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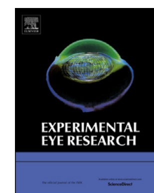

Identification and localization of lamina cribrosa cells in the human optic nerve head

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A B S T R A C T

One of the central features of glaucoma is progressive cupping and excavation of the optic nerve head (ONH). Unmyelinated retinal ganglion cell (RGC) axons exit the eye through the ONH, which is supported by the lamina cribrosa (LC) consisting of plates of connective tissue with channels for bundles of RGC axons. The LC progressively remodels during glaucoma, but the cellular and molecular mechanisms responsible for this remodeling are poorly understood. Two major cell types have been isolated and cultured from the human ONH, which differ in their characteristics. Glial fibrillary acidic protein (GFAP) positive ONH astrocytes are the major cell type and are reactive in glaucoma. GFAP negative LC cells are the second major cell type isolated from the human ONH, and in contrast to ONH astrocytes, are α-smooth muscle actin (α-SMA) positive. Although a number of *in vitro* studies have been conducted with LC cells, to date there has been no direct evidence for these cells *in situ* in the human ONH. We used GFAP and α-SMA immunofluorescent staining of human eyes to clearly demonstrate the presence of not only ONH astrocytes within the human ONH, but also LC cells within the cribriform (e.g. laminar) plates/beams of the LC region. Both of these cell types likely play important roles in the homeostatic maintenance of the ONH and pathogenic changes that occur in primary open angle glaucoma (POAG).

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Primary open angle glaucoma (POAG) is a heterogeneous group of optic neuropathies affecting 70 million people worldwide (Quigley, 1996) and is a major cause of visual impairment and irreversible blindness. Elevated intraocular pressure (IOP) is a major risk factor in the development and progression of glaucomatous optic neuropathies (Kass et al., 2002) and progression of glaucomatous damage (Heijl et al., 2002). POAG is associated with biochemical (Fuchshofer, 2011) and biophysical (Burgoyne, 2011) changes within the human ONH that can damage the unmyelinated optic nerve axons and eventually lead to apoptosis and death of retinal ganglion cells (RGC).

In POAG patients, hallmarks of damage in the ONH include excavation, cupping (Crawford Downs et al., 2011) and alterations in the extracellular matrix (ECM) (Fuchshofer, 2011; Hernandez, 1992; Pena et al., 1998). The LC region of the ONH is composed of glial columns and connective tissue plates that align to form channels, which is the main structural component through which RGC axons exit the eye (Anderson, 1969). The LC is now recognized as a major site of RGC damage in POAG. Cells within the LC have profound effects on the ECM environment and RGC survival. Autocrine and/or paracrine signaling of growth factors between cells of the LC may play a role in maintaining homeostatic mechanisms within the human LC (Lambert et al., 2001a).

Two cell types were initially isolated and cultured from the human ONH by Hernandez and colleagues (Hernandez et al., 1988). One cell type identified as LC cells were characterized as large, flat polygonal cells that were negative for GFAP. The second isolated cell type was characterized as large cells that were positive for GFAP with multiple long and thin cellular processes. These GFAP positive cells could be identified as type 1A or type 1B astrocytes, with type 1B astrocytes considered the major glial cell type in the ONH (Hernandez, 2000). Since this original report, several laboratories have furthered characterized human LC cells as α-SMA positive and GFAP negative, while ONH astrocytes were GFAP positive and α−SMA negative (Lambert et al., 2001a, 2004a, b; Rogers et al., 2012a; Rogers et al., 2012b).

Human ONH astrocytes and human LC cells have been utilized

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for numerous *in vitro* studies (Fuchshofer, 2011; Irnaten et al., 2009; Kirwan et al., 2004, 2005; Lambert et al., 2001a; Lambert et al., 2004a, b; Rogers et al., 2012a; Rogers et al., 2012b). It is apparent from these *in vitro* studies that ONH astrocytes and LC cells are distinct cell types that respond differently to various cellular conditions and stimuli (Lambert et al., 2004a, b). However, there has been no morphological confirmation that LC cells are present in the LC region of the human ONH. The purpose of this study was to identify α-SMA positive LC cells and GFAP positive astrocytes within the human ONH.

Three pairs of normal human eyes were used for each experiment, and ages ranged from 76 to 82 years old. Donor eyes were obtained from regional eye banks within 6–8 h of death and fixed in 10% formalin. The eyes were obtained and managed in compliance with the Declaration of Helsinki. Eyes were embedded in paraffin and stored until further use. Posterior segments were dissected and human ONH tissues were immunostained to detect the presence of α-SMA (e.g. LC cells) and GFAP (e.g. astrocytes). Paraffin sections were deparaffinized, rehydrated with PBS, and placed in 0.01% Trition-X100 for 20 min or citrate buffer (pH 6) for antigen retrieval, followed by 20 mM glycine for 15 min. Sections were blocked in 10% normal serum, rinsed in PBS and then incubated overnight at 4° C with mouse monoclonal GFAP antibody (NeoMarkers; Catalog # Ab-6) at a final dilution of 1:100, rabbit polyclonal α-SMA antibody (Abcam; Catalog # ab5694) at a final dilution of 1:100, goat polyclonal platelet-derived growth factor receptor beta (PDGFRβ) (Abcam; Catalog # ab10848) at a final dilution of 1:50, and mouse monoclonal laminin (Developmental Studies Hybridoma Bank; Catalog #2E8) at a final dilution of 1:100.

The slides were washed, and the primary antibodies subsequently were detected by incubation with either Alexa 488 goat anti-mouse (1:200), Alexa 633 goat anti-rabbit (1:200), Alexa 488 donkey anti-mouse (1:200), Alexa 594 donkey anti-rabbit (1:200), and Alexa 488 donkey anti-goat (1:200) for 1 h (Molecular Probes,

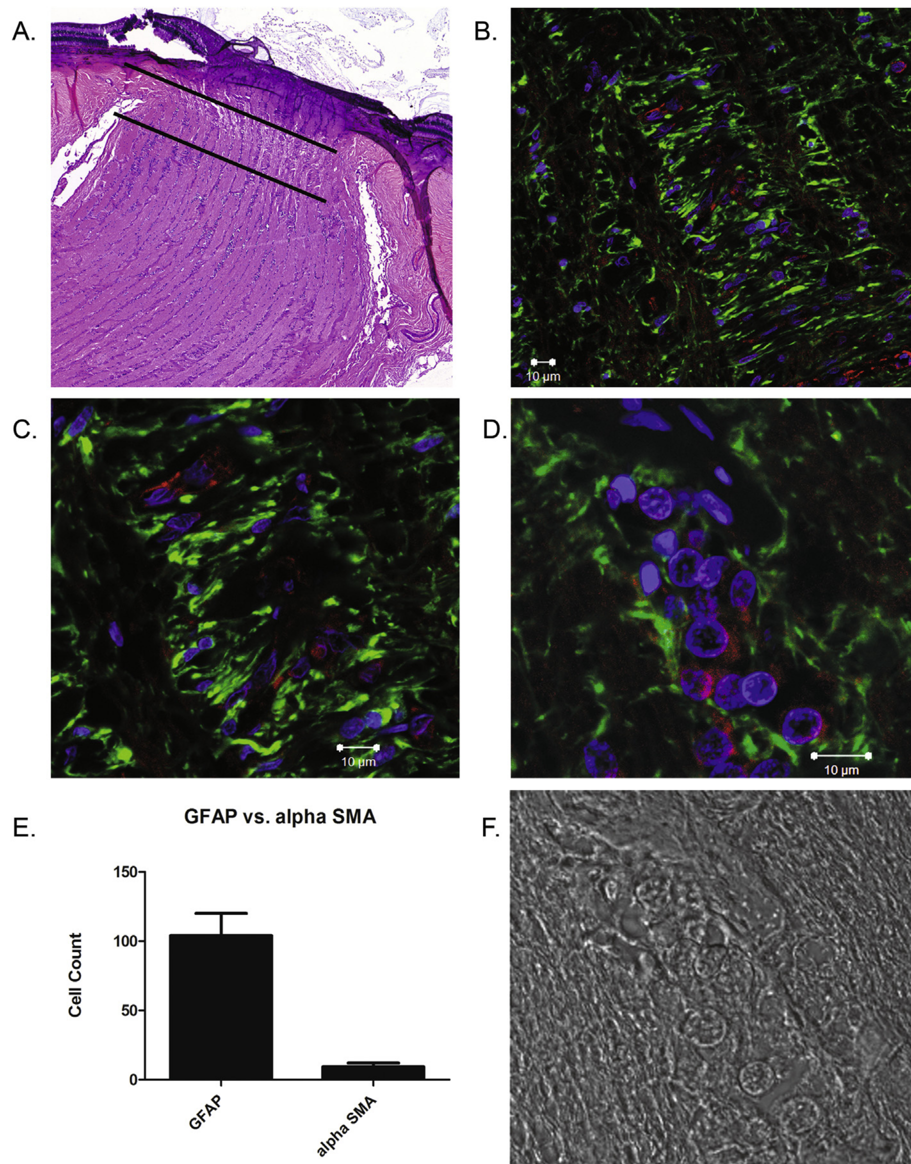

Fig. 1. Localization of α-SMA and GFAP in the human ONH. Hematoxylin and eosin staining of the human ONH (A) identifying the LC region delineated by black bars. LC cells are oval in shape, localized inside cribriform plates and stained positive (red) for α-SMA (B–D). GFAP positive astrocytes (green) were more numerous and extended thin processes into the core of the cribriform plates (B–D). Scale bar equals 10 μm. GFAP ONH astrocytes were significantly more numerous compared to the α-SMA LC cells, $^*p < 0.05$ (E). Differential interference contrast image of the LC region of image d (F).

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