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Commentary

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Schlemm's canal: more than meets the eye, lymphatics in disguise

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Schlemm's canal (SC) is a unique vascular structure that functions to maintain fluid homeostasis by draining aqueous humor from the eye into the systemic circulation. The endothelium lining the inner wall of SC has both blood and lymphatic vascular characteristics, thus prompting exploration of the development and regulation of this unique channel. In this issue of the *JCI*, back-to-back papers by Aspelund et al. and Park et al. detail the mechanisms of SC development, which includes a lymphatic reprogramming that is necessary to maintain proper function. Furthermore, both groups exploit the lymph-like qualities of this canal: they identify VEGF-C as a potential therapeutic for glaucoma and suggest that expression of PROX1, a marker of lymphatic fate, could also serve as a biosensor for monitoring SC integrity. These studies provide substantial insight into the molecular and cellular pathways that govern SC development and reveal that ocular pathology is associated with deregulation of the lymph-like characteristics of SC.

Schlemm's canal: structure and function

To understand the onset of ocular pathologies, it is important to review how the eye maintains proper fluid homeostasis and, more specifically, how the endothelial monolayer of Schlemm's canal (SC) contributes to this process. Aqueous humor is a gelatinous fluid that occupies the space between the lens and the inside surface of the cornea and provides nutrients to these avascular tissues. Aqueous humor is continually being produced by the epithelium of the ciliary body; therefore, this rate of secretion must be balanced by sufficient aqueous humor drainage. Drainage of aqueous humor through a porous tissue called the trabecular meshwork (TM) into SC and then back to the systemic circulation is termed the conventional outflow pathway (Figure 1 and ref. 1). This pathway provides the resistance to fluid outflow, which in turn builds intraocular pressure (IOP) to help push fluid across the TM into SC. Alternatively, in the nonconventional "uveoscleral" pathway, aqueous humor leaves the eye through the interstitial spaces of the ciliary body, and this flow is pressure independent (Figure 1 and ref. 2). Studies in animals estimate that about 80% of aqueous outflow passes through the TM and SC (3), with the remainder utilizing the uveoscleral pathway (4).

Fluctuations in either the production or the drainage of aqueous humor can elevate IOP, a known risk factor for glaucoma — a degenerative, age-related eye disease that is the second leading cause of blindness worldwide. Current treatment strategies for glaucoma attempt to decrease IOP by either reducing aqueous humor production or enhancing its outflow. Because SC constitutes the major resistance to aqueous humor outflow, there has been considerable interest in characterizing this specialized structure in the hopes of identifying potential therapeutic opportunities. Thus, the endothelial lining of SC has been extensively studied and shown to possess characteristics of blood vasculature (5). Yet there are also remarkable similarities between the function and structure of SC and lymphatic vessels, both of which function to filter fluid back to the systemic circulation and are structured as a continuous monolayer of endothelial cells (ECs), lacking pericytes, on a discontinuous basement membrane that is subject to flow stress (5).

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Lymphatics in the eye?

These remarkable similarities between lymphatic vessels and SC have called into question the longstanding dogma that the eye lacks lymphatic vessels (6). Fortunately, over the past 15 years, there has been outstanding progress toward the identification of lymphatic-specific markers and the development of sophisticated genetic tools, collectively enabling broader knowledge of the lymphatic system in development and disease (7). Furthermore, these tools have allowed lymphatic function to be subsequently probed in understudied tissues and organs, such as the eye (8). Several years ago, two independent groups identified the presence of lymphatic markers in the anterior segment and ciliary body of the human eye (9, 10), although it was not clear whether and how these vessels contribute to ocular fluid homeostasis. In 2011, Cao et al. demonstrated the elegant power of the corneal micropocket assay (11), where pellets of (lymph)angiogenic growth factors or corneal sutures can induce pathological lymphatic vessel outgrowth into the mouse cornea. This methodology is now widely used to study in vivo lymphangiogenesis from preexisting corneolimbal lymphatic vessels (Figure 1). Recently, our laboratory showed that the maintenance and function of these corneolimbal lymphatics is essential, as adult mice with global deletion of *Calcrl*, encoding the G protein–coupled receptor for the lymphangiogenic peptide adrenomedul-

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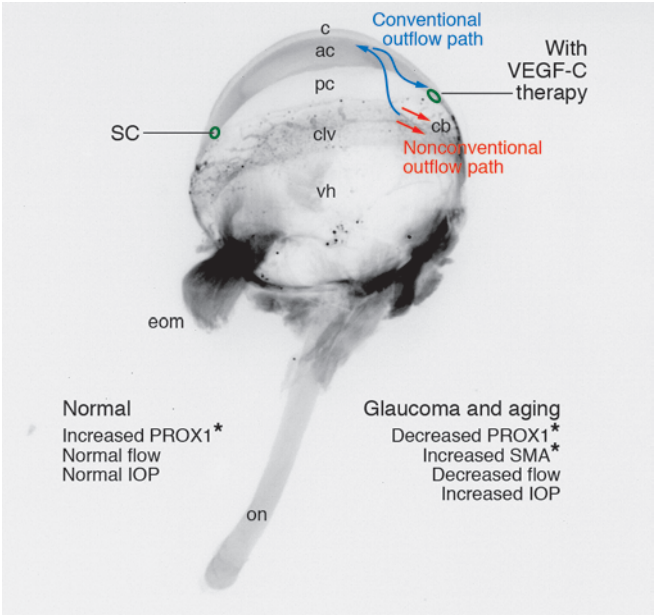

Figure 1. Optical projection tomography (OPT) reconstruction of a mouse eye. Whole-mount immunostaining with LYVE-1 reveals corneolimbal lymphatic vessels (clv) on the backdrop of OPT autofluorescence. SC (LYVE-1 negative) is illustrated in green at the corneoscleral junction. Red and blue arrows denote the two drainage pathways for vitreous humor (vh), originating from the ciliary body (cb). Low-dose VEGF-C therapy stimulates proliferation of SC and improves aqueous humor drainage pathways. Normal and pathological eye phenotypes are contrasted; asterisks denote vascular marker staining specific to SC. c, cornea; ac, anterior chamber; pc, posterior chamber; eom, extraocular muscle; on, optic nerve; SMA, smooth muscle actin. Image credit: S.T. Espenschied.

lin, exhibited corneal edema, inflammation, and dilated corneoscleral lymphatic vessels (12). Earlier this year, Truong et al. discovered high expression of the lymphatic transcription factor prospero-related homeobox 1 (PROX1) on SC endothelium in the eye (13), linking expression of the lymphatic fate determinant PROX1 to a structure within the eye. In this issue of *JCI*, two independent yet complementary studies by Park et al. (14) and Aspelund et al. (15) use genetic mouse models and sophisticated lymphatic imaging techniques to offer insight into the developmental origin of SC and the pathology associated with its improper regulation.

Development of SC: blood or lymphatic?

Studies dating back to the early 1900s suggest that lymphatic ECs arise from preexisting blood ECs in the cardinal vein (16). More recent work shows that induction of PROX1 expression in the lymphatic progenitors commits them to a lymphatic lineage fate, causing them to bud from the veins to form the primitive lymph sac (17). Importantly, although expression of PROX1 is essential for specification of lymphatic fate, additional changes in lymphatic marker expression are necessary to initiate the budding of the lymphatic progenitors from the veins. For example, podoplanin expression as well as signaling through the VEGFR3/VEGF-C pathway is also required for proper budding. To complicate matters, EC fate is known to be plastic, and blood-to-lymphatic differentiation is dynamic depending on PROX1 expression (18). To date, induction of PROX1 expression is unequivocally viewed as the master regulator for commitment to a lymphatic fate (19). These details in developmental origin are important, because they are reminiscent of SC development. This includes the involvement of progenitor cell migration from the veins and a lymphatic fate specification induced by PROX1.

Importantly, both Park et al. and Aspelund et al. confirmed PROX1 expression in SC (14, 15), reinforcing its lymphatic fate characteristics. Immunohistochemical staining to characterize the ECs of SC revealed that they were positive for certain lymphatic and blood endothelial markers, including PROX1, integrin α9, CD31, and VE-cadherin, but negative for (or expressed at very low levels) the differentiated lymphatic markers LYVE-1 and podoplanin and the blood marker smooth muscle actin. Interestingly, SC also showed high levels of CCL21, which promotes lymphatic drainage function (20), and transient or low levels of the lymphatic fate transcription factors FOXC2 and SOX18. Mutations in both *FOXC2* and *SOX18* have been linked to congenital lymphatic diseases in humans (7). The finding that the lymphatic marker expression profile of SC is either incomplete or transient suggests that SC should not be considered a fully differentiated lymphatic vessel, but rather a specialized and unique class of dynamically regulated endothelium.

Elegant lineage tracing studies by Aspelund et al. validated that, like lymphatic vessels, SC has a blood vascular origin and does not originate from preexisting lymphatic vasculature (15). Instead, the ECs of SC acquire lymph-like properties as development progresses. This finding nicely complements the serial intravital imaging of SC in *Prox1-GFP* mice by Park et al., which uncovered a lymphatic reprogramming during postnatal development whereby expression of blood vessel markers decreases as expression of certain lymphatic markers increases (notably, PROX1 and VEGFR3; ref. 14).

The lymphatic fate and targeting of SC in disease

Park et al. considered that the regulatory mechanisms and lymphatic expression profiles that are in place during development might be altered or lost during pathological conditions (14). Similarly, Aspelund and colleagues hypothesized that because SC shares both structural and functional similarities with lymphatic vessels, perhaps it can be targeted with lymphatic-specific therapies, such as VEGF-C (15). Therefore, both groups used various techniques to model changes in the eye associated with glaucoma or aging.

Park et al. showed that the initiation of flow within the primitive SC is the stimulus for increased PROX1 expression and the acquisition of a lymphatic identity; therefore, they proposed and successfully validated in their injury models that as fluid outflow decreased, PROX1 expression in SC ECs also decreased, serving as a bio-

sensor for SC integrity and functionality (14). These data underscore the unique and stochastic nature of SC vascular identity and suggest that changes in PROX1 expression can signal progression of disease. While these data are conceptually compelling, rigorous preclinical studies will be imperative to design strategies by which to monitor in situ PROX1 expression in human patients.

Aspelund and colleagues provided promising data that a single, low-dose injection of intraocular VEGF-C increased sprouting and proliferation of SC ECs and, importantly, showed a trend toward normalized IOP. In contrast, adenoviral or adeno-associated virus (AAV) delivery of VEGF-A induced massive increases in IOP and obliterated SC (15). These contrasting results of VEGF-mediated vascular growth in the eye are perhaps best explained by the observation that the preferential VEGF-C receptor, VEGFR3, is restricted to the ECs of SC, while the canonical VEGF-A receptor, VEGFR2, is ubiquitously expressed throughout the vascular structures of the eye. It is worth noting that the reductions in IOP with VEGF-C–mediated proliferation of SC were rather modest. This modest reduction might be partially explained by species-dependent differences in aqueous humor drainage. Whereas in humans, the conventional SC outflow pathway accounts for approximately 80% of aqueous humor drainage (5), in mice (21), the conventional SC outflow pathway accounts for only approximately 20%, with the nonconventional uveoscleral pathway handing the majority of drainage. Therefore, stimulation of SC with VEGF-C as a means of lowering IOP remains a promising approach to test in preclinical, nonrodent models, with a sharp focus on modes of delivery and precise dosing in order to avoid pathological corneal lymphangiogenesis. It would also be interesting to test, as the Park et al. study suggests (14), whether PROX1 expression can serve as a potential biomarker for IOP-induced pathologies in VEGF-C–treated animals.

In addition to glaucoma, elevated IOP is commonly associated with increased age, making this a critical healthcare issue that requires early detection and comprehensive eye exams to ensure excellent eye health in the aging population. The goal of any therapeutic intervention would be to restore basal physiological mechanisms that would enhance outflow and ultimately decrease IOP. The two featured papers in this issue have provided a rational strategy for exploiting the lymph-like characteristics of SC for the early detection and treatment of glaucoma and age-related disturbances in IOP. The studies are certainly "transforming," and more than meets the eye!

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