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Review

Extracellular matrix in the trabecular meshwork: Intraocular pressure regulation and dysregulation in glaucoma

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ABSTRACT

The trabecular meshwork (TM) is located in the anterior segment of the eye and is responsible for regulating the outflow of aqueous humor. Increased resistance to aqueous outflow causes intraocular pressure to increase, which is the primary risk factor for glaucoma. TM cells reside on a series of fenestrated beams and sheets through which the aqueous humor flows to exit the anterior chamber via Schlemm's canal. The outer trabecular cells are phagocytic and are thought to function as a pre-filter. However, most of the outflow resistance is thought to be from the extracellular matrix (ECM) of the juxtacanalicular region, the deepest portion of the TM, and from the inner wall basement membrane of Schlemm's canal. It is becoming increasingly evident that the extracellular milieu is important in maintaining the integrity of the TM. In glaucoma, not only have ultrastructural changes been observed in the ECM of the TM, and a significant number of mutations in ECM genes been noted, but the stiffness of glaucomatous TM appears to be greater than that of normal tissue. Additionally, TGFβ2 has been found to be elevated in the aqueous humor of glaucoma patients and is assumed to be involved in ECM changes deep with the juxtacanalicular region of the TM. This review summarizes the current literature on trabecular ECM as well as the development and function of the TM. Animal models and organ culture models targeting specific ECM molecules to investigate the mechanisms of glaucoma are described. Finally, the growing number of mutations that have been identified in ECM genes and genes that modulate ECM in humans with glaucoma are documented.

1. Trabecular meshwork cells and extracellular matrix

The trabecular meshwork (TM) is a series of fenestrated beams and sheets of extracellular matrix (ECM) covered with endothelial-like TM cells, which is immediately adjacent to Schlemm's canal (SC) (Hogan et al., 1971). Aqueous humor continuously drains through the TM at approximately 2.75 μl/min to exit the anterior chamber of the eye (Brubaker, 1991). Aqueous humor inflow is relatively constant and pressure-insensitive up to high pressure levels, thus intraocular pressure (IOP) is regulated by the resistance to aqueous humor outflow (Acott and Kelley, 2008; Brubaker, 1970, 1975; Johnson, 2006; Johnson and Erickson, 2000). Most of the resistance to aqueous humor outflow is thought to be due to ECM within the deepest portion of the TM and the basement lamina of Schlemm's canal (SC) inner wall endothelium (Acott and Kelley, 2008; Johnson, 2006).

TM cells have different functions and ECMs depending on location within the tissue. The ECM beams in the outer uveal and corneoscleral regions are normally covered with confluent layers of TM cells and contain relatively large intratrabecular spaces, which form a convoluted fluid flow pathway to the deeper layers (Gong et al., 1996; Hogan et al., 1971; Lutjen-Drecoll and Rohen, 1996). These TM cells maintain the collagen-elastic fibers embedded in a ground substance of proteoglycans, glycosaminoglycans (GAGs), non-fibrillar collagens and several families of extracellular glycoproteins, all of which comprise the trabecular beams (Acott and Kelley, 2008; Borras, 2003; Keller et al., 2009a; Lutjen-Drecoll, 1999; Lutjen-Drecoll et al., 1989; Lutjen-Drecoll and Rohen, 1996). The cells on these beams have a robust basement membrane, and long cellular projections often traverse adjacent beams (Gong et al., 2002, 1996; Grierson and Lee, 1974; Grierson et al., 1978; Johnstone, 1979; Johnstone and Grant, 1973). These outer beam cells are aggressively phagocytic and are thought to function as a pre-filter, removing debris from the aqueous humor (Buller et al., 1990; Sherwood and Richardson, 1988; Tamm, 2009). Recent studies have shown that phagocytic challenge of TM cells increases the

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expression and activity of ECM remodeling genes (Porter et al., 2013, 2012).

The deepest portion of the TM, called the juxtacanalicular (JCT) or cribriform region, is approximately 7–20 μm thick and abuts the inner wall endothelium of Schlemm's canal (SC), hence the name juxtacanalicular (Gong et al., 1996; Keller and Acott, 2013). The JCT cells reside on basement membrane and are partially embedded in an amorphous and porous 3-D ECM with some areas exposed to the open spaces of the fluid egress pathways (Acott and Kelley, 2008; Fuchshofer et al., 2006; Keller and Acott, 2013). This mixed organization has produced some controversy about the endothelial-like vs. fibroblast-like nature of JCT cells. The cells touch each other via relatively long cellular processes and frequently contact the SC inner wall cells as well (Grierson and Lee, 1974; Grierson et al., 1978; Lutjen-Drecoll, 1999). The inner wall SC cells are endothelial, forming a monolayer with very tight junctions. Interestingly, the basement membrane of SC cells is often not continuous and the degree of coverage varies somewhat between species (Lutjen-Drecoll, 1999).

Gene expression differences between TM beam cells, JCT cells and SC inner wall cells have been reported (Fuchshofer et al., 2006; O'Brien et al., 2014; Perkumas and Stamer, 2012). Many ECM proteins are present throughout the TM including in the JCT and basal lamina of the SC inner wall. Collagens, elastic fiber components, proteoglycans, GAGs, fibronectin, matricellular proteins, and many others are found at different levels in all layers. Matrix and contractile genes were highly expressed in JCT cells compared to SC cells, while ICAM-1 and fibulin-1 were relatively enriched in SC cells (O'Brien et al., 2014). SC cells, but not TM, express PECAM-1, VE-cadherin and integrin α6 (Heimark et al., 2002; VanderWyst et al., 2011).

A number of reviews focused on various aspects of the ECM of normal TM/SC have appeared in recent years and these address numerous specific details of the outflow pathway, hence the reader is referred to these sources for additional understanding of the normal ECM (Acott and Kelley, 2008; Ethier, 2002; Filla et al., 2004; Hernandez and Gong, 1996; Johnson, 2006; Keller and Acott, 2013; Keller et al., 2009a; Lutjen-Drecoll, 1999; Tamm, 2009; Yue, 1996).

2. Development of the TM

There are several studies that have investigated TM during embryonic development. When the anterior segment of the eye is developing, migrating cells from the neuroepithelium or the surface epithelium must interact with mesenchymal stem cells from the neural crest (Fig. 1). Important to this process is the temporal and spatial coordination of transcription factor expression in conjunction with signaling pathways, various cytokines, and the synthesis and remodeling of unique ECM. When the required interaction does not occur, or does not occur at the appropriate time, complex developmental disorders may occur (Cvekl and Tamm, 2004). It is now thought that the migration of periocular mesenchymal cells, which partially or wholly develop into many structures of the anterior segment, occurs in three waves. The third and last migratory wave of periocular mesenchymal cells, which is not well characterized, is involved with the formation of eyelid stroma and TM (Kao et al., 2013). For a detailed review of both the embryological development of the TM and of anterior segment dysgenesis disorders, see (Cvekl and Tamm, 2004) and (Gage et al., 2005). Both neural crest and mesoderm are represented in the ocular mesenchyme (Fig. 1). Early studies showed that TM cells are likely derived from the neural crest based on immunostaining with neuron-specific enolase antibodies (Tripathi and Tripathi, 1989). Fate mapping developmental studies later confirmed this by studying rats with a mutation in the transcription factor *PAX6* (Matsuo et al., 1993). The anterior midbrain neural crest cells in these rats did not migrate beyond the eye rudiments suggesting that the *PAX6* gene is involved in conducting migration of neural crest cells. In heterozygous *PAX6* knockout mice, the anterior angle tissues do not differentiate and the mice do not develop an outflow pathway (Baulmann et al., 2002).

Other human developmental disorders that affect structures in the anterior segment of the eye are known as anterior segment dysgenesis disorders and include aniridia, Peters' anomaly and Axenfeld-Rieger's syndrome (Cvekl and Tamm, 2004; Ito and Walter, 2014; Sowden, 2007). Other groups have examined anterior segment dysgenesis in murine models (Gould and John, 2002). Understanding why these disorders occur during development has aided in elucidating the function and migration patterns of cells in the normal eye. One possibility is that transcription factors involved in the control of anterior eye morphogenesis modulate expression of signaling molecules. Mutant mouse model studies suggest that bone morphogenetic protein 4 (BMP4) and/or transforming growth factor-β (TGF-β) are directly involved in control of mesenchymal morphogenesis in the anterior segment of the eye (Chang et al., 2001; Ittner et al., 2005). The ciliary body, retinal pigment epithelium, and the iris of both embryonic and adult mouse eyes express BMP4. Haploinsufficient *Bmp4* mice demonstrate various abnormalities of the ocular segment such as opacity of the cornea at the periphery, diffuse corneal haze, irregularly shaped pupils (iris), small or absent Schlemm's canal, and hypoplastic or absent TM (Chang et al., 2001). *BMP4* and *OTX2* have also been implicated in TM development in humans since their absence by gene deletion causes microcornea (Takenouchi et al., 2013). Other transcription factors such as pituitary homeobox 2 (*PITX2*) and the Forkhead box C1 (*FOXC1*) are also critical to anterior segment development (Acharya et al., 2011). Mutations in these genes in humans lead to anterior segment dysgenesis, and approximately 50% of patients with these mutations will develop glaucoma. In humans, *PITX2* or *FOXC1* mutations during anterior eye development cause a wide variety of abnormalities with different specific clinical phenotypes (Ito and Walter, 2014). Collectively, these studies show that TM development is governed by a complex regulatory network of transcription factors and growth factors.

Although there are a number of known genes that affect TM development as described above, the exact effect of many of these mutations on ECM is not yet clear. Much of ECM development, including timing, differentiation of the tissue, and molecular interactions in the ECM, has not been fully studied. Of interest, however, is an extracellular matrix-associated protein, peroxidasin (PXDN), with peroxidase catalytic activity, which has been localized to the cornea and lens epithelial layers (Khan et al., 2011). *PXDN* is critical in the normal development of the lens and cornea and evidence suggests that peroxidasin may have a functional role as an anti-oxidant in protecting lens, cornea, and TM from oxidative damage (Khan et al., 2011). In other systems, mammalian peroxidasin has been localized to the endoplasmic reticulum and is secreted into the extracellular space after TGF-β-induced differentiation of fibroblasts into myofibroblasts (Nelson et al., 1994). Here it forms part of a fibril-like network with fibronectin and other ECM proteins and has been suggested to be involved in ECM, phagocytosis, and defense.

3. Function of the TM

The primary function of the TM is to regulate bulk aqueous humor outflow from the anterior chamber. To do this, resistance to outflow is generated in the TM. In response to sustained elevated pressure, the TM is modified to allow greater outflow in order to reduce the pressure. IOP homeostasis is therefore defined as