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Life under pressure: the role of ocular cribriform cells in preventing glaucoma

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Abstract

Primary open-angle glaucoma is a multifactorial blinding disease often impacting the two pressure-sensitive regions of the eye: the conventional outflow pathway and the optic nerve head (ONH). The connective tissues that span these two openings in the globe are the trabecular meshwork of the conventional outflow pathway and the lamina cribrosa of the ONH. Resident cribriform cells of these two regions are responsible for actively remodeling and maintaining their connective tissues. In glaucoma, aberrant maintenance of the juxtacanalicular tissues (JCT) of the conventional outflow pathway results in ocular hypertension and pathological remodeling of the lamina cribrosa results in ONH cupping, damaging retinal ganglion cell axons. Interestingly, cells cultured from the lamina cribrosa and the JCT of the trabecular meshwork have similarities regarding gene expression, protein production, plus cellular responses to growth factors and mechanical stimuli. This review compares and contrasts the current knowledge of these two cell types, whose health is critical for protecting the eye from glaucomatous changes. In response to pressure gradients across their respective cribriform tissues, the goal is to better understand and differentiate healthy from pathological behavior of these two cell types.

Keywords

Primary open-angle glaucoma; lamina cribrosa; juxtacanalicular tissue; trabecular meshwork; astrocyte; Schlemm's canal

1. Introduction

Primary open-angle glaucoma (POAG) is defined as a chronic optic nerve disease that is characterized by loss of retinal ganglion cells (RGC) and “cupping” of the optic nerve head (ONH). Cupping is a result of RGC axon loss and remodeling of the lamina cribrosa (LC), a

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reticulated meshwork of extracellular matrix (ECM) that spans the optic disc opening and provides support to RGC axons as they leave the eye. Often coincident with these glaucomatous changes at the ONH is an elevation in intraocular pressure (IOP) caused by an increased resistance to conventional outflow.(Weinreb and Khaw, 2004; AGIS investigators., 2000) Elevated outflow resistance is thought to be primarily caused by dysfunctional remodeling of extracellular matrix materials in the juxtacanalicular tissues (JCT) of the pressure-sensitive conventional outflow pathway.(Keller and Acott, 2013; Stamer and Acott, 2012) While glaucoma progresses in many when IOP is in the normal range, effective IOP lowering in these people slows or stops vision loss.(Collaborative Normal-Tension Glaucoma Study Group, 1998; Heijl et al., 2002; Kass et al., 2002) Moreover, provocative elevation in IOP of normal eyes induces characteristic glaucomatous changes at the ONH in different animal models, including non-human primates.(Quigley et al., 2000; Yan et al., 2015) Thus, it appears that glaucoma involves pathology that may be attributed to the dysfunctional responsiveness to mechanical stimuli (normal or excessive) of the two cell types from cribriform regions of the eye, the JCT and LC. These areas have been named “cribriform” due to their tridimensional, reticulated, and multilamellar sieve-like structure. Moreover, responsiveness and susceptibility of both cribriform tissues to glaucoma may be in the context of genetic background and/or factors found up-regulated in different parts of the eye, such as reactive oxygen species or transforming growth factor-beta-2 (TGF-beta2). (Agarwal et al., 2015; Awai-Kasaoka et al., 2013; McElnea et al., 2011; O'Reilly et al., 2011; Pena et al., 1999; Tamm et al., 2015).

For over three decades, few studies have focused on the potential pathophysiological links between these two connective tissues in glaucoma.(Rehnberg et al., 1987; Tengroth and Ammitzbøll, 1984; Tengroth et al., 1985) The biochemical similarities of the ECM of both regions(Hernandez and Ye, 1993; Hernandez, 1992; Hernandez et al., 1990, 1987; Keller and Acott, 2013; Rehnberg et al., 1987; Steely et al., 2000; Tengroth and Ammitzbøll, 1984; Tengroth et al., 1985; Vranka et al., 2015b) supports the notion that POAG is a complex disorder involving distinct tissues of the eye, but with analogous (re) actions (Figure 1). Descriptions of pathobiological activity of the LC cells in the optic nerve and the JCT cells in the TM are hauntingly similar.(Clark et al., 1995; Fuchshofer et al., 2006; Hernandez et al., 1988; Rohen et al., 1982) For instance, both cells maintain complex ECM structures that are orchestrated by a number of autocrine and paracrine signaling molecules, some of which are related to the pathologic processes of glaucoma. However, no previous work has either concomitantly looked at the cellular and ECM responses of both cells *in vitro* or summarized all current unconnected reports.

Here, we present a critical review of the literature assessing the similarities and differences between LC and JCT cells, with particular attention to work using cultured cells. Our goal is to take a unique perspective that is intended to provide insight into how these two cell types when healthy prevent progression to POAG, conditions that may set up the development of POAG and ideas that may catalyze future research. A systematic search on PubMed and Google Scholar was performed using the terminology related to POAG presented in this review, with no restriction on publication dates until April 2016. Ninety-nine selected full articles and abstracts published in English were reviewed, using the keywords: trabecular meshwork tissue, trabecular meshwork cells, juxtacanalicular tissue, juxtacanalicular cells,

cribriform tissue, cribriform cells, optic nerve tissue, optic nerve cells, lamina cribrosa tissue, lamina cribrosa cells (Supplemental table 1).

2. Lamina Cribrosa Cells

2.1. Morphological Characterization

The optic disc, or ONH, is the anterior part of the optic nerve and consists of bundled axons from the retinal ganglion cells, plus support tissues and cells.(Weinreb and Khaw, 2004) Before emerging as the extraocular optic nerve, unmyelinated fibers traverse a perforated connective tissue diaphragm called the LC.(Burgoyne, 2011; Weinreb and Khaw, 2004) Through the three-dimensional fibroelastic meshwork of the LC, laminar capillaries deliver nutrition to the axonal bundles and all local cells.(Burgoyne, 2011; Wallace and O'Brien, 2016) Resident cells cultured from the ONH include astrocytes, scleral fibroblasts, oligodendrocytes, microglia, vascular endothelial cells, pericytes, and LC cells.(Clark et al., 1995; Hernandez et al., 1988; Kennedy and Lisak, 1980; Neufeld, 1999; Wallace and O'Brien, 2016; Yuan and Neufeld, 2001) LC cells are located within the LC plates, which are composed of elastin, collagen type I, III, IV, and VI, laminin, and heparan sulfate proteoglycan.(Wallace and O'Brien, 2016; Hernandez and Pena, 1997; Tovar-Vidales et al., 2016)

In the first reports, a “fibroblastoid” glial fibrillary acidic protein (GFAP)-negative (–) cell type cultured from dissociated optic nerve of adult rats was described.(Kennedy and Lisak, 1980) Later on, Hernandez et al. worked extensively with these GFAP(–) cells from dissected human ONH in culture, and denominated them as LC cells.(Hernandez et al., 1988) LC cells have been described as large, flat, broad, polygonal GFAP(–)/alpha-smooth muscle actin (alpha-SMA)+ cells that possess multiple cell processes.(Clark et al., 1995; Hernandez et al., 1988; Wallace and O'Brien, 2016), but take on a more oval shape *in situ*.(Tovar-Vidales et al., 2016) Although LC cells reside within the LC cribriform plates, GFAP positive astrocytes are located in the plate openings alongside axon bundles as they transverse multiple plates.(Clark et al., 1995; Hernandez, 2000; Hernandez et al., 1988; Wallace and O'Brien, 2016) Regarding their embryonic origin, the glial elements of the ONH are derived from ectodermal cells, but the mesenchymal cells of neural crest account for the development of the LC tissue, and cells.(Cook et al., 2012)

Ultrastructural evaluations show that LC cells are elongated with abundant cytoplasmic actin microfilaments and organelles, such as an active rough endoplasmic reticulum, a well-developed Golgi apparatus and a dense band of heterochromatin along the nuclear membrane. When cultured in monolayers, LC cells generate a non-continuous basement membrane-like material, surrounded by an uninterrupted layer of ECM.(Clark et al., 1995; Hernandez et al., 1988; Wallace and O'Brien, 2016) LC cells cultured from glaucomatous donors have a higher number of accumulated peri-nuclear organelles, suggested to be lipofuscin granules.(McElnea et al., 2014)

LC cells are cultured from dissected LC tissue. Explants are placed in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum, buffered in a humidified atmosphere containing 5% CO₂ at 37° C. Cells can be passaged after 2–3 months of

outgrowth using trypsin or Cytodex 3 microcarrier beads, which was proposed as better option for reaching healthy, higher-passage cells.(Clark et al., 1995; Hernandez et al., 1988) The addition of platelet-derived growth factor to the initial LC explant culture stimulates cells to start migrating away from the dissected tissue and divide in culture (Steely et al., 2000) Not surprisingly, cell cultures from older donors display a longer time to reach complete confluency, and have difficulties in subsequent cultivation.(Hernandez et al., 1988)

2.2. Cell pathobiology

A consistent pattern of protein expression of the LC cells in culture includes absence of GFAP, and presence of alpha-SMA, fibronectin, laminin, elastin, proteoglycans, and collagen type I, III, and IV expression.(Clark et al., 1995; Hernandez and Ye, 1993; Hernandez et al., 1988, 1987; Wallace and O'Brien, 2016; Wang et al., 2015; Job et al., 2010) These constitute some of the major proteins observed in the ECM of LC in humans, nevertheless higher or lower expression of individual proteins were demonstrated depending on the cellular maturation in culture and on the different conditions affecting the LC tissue. (Clark et al., 1995; Hernandez and Ye, 1993; Hernandez, 1992; Hernandez et al., 1990, 1989, 1988, 1987, 1986; Morrison et al., 1989) In culture, LC cells must be differentiated from potential local cellular contaminants such as astrocytes, scleral fibroblasts, vascular endothelial cells, and pericytes (Table 1). Morphological characteristics and biochemical markers have been described for all of these cells, as follows: a) astrocytes – large cells with long, thin, and varicose processes positively staining for GFAP and collagen type IV; b) scleral fibroblasts – long, thin, spindle-shaped cells growing in multiple layers, expressing fibroblast-specific protein 1, but not collagen type IV; c) vascular endothelial cells – polygonal cells with smooth borders, and cobblestone appearance presenting typically positive expression of CD31, CD34(Feilchenfeld et al., 2008), collagen IV, laminin, and Factor VIII; d) pericytes – large cells with a dendritic shape and thin processes, that stain with the antibody (3G5)-defined ganglioside antigen, but not collagen IV, laminin, and Factor VIII.(Clark et al., 1995; Strutz et al., 1995)

Since a mechanical failure and fibrosis of the LC has been postulated as major susceptibility events that underlie ONH damage observed in patients with POAG, recent studies focus on LC responses to IOP or simulated IOP in cell culture with stretch.(Hernandez et al., 1990) It is important here to note that early studies with LC cells simulated elevated IOP by enclosing cultured cells on plastic in pressure chambers (Wax et al., 2000; Yang et al., 1993). These studies have been subsequently shown not to model elevated IOP, but hypoxia (Lei et al., 2011). So data generated in these early studies with LC cells and astrocytes need to be evaluated with this in mind.

In advanced glaucoma, the fibrotic LC tissue is displaced posteriorly, collapsed, and thickened (Burgoyne, 2011); displaying increased elastin and collagen.(Hernandez and Pena, 1997) ECM remodeling in the glaucomatous ONH includes increased density of collagen type IV(Hernandez et al., 1994b, 1990), VI(Hernandez et al., 1990), compacted collagen type I(Hernandez et al., 1990), abnormal and disorganized elastin(Hernandez, 1992; Hernandez et al., 1994a). Interestingly, no apparent alterations in collagen type III or fibronectin were detected.(Hernandez and Pena, 1997; Hernandez and Ye, 1993; Hernandez

et al., 1990) Moreover, LC cells from donors with POAG express pro-fibrotic genes differently than LC cells from normal donors.(Kirwan et al., 2009) Thus, POAG LC cells show higher expression of periostin, tissue inhibitor of metalloproteinase 3 (TIMP-3), versican, secreted protein acidic and rich in cysteine (SPARC), thrombospondin (TSP) -1, lysyl oxidase (LOX), TGF-beta, collagen I, V, and XI.(Kirwan et al., 2009; Wallace and O'Brien, 2016)

LC cells produce increased expression of ECM proteins if exposed to mechanical stimulation(Kirwan et al., 2005a) or treatment with growth factors such as TGF – beta1. (Kirwan et al., 2005b) In fact, TGF –beta1, and –beta2, has been extensively associated with glaucoma, including the higher expression of TGF-beta2 by cells of the LC in glaucomatous patients.(Pena et al., 1999) LC cells stimulated by TGF-beta1, –beta2, or stretch increased the expression of genes involved in ECM remodeling, such as connective tissue growth factor (CTGF), TGF-beta-1, TGF-beta-2, TIMP-1, matrix metalloproteinase (MMP)-2, elastin, fibronectin, LOX, TSP-1 and -2, collagen I, IV, VI, VIII, and IX.(Kirwan et al., 2005a, 2005b, 2004; Zode et al., 2011) Using a proteomics approach, cyclic stretch of LC cells resulted in increased levels of TGF-beta1, bone morphogenetic proteins (BMP)-7, tumor necrosis factor, caspase-3, and tumor protein p53.(Rogers et al., 2012; Kirwan et al., 2005a) Irnaten et al. proposed that the mechanism by which LC cells modify their protein production is through calcium sensitive-K⁺ ion channel activity in response to the cyclical stretch.(Irnaten et al., 2013; McElnea et al., 2011) A prominent change in LC cells of the glaucomatous optic nerve is an altered arrangement of stress fibers bundles.(Job et al., 2010) LC cells, but not astrocytes, present with more frequent cytoskeletal structures called cross-linked actin networks (CLANs). Located mostly around the nucleus, CLANs have a typical triangular “hub and spoke” arrangement of actin and actin binding proteins that assume a geodesic structures.(Job et al., 2010) The presence of CLANs in normal ONH specimens likely correspond to LC cells under tension in the mechanically demanding LC environment. However, it has been hard to describe a CLAN distribution and alignment pattern considering the complex tridimensional architecture of the LC.(Job et al., 2010)

3. Juxtacanalicular Tissue Cells

3.1. Morphological Characterization

The conventional drainage pathway for aqueous humor consists of the TM tissue, which is located circumferentially in the scleral sulcus at the peripheral angle of the anterior chamber. The TM tissue can be divided into three regions: the uveal meshwork, the corneoscleral meshwork and the JCT, where Schlemm’s canal (SC) inner wall cells interact with JCT cells.(Stamer and Acott, 2012; Tamm et al., 2015) Here in the JCT region is where resistance to outflow is generated and IOP is determined.(Grant, 1963; Tamm et al., 2015) Increased resistance in the JCT is responsible for ocular hypertension frequently observed in POAG.(Grant, 1951) While resistance generation is complicated, the primary contributors are the contraction status of JCT cells, ECM abundance/content of the JCT and permeability of the inner wall of SC.(Keller and Acott, 2013; Stamer and Acott, 2012) The ECM in the JCT, also known as the cribriform region of TM (formerly described as the “trabeculum cribriforme”)(Vegge, 1963), is a three-dimensional porous matrix of fibrillar components

and amorphous ground substances of proteoglycans. Interspersed is an elastic fiber network, the cribriform plexus, which extends from the ciliary muscle tendons to the SC basement membrane.(Johnson et al., 1997) Specimens of POAG TM tissue display thickening of tissue lamellae, increased amount of sheath-derived plaques, and “lattice” collagen in the JCT region.(Lütjen-Drecoll et al., 1986) Shortening of the connecting fibrils in the surrounding elastic network was also described in the JCT region, but changes in the elastic fibers content of the JCT cells isolated in culture have not been examined.(Lütjen-Drecoll et al., 1986; Rasmussen and Kaufman, 2014)

TM cells have been isolated and cultured over the last 40 years(Polansky et al., 1979; Rohen et al., 1982, 1975; Stamer and Clark, 2016), with efforts attempting to identify specific JCT cell subcultures that were first described in the 1980's.(Rohen et al., 1982) JCT cells show higher growth kinetics than corneoscleral cells isolated from dissected TM tissue, which means that they are likely the major cellular component of “mixed TM” cell cultures.(Rohen et al., 1982) Different media contents have been used to grow JCT cells, from Medium 199 to DMEM plus 10–15% fetal bovine serum, with addition or not of glutamine and growth factors.(Lütjen-Drecoll, 1999; O'Brien et al., 2014, 1999) However, isolation techniques vary, including blunt dissection and sharp dissection of all TM tissue or separate regions. (O'Brien et al., 2014, 1999; Polansky et al., 1979; Stamer et al., 1995; Welge-Lüssen et al., 1998) O'Brien et al. successfully isolated JCT cells in culture, progressively dissecting away TM tissue from the uveal beams to cribriform plates, revealing the JCT. When JCT and SC inner wall tissues were removed together, only the oriented side of JCT was use to culture JCT cells.(O'Brien et al., 2014)

In culture, JCT cells have been characterized as small, elongated, somewhat “triangular” cells with long, overlapping cellular processes. Like other primary cultures of cells, JCT cells enlarge with passaging.(O'Brien et al., 2014) JCT cells have a prominent Golgi complex, around a nucleus with a great amount of nucleolar material, as well as a conspicuous rough endoplasmatic reticulum with many ribosomes and some lysosomes, indicating avid protein metabolism.(Lütjen-Drecoll, 1999; Rohen et al., 1982) Ultrastructural studies of JCT cells in situ showed a discontinuous basement membrane associating with elastic fibers at points of thickened plasma membrane.(Fuchshofer et al., 2006; Lütjen-Drecoll, 1999; Rohen et al., 1982) These cells also have abundant characteristic cell-to-cell contacts to both SC and other TM cells through communicating processes.(Grierson et al., 1978; Inomata et al., 1972; Keller and Acott, 2013)

Considering the limited number of studies describing preferentially isolated JCT cells in culture, in this review we have included studies that evaluated functions of “TM cells” that are attributable to JCT cells such as mechanical responses, ECM turnover, and contractility. Due to very similar expression patterns and lack of differential markers, evidence suggest that JCT and TM cells are the same cell “type”, but JCT cells may be a differentiated form of TM cells, assuming unique responsibilities based on their location and role in resistance generation.(Stamer and Clark, 2016) This is in agreement with their common embryological origin at the neural crest evidenced by immunohistochemical studies.(Keller and Acott, 2013; Overby et al., 2009; Rasmussen and Kaufman, 2014; Stamer and Acott, 2012; Tripathi and Tripathi, 1989) We hypothesize that a combination of local stimuli such as ECM

substrates, soluble factors and/or mechanical stress induces a differentiation of TM cells to a JCT phenotype.(Park and Latina, 1993; Tamm et al., 1996) For example, a JCT “marker”, alphaB-crystallin is upregulated by TM cells under stressful conditions such as dexamethasone treatment (Siegnier et al., 1996) or stress caused by heat shock or hydrogen peroxide treatment.(Tamm et al., 1996) The expression of alphaB-crystallin by JCT cells *in vitro* and *in situ* likely reflect their existence in a mechanically and metabolically dynamic environment.(Fuchshofer et al., 2006; Lütjen-Drecoll, 1999; Siegnier et al., 1996; Tamm et al., 1996)

While it is difficult to distinguish TM cells from JCT cells, possible cellular contaminants in culture such as corneal endothelium, scleral fibroblasts, scleral spur cells, and SC cells are usually easier to detect. Cells can be discriminated primarily via *in vitro* morphology and cell-specific markers (Table 1). For example, contaminant cells have the following characteristics: a) corneal endothelium cells – polygonal shape, honey-comb confluence pattern, non-proliferative and expression of zona occludens 1(Palchesko et al., 2015; Tripathi and Tripathi, 1982); b) scleral fibroblasts – elongated, spindle-shaped, and disorganized, positive for fibroblast-specific protein 1, and frequent multilayered foci in culture (Stamer et al., 1998; Strutz et al., 1995); c) scleral spur cells – elongated, spindle-shaped cells with long, thin processes, and hill-valley-growth aspect, immunostaining for vimentin, and alpha-SMA(Welge-Lüssen et al., 1998); d) SC cells – fusiform cells, with linear “railroad track” monolayer formation, isolation based on cannulation of SC lumen, dissection of inner wall (Underwood et al., 1999) or immunopanning using CD31 (Karl et al., 2005; Braakman et al., 2015), vascular-endothelial-cadherin expression (Heimark et al., 2002), transendothelial electrical resistance >10 Ohm cm², and absence of increased myocilin expression with dexamethasone treatment. (Perkumas and Stamer, 2012; Stamer et al., 1998) In contrast, both JCT and TM cells display induction of myocilin protein with dexamethasone treatment.(Stamer and Clark, 2016)

3.2. Cell pathobiology

Although altered functions of the JCT and SC cells is related to increase outflow resistance, the ECM milieu in the JCT region next to the basement membrane of SC is considered the critical part of the matrix for generating outflow resistance, and appears altered in several glaucomatous conditions.(Overby et al., 2009; Vranka et al., 2015b; Last et al., 2011) The ECM of the JCT region is comprised of elastic microfibrils, fibrillar and non-fibrillar collagens, proteoglycans, and matricellular proteins.(Acott and Kelley, 2008; Vranka et al., 2015a, 2015b) For instance, the matricellular proteins shown altered in glaucoma specimens include SPARC, myocilin, CTGF, TSP-1, and -2.(Chatterjee et al., 2014) Changes in these and other proteins can be observed following treatment of cultured cells with stretching, dexamethasone, or TGF-beta stimuli. For example, increased expression of fibronectin, tenascin C, Versican, CD 44, syndecan, periostin, TSP-2, VCAM-1, GLA protein, and MMP-2 have been observed in response to stretch.(Acott and Kelley, 2008)

In line with increased/altered ECM, it appears that the JCT region in glaucomatous eyes are significantly stiffer than age-matched “normal” eyes. (Last et al., 2011) Correspondingly, increased rigidity of the culture substratum induces TM cells to express several molecules

involved in the glaucomatous process, including myocilin, SPARC, transglutaminase-2, alpha-SMA, and alphaB-crystallin. (Vranka et al., 2015b) To get a better idea of altered protein expression in the JCT tissue of glaucomatous eyes in an unbiased way, O'Brien et al. evaluated genes overexpressed by cultured JCT cells from trabeculectomy specimens. They found that most of the upregulated genes in these cells are related to cellular contractility, and matrix function. Particular attention was given to the expression of heparan sulfate 3-O-sulfotransferase-1 precursor, which may affect ECM remodeling. (O'Brien et al., 2014)

In general, cells of TM are contractile, and actin fibers are directly involved in normal and pathological conditions related to glaucoma. (Stamer and Acott, 2012) The ECM of the JCT region contains an elaborate elastin fiber system that supports connections to the scleral spur and ciliary body, and allows tissue distensibility during ciliary muscle contractions and IOP fluctuations. (Hann and Fautsch, 2011) Contractile TM cells oppose ciliary muscle contraction, resulting in a combination of force vectors that modulate the TM architecture and outflow function. (Tamm, 2009) A bidirectional interplay between ECM synthesis and contractile activity is a result of the two-way interplay between TM cell cytoskeleton and components of the ECM. (Pattabiraman and Rao, 2010) In pathological conditions of ocular hypertension, JCT cells may present myofibroblast-like contractile features and increase their connections to the ECM, leading to an overall increase in stiffness of TM tissue, and subsequently decreased outflow. (Tamm, 2009; Tamm et al., 2015)

In this context, some spatial chord-like and “sheet” orientations of actin fibers have been described in normal uveal, and corneoscleral TM respectively, as well as a complex tridimensional arrangement of fibers in the JCT region, also called CLANs. (Hoare et al., 2009) Similar to LC cells of the ONH, CLANs are considered a sign of mechanical stimulation, and are observed in normal TM tissues; however, they are more prominent in glaucomatous eyes. (Hoare et al., 2009; O'Reilly et al., 2011)

4. Similarities between LC and JCT Cells related to Glaucoma

The LC and the TM regions possess several similar characteristics in normal and glaucoma-related conditions. The ECM of these two cribriform plates regions are somewhat alike, built to withstand a pressure gradient created by IOP, but allow passage of aqueous humor in the conventional tract or axon fibers in the ONH. Here we more closely examine the mechanical and biochemical responsibilities of resident cells of these two ocular tissues and how they may be similarly altered in POAG. (Clark et al., 1994; Hernandez et al., 1987; Kirwan et al., 2009; Morrison et al., 1989; Rehnberg et al., 1987; Steely et al., 2000) Not surprisingly, LC and JCT cells share several characteristics. (Fuchshofer et al., 2006; Hernandez et al., 1988; Siegner et al., 1996) We present in Table 2 the similarities of both cell types, from their embryological origin and environmental characteristics to protein expression. Several details presented are striking such as origin, ultrastructural findings, cellular behaviors in culture and functional relationship with the surrounding ECM. They also are remarkably alike in terms of the type and protein components of their basement membrane and ECM, which are altered in glaucoma (Acott and Kelley, 2008; Chatterjee et al., 2014; Kirwan et al., 2009, 2005b, 2004; Liton et al., 2006; Rehnberg et al., 1987; Steely et al., 2000; Wallace and O'Brien, 2016).

Oxidative stress has been described as a potential glaucoma-related pathological event for both the LC and TM. In the glaucomatous LC, damage to the axonal bundles of the optic nerve has been partially attributed to decreased ONH perfusion and a hypoxic environment. (Kerr et al., 1998; Tezel and Wax, 2004; Weinreb and Khaw, 2004) Altered cellular processes associated with ischemia and oxidative stress has been described in LC cells from glaucoma donors, such as diminished antioxidant capacity (McElnea et al., 2011), mitochondrial dysfunction (McElnea et al., 2011), and impaired calcium homeostasis (Irnaten et al., 2013; McElnea et al., 2011). Enzymatic counteraction to the oxidative stress seems to be diminished in both glaucoma astrocytes and LC cells. (Hernandez et al., 2002; McElnea et al., 2011; Wallace and O'Brien, 2016) Increased lipofuscin granules found in such conditions could be also related to this ischemic processes and cell autophagy. (McElnea et al., 2014) Still, LC cells show higher expression of neurotrophic factors in a glucose-oxygen-deprived environment, which might be important for supporting traversing axonal bundles. (Lambert et al., 2001, 2004) Other genes involved in angiogenesis, mitochondrial function, neurogenesis, ECM maintenance and apoptosis show increased expression in LC cells exposed to reduced oxygen levels conditions. (Kirwan et al., 2012) Moreover, altered expression of ECM fibrosis-related proteins in LC cells was attributed to oxidative stress (Wallace et al., 2013), but has also been associated with other stimuli, such as mechanical strain, and TGF-beta stimulation. (Kirwan et al., 2005a, 2005b; Wallace and O'Brien, 2016)

Similarly, the TM tissue of patients with POAG have increased expression of oxidative stress markers. (Rönkkö et al., 2007; Izzotti et al., 2006) Moreover, recent studies have proposed that the oxidative damage of the TM could be caused by an increased oxygen tension in the anterior chamber angle of glaucoma-predisposed patients with diminished central corneal thickness. (Siegfried et al., 2015, 2010) In POAG and exfoliative glaucoma, several components of the TM are also altered due to oxidative stress, as shown by protein and gene analyses. These include elastin, fibronectin, glycoprotein 39, TIMP-2, MMP-1, -3 and -10, carboxy glutamic acid (GLA), cochlin, myocilin, periostin, TSP-3, collagen I, III, V, VI, XI, XII, and XIV. (Acott and Kelley, 2008; Liton et al., 2006) Besides, TM cells exposed to hyperoxic culture conditions or treated with hydrogen peroxide display autophagic dysfunction (Porter et al., 2015), plus activation of the protein kinase B (Akt), p38, and extracellular regulated kinase (ERK) 1/2 signaling pathways (Awai-Kasaoka et al., 2013).

Another common feature experienced by both the LC and JCT cells is the presence of elevated TGF-beta in glaucoma, dramatically impacting their behavior. TGF-beta (particularly the isoforms 1 and 2) stimulates the production of proteins involved in pathological remodeling such as fibronectin, collagen I, and VI, elastin, LOX, CTGF, TSP-1, transglutaminase. (Fuchshofer and Tamm, 2012; Fuchshofer et al., 2005; Gasiorowski and Russell, 2009; O'Reilly et al., 2011; Pena et al., 1999; Sethi et al., 2011; Wallace and O'Brien, 2016; Wordinger et al., 2014) TGF-beta treatment has also been associated with increased CLAN formation in both tissues. (O'Reilly et al., 2011; Wallace and O'Brien, 2016) These findings point to a central role for TGF-beta in these two anatomically separated tissues, turning their cellular and extracellular components into a fibrotic-like pathological pattern observed in glaucoma. (Agarwal et al., 2015; Fuchshofer and Tamm, 2012; Wallace and O'Brien, 2016) At the end-stage of glaucomatous disease, both tissues

become deformed, less stretchable and exhibit features of fibrosis; which seems to occur through the upregulation of CTGF, fibronectin, elastin, and collagen.(Agarwal et al., 2015; Fuchshofer and Tamm, 2012; Junglas et al., 2009; Wallace and O'Brien, 2016)

Both LC and JCT cells are under constant dynamic mechanical stress as evidenced by the prominence of CLANs. Known to be involved in contractile changes in TM tissues(Read et al., 2007), CLANs are also markedly increased in LC cells from glaucoma donors, and in cells treated with dexamethasone.(Job et al., 2010; Steely et al., 2000). CLANs in LC cells are considered similar to those observed in TM cells but tend to locate more frequently around the nucleus.(Job et al., 2010) Although CLANs have also been found in normal TM and LC tissues, specimens or cells from patients with glaucoma display an increased incidence of CLANs.(Hoare et al., 2009; O'Reilly et al., 2011) The induction of CLANs observed in cultures of TM cells, in both the non-stimulated glaucoma samples and those under glucocorticoid treatment, probably involves a fibronectin-binding integrin mechanism. (Clark et al., 1994; Filla et al., 2011), dependent upon the cell surface laminin 5-derived syndecan-4 peptide.(Filla et al., 2014; Read et al., 2007).

Fibrotic changes in both the LC and JCT regions are associated with a “myofibroblast-like” transition of resident LC and JCT cells that appears to occur during the degenerative glaucomatous processes. (Clark et al., 1995; Kirwan et al., 2009; Lambert et al., 2001; Tamm, 2013; Tamm et al., 2015; Wallace and O'Brien, 2016) In JCT cells, increased expression of alpha-SMA and fibronectin point to an epithelial to mesenchymal-transition-like phenomenon resulting in modifications of their phenotypes, such as an increase of motility, as suggested by Takahashi et al.(Takahashi et al., 2014) Moreover, TGF-beta2-induced fibrotic responses of JCT cells, includes increases in both the alpha-SMA expression and collagen I production, involves the Rho-GTPase signaling; which is being exploited as a treatment for glaucoma.(Pattabiraman et al., 2014) However, these avenues have not yet been explored in LC cells.

Stretching stimuli applied to cultures of both cells have been used to mimic some conditions observed in the LC and JCT region of patients with glaucoma, and the nature of the applied stimulus varies somewhat between studies. Cycles of 1 Hz frequency with 3–20% average strain applied up to 24 h are the most common parameters associated with cyclical stretch (Quill et al., 2011; Rogers et al., 2012; Wallace and O'Brien, 2016). Other studies used constant stretch protocols with cells cultured on membranes subjected to sustained mechanical distention with an average non-cycling strain of 10%. (Bradley et al., 2001, 2003; Vittal et al., 2005)

Tamm et al. speculated that altered ECM contents in the JCT region along with presentation of myofibroblast characteristics by JCT cells may result in higher release/activity of TGF-beta/CTGF signaling in POAG. (Tamm et al., 2015). In a pathological setting, ocular tissues of patients with glaucoma may respond differently to long-term repetitive and variable stretch caused by several daily processes such as awakening, blinking, accommodation, and ocular blood pulse (Coleman and Trokel, 1969). In such circumstances, both resident JCT and LC cells may over time acquire a profibrotic phenotype either as a direct consequence of the mechanical stimuli or indirectly after being stimulated by TGF-beta.

Analogous activity of LC and JCT cells are also supported by some common findings regarding cellular, and extracellular changes observed in other forms of glaucoma. (Job et al., 2010; Read et al., 2007; Stamer and Acott, 2012) Steroid induced glaucoma (SIG) is a secondary form of glaucoma, which also presents with increased outflow resistance in TM tissue. Both JCT and LC cells display cytoskeletal and ECM expression alterations when treated with glucocorticoids. (Clark et al., 1995, 1994; Filla et al., 2011; Job et al., 2010; Karali et al., 2000; Lo et al., 2003; Stamer and Acott, 2012; Vranka et al., 2015b) Increased fibronectin and reduced versican amounts were observed following corticosteroid exposure, but the results were variable depending on the JCT cell strain tested. (Lo et al., 2003; Steely et al., 1992) In addition to increased number of CLANs, cultured LC cells treated with dexamethasone increased their expression of fibronectin and laminin. (Clark et al., 1995; Job et al., 2010)

Another prominent form of glaucoma, exfoliative glaucoma (XFG) shows common ECM defects in the ONH and the TM, indicative of equivalent (dys)functions of LC and JCT cells in ECM maintenance of their respective cribriform regions. XFG is a prevalent identifiable cause of glaucoma in which disturbance of aqueous humor outflow is related to the deposition of exfoliative material and degenerative elastic fibers. Polymorphisms in the lysyl oxidase-like-1 (LOXL-1) gene is the major genetic risk factor for developing XFG. (Rasmussen and Kaufman, 2014; Ritch et al., 2015) Importantly, sufficient elastic fiber formation, and maintenance depends on LOXL-1 enzyme crosslinking activity. The hallmark feature of XFG is the appearance of aggregates of pathologic proteinaceous extracellular material in the eye, which accumulates in the JCT region of XFG patients. (Schlötzer-Schrehardt, 2009; Schlötzer-Schrehardt et al., 2012; Zenkel and Schlötzer-Schrehardt, 2014) Besides the accumulation of exfoliative material in the TM tissue, a decreased stiffness of the ONH has been reported. (Braunsmann et al., 2012) In this context, the localization of altered fibrillin-1 and LOXL-1 and sites of an elastotic degeneration in both the LC and TM tissues was observed. (Schlötzer-Schrehardt, 2009; Schlötzer-Schrehardt et al., 2012; Zenkel and Schlötzer-Schrehardt, 2014) Moreover, the optic nerve head is thinner by OCT in exfoliation syndrome patients having IOPs in the normal range. (Moghimani et al., 2016) Taken together, these findings emphasize the relevance of elastin homeostasis in the functionality of both regions. Nevertheless, additional nongenetic factors possibly contribute to the pathology and accumulation of exfoliative aggregates and alteration of elastic fibers, and LOXL1 in the LC tissue. (Ritch et al., 2015; Zenkel and Schlötzer-Schrehardt, 2014) For example, astrocytes, but not LC cells express LOXL1 (Schlötzer-Schrehardt et al., 2012), and TGF-beta induces LOXL1 expression in cultured JCT cells (Sethi et al., 2011). However, there is no published work evaluating TGF-beta effects on LOXL1 in cultured LC cells.

5. Conclusions and Perspectives

We present and discuss currently available information concerning the anatomy and cell biology of JCT and LC cells in normal and diverse glaucoma-related circumstances. The similarities between LC and JCT cells described in this review, mainly related to cell contractility and interaction with ECM, create a substantial background for further investigations on the cell-mediated pathologic features of POAG, but also SIG, and XFG. Events linked to the bidirectional mechanochemical interplay between these cribriform cells

and their ECM presented here need to be detailed in the future by testing both cells in parallel, if possible from the same donor eyes.

As shown, LC and JCT cells are under continual mechanical strain and are active metabolizers/synthesizers of ECM materials, with constituents and elements linked to a discontinuous basement membrane. Mechanical stress and TGF-beta stimuli constitute a context for these environmental changes, as well as for biomechanical modification in actin fiber organization, possibly leading to CLAN formation. Active TGF-beta, considered as an essential modulator of several alterations observed in glaucoma tissues, is elevated as a consequence of a potential disturbance in the latent TGF-beta-binding sites at the elastic microfibrils of the LC and TM. (Kuchtey and Kuchtey, 2014) In cases of genetic predisposition/susceptibility of tissues, otherwise physiological and non-injurious mechanical stress may unnaturally deform or provoke abnormal remodeling of cribriform tissues. (Feola et al., 2016) For example the LC of patients with exfoliation syndrome is thinner by OCT (Moghimi et al., 2016), and corneas of some people of African descent are thinner, imposing increased risk of developing POAG. (Gordon et al., 2002) Extensive distention of these tissues may lead to a progressive alteration in the ECM content, releasing unusually high levels of TGF-beta. As LC and JCT cells have the closest interaction with the elastin-microfibril bundles, they lay in harm's way of released TGF-beta, and would initiate a vicious circle driving several local changes related to glaucoma. Thus, either a disturbance in the elastin-fibrillin biogenesis leading to an improper extracellular TGF-beta activation, or altered cell-matrix binding proteins facilitating TGF-beta production/release may account for the end-stage fibrotic features of glaucoma in those tissues. These varied processes have been associated with some human basement membrane, vascular, skin and kidney diseases, such as Marfan syndrome, and scleroderma. (Olivieri et al., 2010) This scenario needs to be explored further in controlled fashion to identify potential targets for intervention.

Results observed in both tissues related to cellular contractility urge further studies on myofibroblast-like actions, most of them resembling the functioning of cells from the intimal layer of blood vessels and their pathological involvement in some vascular diseases. For example, atherosclerosis presents features of ECM damage, which are associated with high arterial pressure and involves activated intima layer cells. Thus, results from experimental assays forcing LC and JCT cells towards a differentiation into a myofibroblast-like phenotype may facilitate novel strategies for intervention.

Aiming new therapeutic approaches to control or reprogram the cells, various *in vitro* scenarios of escalating levels of differentiation can be proposed using stimuli associated with both myofibroblast differentiation and the glaucoma degenerative process, such as TGF-beta, oxidative stress and mechanical stretching.

While we have techniques to specifically isolate LC cells, studies directly focused toward the specific isolation and culture of JCT cells are needed. Moreover, we need new pharmacological and/or genetic approaches to target both JCT and LC cells geared toward modifying their vulnerable elastic tissues. Targeted studies to further characterize LC and TM elasticity in POAG should be performed using atomic force microscopy, and cryoelectron microscopy, as well as advanced protein-protein interaction analyses. In

particular, looking at the interaction between elastic-microfibril, latent TGF-beta, and LC and JCT cell reactivity to challenges, such as stretching, may help to elucidate the primordial pathological events in glaucoma. That is, under predisposing elastic malfunctioning and repetitive mechanical stretching, both regions would be subjected to alterations in the TGF-beta release, increases in alpha-SMA expressing cells, and production of collagen types I, and IV, which altogether will likely induce the local fibrotic features observed. More information about the biology and pathobiology of these two ocular cell types that live and work under constant mechanical strain are critical for a better understanding of their role in the development of different forms of glaucoma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

The resident cells of the cribriform tissues that span two openings in the eye, the optic nerve head and conventional outflow pathway, are pressure sensitive/responsive.

Lamina cribrosa and juxtacanalicular cells are responsible for maintaining their respective cribriform, connective tissues.

In glaucoma, pathological remodeling of cribriform tissues results in optic nerve head cupping and increased outflow resistance generation.

Figure 1. Schematic showing human eye in cross section, highlighting the two cribriform regions
 In the posterior eye, the lamina cribrosa, its structure and resident cells are depicted (blood vessels are not shown for simplicity). In the anterior eye, the conventional outflow pathway is shown, zooming in on the juxtacanalicular region of the trabecular meshwork where juxtacanalicular cells reside.

Table 1

Main morphological and immunohistochemical characteristics of major cellular contaminants in cultures of Lamina Cribrosa, and Juxtacanalicular tissue cells.

	Potential contaminant	Morphology	Immunostaining
LC cells	Astrocytes	Large cells; long, thin, varicose processes	GFAP + Collagen IV + Alpha-SMA –
	Scleral Fibroblasts	Multiple layers of long, thin, spindle-shaped cells	FSP1+ Collagen IV –
	Vascular Endothelial cells	Polygonal cells; cobblestone appearance in culture	CD31 +/CD 34 + Collagen IV + Laminin + Factor VIII +
	Pericytes	Large, dendritic-shaped cells; thin processes	3G5-ganglioside antigen + Collagen IV – Factor VIII –
JCT cells	Corneal Endothelium cells	Polygonal cells; honey-comb confluence	ZO1 +
	Scleral Fibroblasts	Multiple layers of long, thin, spindle-shaped cells	FSP1+ Collagen IV –
	Scleral Spur cells	Elongated, spindle-shaped cells; long, and thin processes Hill-valley aspect	Vimentin + Alpha-SMA + Alpha B-crystallin – Desmin –
	Schlemm's Canal cells	Fusiform cells, monolayer formation in culture	CD31 + VE cadherin +

LC: Lamina Cribrosa; JCT: Juxtacanalicular tissue; GFAP: glial fibrillary acidic protein; FSP1: fibroblast-specific protein 1; CD: cluster of differentiation; VE: vascular endothelial; ZO: zona occludens; SMA: smooth muscle actin.

Table 2

Comparative findings of critical similarities and differences related to LC and JCT cells.

	Lamina Cribrosa Cells	Juxtacanalicular Tissue Cells
Origin	Neural Crest (mesenchyme)	Neural Crest (mesenchyme)
Cellular Morphology	Large, broad, polygonal, with multiple branches	Variable size, broad, elongated, with multiple branches
Ultrastructural characteristics	Abundant cytoplasmic microfilaments, perinuclear active RER and Golgi complex; external non-continuous basement membrane-like surrounding	Abundant cytoplasmic microfilaments, conspicuous active RER and Golgi complex, increased ribossomos and lysossomos; external non-continuous basement membrane-like surrounding
<i>In vivo</i> environment	Interspersing a 3-D fibroelastic meshwork of the cribriform plates of the ONH Lamina Cribrosa	Interspersing a 3-D fibroelastic meshwork of the cribriform region of the Trabecular Meshwork
Characteristic markers	Glial fibrillary acidic protein –	Alpha B-crystallin + (<i>in situ</i>)
Cellular functions	Contractility (alpha-SMA+ with CLAN formation), autophagocytosis, production of ECM proteins	Contractility (alpha-SMA+ with CLAN formation), phagocytosis *, production of ECM proteins
<i>In vitro</i> protein expression	Glial fibrillary acidic protein, alpha-SMA, FN, laminin, proteoglycans, elastin, collagen I, III, and IV	Alpha B-crystallin, alpha-SMA, nidogen, FN, laminin, collagen IV and VIII
Altered gene expression in isolated cultures from POAG samples	Periostin, TIMP-3, versican, SPARC, TSP-1, LOX, TGF-beta-2, collagen I, V, and XI	Heparan sulfate 3-O-sulfotransferase-1. (Other genes related to ECM, contractility, and neural features)
Common altered features	TGF-beta1, alpha-SMA, collagen types I, and IV	
Due to mechanical stretch Due to TGF-beta stimulus	CTGF, Fibronectin, elastin, LOX, TSP-1, collagen types I, and VI, and CLAN	

* Not described in isolated JCT cells cultures; RER: Rough endoplasmic reticulum; 3-D: three-dimensional; ONH: Optic nerve head; SMA: Smooth muscle actin; ECM: Extracellular matrix; FN: fibronectin; POAG: Primary open-angle glaucoma; TIMP: Tissue inhibitor of metalloproteinase; SPARC: Secreted protein acidic and rich in cysteine; TSP: Thrombospondin; LOX: Lysyl oxidase; TGF: Transforming growth factor; CTGF: Connective tissue growth factor; CLAN: Cross-linked actin network.