. Progenitor cells formed extensive processes when grown in serum and proliferated from the explant when grown in secreted proteins of RPE cells as demonstrated by bromodeoxyuridine (BrdU). All progenitor cells at early and late passages including a cloned cell line (D4) expressed Pax6, a transcription factor essential for eye development, which was verified by Western blotting. All cells expressed nestin, an early neuroepithelial cell marker. These two traits showed the immature character of these rat retinal progenitor cells. All cells expressed the intermediate filament protein vimentin, an intermediate filament protein. Interestingly, most progenitor cells grown in serum expressed the mature cell markers opsin, but few cells expressed glial fibrillary acidic protein (GFAP). The progenitor cells responded to proteins secreted by cultured RPE cells by forming large clusters, while cells grown in retinoic acid formed long thin processes that extended from a round cell body. These progenitor cells, following treatment with secreted proteins of the RPE, will be tested for their therapeutic effect in diseased rat retinas.



## 1. Introduction

Progenitor cells are derived from other more immature progenitor cells or stem cells. However, progenitor cells have a more limited mitotic activity and a narrow cell fate (Gage et al., 1995; Kendroti and Vernadakis, 1995). Neurons and glial cells in the retina have a common progenitor cell late in development (Turner and Cepko, 1987). One difficulty regarding the study of progenitor cells relates to the number of cells that can be generated for biochemical and genetic analyses and to determine the factors necessary for cell fate determination. This limitation has been overcome for retinal progenitor cells by treatment of retinal explants with proteins secreted by cultured retinal pigment epithelial (RPE) cells (Sheedlo and Turner, 1996a,b).

Recently, a procedure was developed to produce pure populations of retinal progenitor cells that were harvested from embryonic and neonatal rat retinal explants stimulated by RPE-secreted proteins (Sheedlo and Turner, 1996a,b). As shown by immunocytochemistry, approximately 80% of the rat retinal progenitor cells from embryonic rat retinal explants expressed a photoreceptor cell phenotype (opsin and arrestin), while approximately 20% expressed a Müller cell marker, cellular retinaldehyde binding protein (CRALBP) (Sheedlo and Turner, 1996b). Slightly more progenitor cells from neonatal rat retinas were opsin-expressing (90%), while fewer cells

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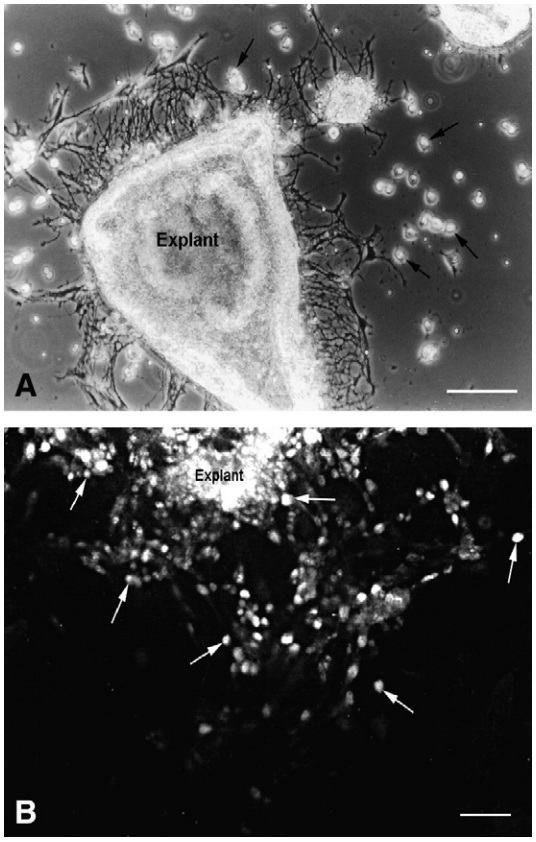


**Fig. 1 – Phase contrast microscopic examination of retinal progenitor cells from postnatal day 2 rats. (A) P2 retinal explants grown in RPE-secreted proteins for 7 days exhibited round cells (arrowheads) emerging from their periphery. (B) Proliferating cells were detected within and emerging from retinal explants grown in RPE-secreted proteins showed as shown by BrdU immunolabeling. Scale bars = 100 μm (A and B).**

expressed CRALBP (20%) (Sheedlo and Turner, 1996b). However, these progenitor cells, even though expressing mature-cell proteins, still retained the potential to undergo mitosis; thus, these cells were not completely differentiated. Specifically, progenitor cells from P2 rat retinas divided 2 fold over a three-day period when grown in RPE factors, but were not similarly stimulated by known growth factors, such as nerve growth factor (NGF) or epidermal growth factor (EGF) (Sheedlo and Turner, 1996b).

A factor has been isolated from secreted proteins of human RPE cells, termed pigment epithelial derived factor (PEDF). This factor promoted differentiation of the Y79 neuroblastoma cells (Tombran-Tink et al., 1991). In addition, RPE cells were shown to secrete and possess the message for several factors, including basic fibroblast growth factor (FGF-2) (Campochiaro, 1993; Schweigerer et al., 1987; Sheedlo et al., 1997), epidermal growth factor (EGF), platelet-derived growth factor (PDGF) (Campochiaro et al., 1988) and the neurotrophins (nerve growth factor, brain-derived neurotrophic factor, neurotrophin-3 and -4) (Ishida et al., 1997). Furthermore, fetuin, a plasma protein, primarily synthesized and secreted by hepatocytes, was shown to be secreted by cultured rat RPE cells (Sheedlo et al., 1999). The RPE differentiates prior to cells of the neural retina, thus these cells are most likely the source of proteins essential for retinal cell survival, cell fate determination and maturation (Braekevelt and Hollenberg, 1970; Clark, 1986). Retinoic acid, a molecule stored in the RPE, is an essential molecule involved in retinal development, specifically photoreceptor differentiation (Jean et al., 1998; Kelley et al., 1994; Soderpalm et al., 2000; Wightman et al., 2001) and development of high visual acuity (Luo et al., 2006).

Pax6 is a transcription factor that has an N-terminal amino acid DNA binding domain. This factor is expressed in developing ocular tissue, including the retina, lens and cornea. During early development of the retina, the primary role of Pax6 is to maintain the numbers of retinal progenitor cells by allowing proliferation without affecting differentiation. Pax6 regulates the cell fate and potency of retinal progenitor cells by controlling the evolution of retinal progenitor cells toward a specific differentiated cell type. Inactivation of Pax6 affects cell fate of retinal progenitor cells to a single cell type, amacrine cells (Marquardt et al., 2001). Nestin, an early neuroepithelial cell marker, is an intermediate-filament associated protein that plays a role in the cytoskeleton. Nestin expression has been used as a marker for progenitor and stem cells within the

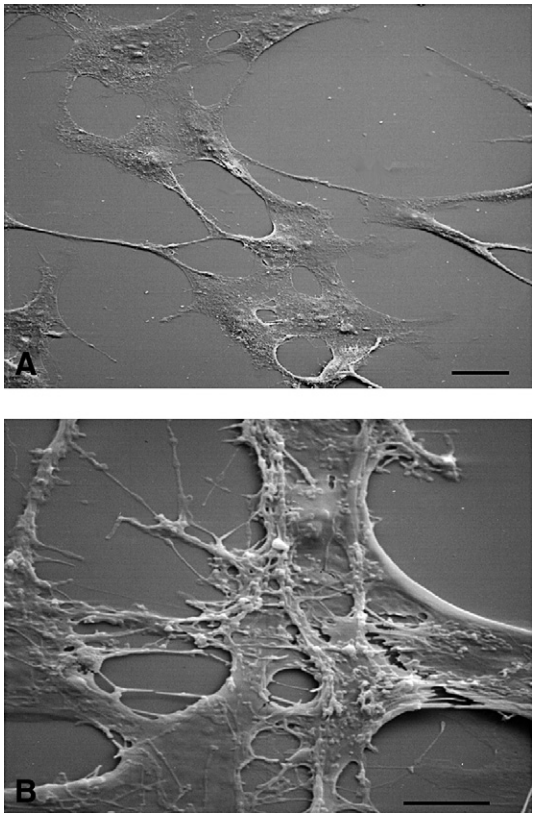


**Fig. 2 – Scanning electron microscopic examination of rat retinal progenitor cells. (A) Progenitor cells, at 13th passage, grown in serum for 1 week appeared flattened and had slender, long processes. (B) When viewed at higher magnification, the cell body of these cells was covered by slender processes from adjacent cells and, possibly, the same cell. Scale bars = 10 μm (A); 20 μm (B).**