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Research article

The many faces of the trabecular meshwork cell

W. Daniel Stamer [a], Abbot F. Clark [b, *]

[a] Departments of Ophthalmology and Biomedical Engineering, Duke University, Durham, NC, United States
[b] North Texas Eye Research Institute, University of North Texas Health Science Center, Ft. Worth, TX, United States

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ABSTRACT

With the combined purpose of facilitating useful vision over a lifetime, a number of ocular cells have evolved specialized features not found elsewhere in the body. The trabecular meshwork (TM) cell at the irido-corneal angle, which is a key regulator of intraocular pressure, is no exception. Examination of cells in culture isolated from the human TM has shown that they are unique in many ways, displaying characteristic features of several different cell types. Thus, these neural crest derived cells display expression patterns and behaviors typical of endothelia, fibroblasts, smooth muscle and macrophages, owing to the multiple roles and two distinct environments where they operate to maintain intraocular pressure homeostasis. In most individuals, TM cells function normally over a lifetime in the face of persistent stressors, including phagocytic, oxidative, mechanical and metabolic stress. Study of TM cells isolated from ocular hypertensive eyes has shown a compromised ability to perform their daily duties. This review highlights the many responsibilities of the TM cell and its challenges, progress in our understanding of TM biology over the past 30 years, as well as discusses unanswered questions about TM dysfunction that results in IOP dysregulation and glaucoma.

1. Trabecular meshwork structure and function

Trabecular meshwork (TM) cells are the primary cell type that occupy and form the proximal portion of the conventional outflow pathway, the primary egress route for aqueous humor from the eye. In this passageway resistance to unobstructed outflow is generated, regulated and responsible for homeostatic intraocular pressure (IOP) control. In coordination with the inner wall of Schlemm's canal (SC), IOP is rigorously maintained within a couple of millimeters of mercury by the TM and SC for about 90% of people over a lifetime (David et al., 1987; Klein et al., 1992). Unfortunately, cellular dysfunction in the conventional outflow pathway, including the TM, results in the generation of "extra" resistance that causes elevated IOP (ocular hypertension) characteristic of most types of primary open-angle glaucoma (POAG) (Grant, 1951, 1963). Thus, in POAG, the most common type of glaucoma, the irido-corneo angle is open and there are no gross abnormalities or clinically visible accumulation of material in the TM.

The TM is an avascular, architecturally complex connective tissue bridging Schwalbe's line to the scleral spur/ciliary muscle; and spanning the entire length of SC. The TM can be anatomically divided into three regions (from inner to outermost): (i) the uveal meshwork, which is closest to the anterior chamber and consists of a network of collagen and elastin lamellae covered by TM cells with large "open spaces" in between individual lamellae; (ii) the corneoscleral meshwork is the "middle layer", which is composed of a series of perforated collagen and elastin plates covered by TM cells; and (iii) the juxtacanalicular tissue (JCT) is a loose connective tissue containing "TM" cells surrounded by extracellular matrix between the outermost corneoscleral plates and the inner wall of SC. The ciliary muscle is anatomically and functionally linked to the SC inner wall via a network of elastin fibers that extend from the tips of the longitudinal fibers through the corneoscleral and JCT TM, anchoring onto the inner wall (reviewed by (Tamm, 2009)). These three divisions are considered part of the "filtering" TM since they lay directly over SC. A fourth division called the "insert" region of the TM is considered "non-filtering" since it resides just below Schwalbe's line, adjacent to, but not in front of SC. Data suggest that this region contains a population of TM stem cells (reviewed by (Kelley et al., 2009)).

The TM tissue has two primary responsibilities, filtration and resistance generation, to assure effective outflow resistance regulation over nearly a century of life. As aqueous humor leaves the

* Corresponding author. North Texas Eye Research Institute/CBH-441, University of North Texas Health Science Center, 3500 Camp Bowie Blvd, Fort Worth, TX 76107, United States.
E-mail address: abe.clark@unthsc.edu (A.F. Clark).

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eye, it first encounters the uveal and corneoscleral TM, which function as a self-cleaning biological filter; intercepting cellular debris and reactive oxygen species before reaching the resistance generating region of the JCT. The JCT region of the TM is populated and maintained by JCT-TM and inner wall of SC cells. The precise manner by which JCT-TM cells and SC cells function to generate and regulate outflow resistance (and thus IOP) is not completely known. However, it appears that the two cell types work together physically and functionally to generate outflow resistance, to restrict flow of aqueous humor into the lumen of SC and onto the systemic venous system (Reviewed by (Overby et al., 2009)).

While TM cells that populate the conventional outflow tissues have two different morphologies, both have a common embryological origin, the neural crest (Tripathi and Tripathi, 1989). Uniquely, these mesenchymal cells display characteristics of four different cell types, supporting the two primary responsibilities of the TM (Table 1). Thus, requisite for an occupation as a biological filter, cells of the inner TM have macrophage-like activity. Acting as professional phagocytes, TM cells clear cellular debris derived from shed pigmented epithelia propelled forward toward the iridocorneo angle by the flow of aqueous humor (Samuelson et al., 1984; Grierson and Lee, 1973; Rohen and van der Zypen, 1968). Importantly, debris is rapidly cleared by the inner TM before reaching deep into the TM where it might accumulate and interfere with resistance generation and regulation. Cultured TM cells as well as TM cells in vivo are actively phagocytic (Johnson et al., 1989; Epstein et al., 1986; Tripathi and Tripathi, 1982), which appears to be an essential part of keeping the “outflow filter” clean. In addition to engulfing pigment granules and debris, TM cells also experimentally phagocytize latex beads and other labeled particles (Johnson et al., 1989; Yue et al., 1987; Grierson et al., 1986). Similar to macrophages, TM cells express scavenger receptors, thought to be used for uptake of foreign and waste materials. In fact, early identification of TM cells was partially dependent upon their unique expression of receptors that mediate acetylated low density lipoprotein uptake (Chang et al., 1991; Stamer et al., 1995b). Interestingly, factors other than defects in phagocytosis are thought responsible for secondary forms of glaucoma that display accumulation of pigment or exfoliation material (Matsumoto and Johnson, 1997; Epstein et al., 1986).

To maintain a clear outflow pathway, the cells of the inner TM completely cover the elaborate collagen-elastin lamellae and plates as thin continuous monolayers. Functioning as endothelia, TM cells produce large quantities of antithrombotic substances, like heparin sulfate and tissue-plasminogen activator (tPA). Incredibly, TM cells produce one hundred times more tPA than vascular endothelia, emphasizing the importance of passageway patency (Snyder et al., 1993; Shuman et al., 1988). Also like endothelia, cells from the inner TM participate in antigen presentation and inflammation mediation, producing major histocompatibility proteins and a wide array of inflammatory cytokines, respectively (Shifera et al., 2010; Tripathi et al., 1990a; Latina et al., 1988; Lynch et al., 1987). In fact, laser trabeculoplasty takes advantage of the TM's role in local inflammatory mediation and resolution to decrease outflow resistance. Hence, delivery of laser energy to the TM results in the rapid secretion of IL-1α and TNFα in response to the injury, stimulating extracellular matrix turnover and debris phagocytosis (Bradley et al., 2000; Latina et al., 1998).

In line with their second responsibility, resistance generation, TM cells in the JCT region have both fibroblastic and smooth muscle-like qualities. For example, TM cells secrete a number of extracellular matrix proteins and their cognate degradation enzymes to support the continual remodeling of extracellular matrix (reviewed by (Keller et al., 2009)). Indeed, the activity of TM cells resembles fibroblasts at a wound site, turning over matrix proteins as rapidly as every 48 h (Acott et al., 1988). In keeping with the interplay between extracellular matrix turnover and cytoskeletal tension, TM cells in the JCT and corneoscleral region are contractile, expressing smooth muscle actin and myosin (reviewed by (Tian et al., 2009)). Cellular contractile force generation by the TM cells counter tension that is imposed by the ciliary muscle, which extends elastic tendons into the TM, connecting with its elastic network that further extends and terminates at the inner wall of SC (Overby et al., 2014; Rohen et al., 1981). Thus, contraction of the ciliary muscle pulls on the TM elastic network, opening spaces between lamellae and cribiform plates plus preventing collapse of the SC lumen (Li et al., 2014; Lutjen-Drecoll, 1973). Together, the relationship between the ciliary muscle, TM and SC modifies/maintains flow pathways to increase outflow facility. Paradoxically, treatment of TM tissues with drugs that decrease contractility, such as actin depolymerizing agents or rho kinase inhibitors, also increases outflow facility (Rao et al., 2001; Kaufman and Erickson, 1982). While the precise mechanism is unknown, rho kinase inhibitors increase the separation distance between the outermost cribiform plate and the SC inner wall (Yang et al., 2013), emphasizing the importance of the relationship between JCT TM and SC inner wall cells in outflow resistance generation and dampening of IOP spikes.

2. TM cells reside in a biologically demanding environment

Owing to their location, pressure-sensitivity, lack of a blood supply and continual exposure to byproducts of UV radiation and cellular metabolism, the cells of the TM weather a combination of stresses not experienced by most other cells in the body. For instance, TM cells on a daily basis experience mechanical stress due to routine daily activities such as eye rubbing, squinting, blinking, ocular pulse and saccades that range from a 20–105% increase of their original dimensions (Coleman and Trokel, 1969; Johnstone and Grant, 1973). In fact, recent studies by Downs show that about 15% of all energy in the eye is due to pressure spikes (on top of steady state IOP) (Downs, 2015). To survive such insults, TM cells possess adaptations such as a prominent cytoskeleton, complex cell-cell and cell-matrix attachments and expression of water channels to facilitate rapid changes in cell volume following stretch (Baetz et al., 2009; Grierson and Lee, 1975; Bhatt et al., 1995;

Table 1
TM phenotypes, tissue location, cell behavior and associated responsibilities.

Phenotype	Location	Cell behavior	Responsibilities
Endothelial	Uveal/corneoscleral	Endothelia	Maintain passageway patency Neutralize reactive oxygen species
		Macrophage	Biological filter/phagocytosis Immune mediation
Fibroblastic	Juxtacanalicular tissue	Fibroblast	ECM turnover/tissue repair
		Smooth muscle	Contractile tone Mechanotransduction

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