All TIEd up: mechanisms of Schlemm's canal maintenance

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Glaucoma is a leading cause of blindness, with an estimated world-wide prevalence of 3.5% in members of the population older than 40 years of age. Elevated intraocular pressure as the result of abnormal resistance to aqueous humor drainage is a major contributing, and the only preventable, factor in glaucoma development. Schlemm's canal (SC), a lymphatic-like vessel encircling the anterior portion of the eye, plays a key role in promoting aqueous humor outflow and maintenance of normal intraocular pressure. The risk of developing glaucoma increases with age; therefore, understanding mechanisms of SC maintenance and how aging affects SC function are of special importance, both for prevention and novel treatment approaches to glaucoma. Using a compelling array of genetic models, Kim et al. report in this issue of the *JCI* that continuous angiopoietin/TIE2 signaling is required for maintaining SC identity and integrity during adulthood and show that its age-related changes can be rescued by a TIE2 agonistic antibody.

A "classical" view of lymphatic vasculature

The lymphatic vasculature serves as a one-way drainage system for extracellular fluid and immune cells from the periphery of the body into the bloodstream. In general, the lymphatic vascular network is composed of capillaries and collecting vessels. Extracellular fluid and immune cells enter lymphatic vessels through permeable capillaries. Lymph then flows into collecting vessels through lymph nodes and into venous circulation. Lymphatic capillary permeability is achieved through discontinuous cell-cell junction organized as flap valves between lymphatic endothelial cells (LECs) that open in response to increases in extracellular pressure, whereas collecting vessels have continuous closed cell-cell junctions. Intraluminal valves and contractions of smooth muscle cells in collecting lymphatic vessels ensure unidirectional propulsion of lymph (1).

The transcription factor PROX1 is an early marker of LEC identity, and PROX1-induced transdifferentiation of blood endothelial cells (ECs) into LECs is a major step of lymphatic vascular development. Signaling via receptor tyrosine kinase VEGFR3 expressed by LEC and VEGFR3 ligand VEGFC produced by surrounding mesenchymal cells promotes lymphatic vessel expansion by inducing LEC proliferation and migration. Developmental lymphangiogenesis is completed by the early postnatal period in most tissues whereby lymphatic vessels mature and specialize to capillaries and collecting vessels and become VEGFC independent (1).

Schlemm's canal: a hybrid vessel important for aqueous outflow

The description above is the classical view of adult lymphatic vasculature and is typical of what is observed in the normal skin. However, new genetic mouse models, lineage-tracing approaches, and advances in tissue clearing and high-resolution microscopy have made possible studies of organ-specific lymphatic vascular beds, including vessels in the small intestine, brain meninges, heart, and lymph node (2–5). The emerging picture is that there is a great deal of diversity in vessel development, patterning, and associated molecular mechanisms; therefore, understanding how such diversity is generated by the specific organ and/or tissue environment and the significance of these differences for maintenance of normal organ function is one of the current key challenges of vascular biology.

A case in point is Schlemm's canal (SC), with recent exciting discoveries having led to the identification of mechanisms controlling development and function of this specialized vessel. SC encircles the cornea and is posteriorly covered by the trabecular meshwork (TM), an intricate array of extracellular matrix fibers and highly specialized fibroblast-like cells of neural crest origin (Figure 1 and refs. 6, 7). SC, together with the TM, plays a key role in the regulation of aqueous humor outflow (AHO) in the eye and, consequently, in the regulation of intraocular pressure (IOP). Normally, ciliary body epithelial cell AH production is balanced by AH drainage through the TM and SC into aqueous veins and episcleral blood vessels (Figure 1). In primary open-angle glaucoma (POAG) — the most common type of glaucoma in the world — trabecular outflow draining is impaired, resulting in resistance to AHO and chronically elevated IOP, which, with time, irreversibly damages optic nerve axons (8). Importantly, reduction in IOP delays or prevents optic nerve damage in POAG and therefore represents a major target for therapeutic interventions (8).

SC was first described by the 19th century German anatomist Friedrich Schlemm

▶ Related Article: p. 3877

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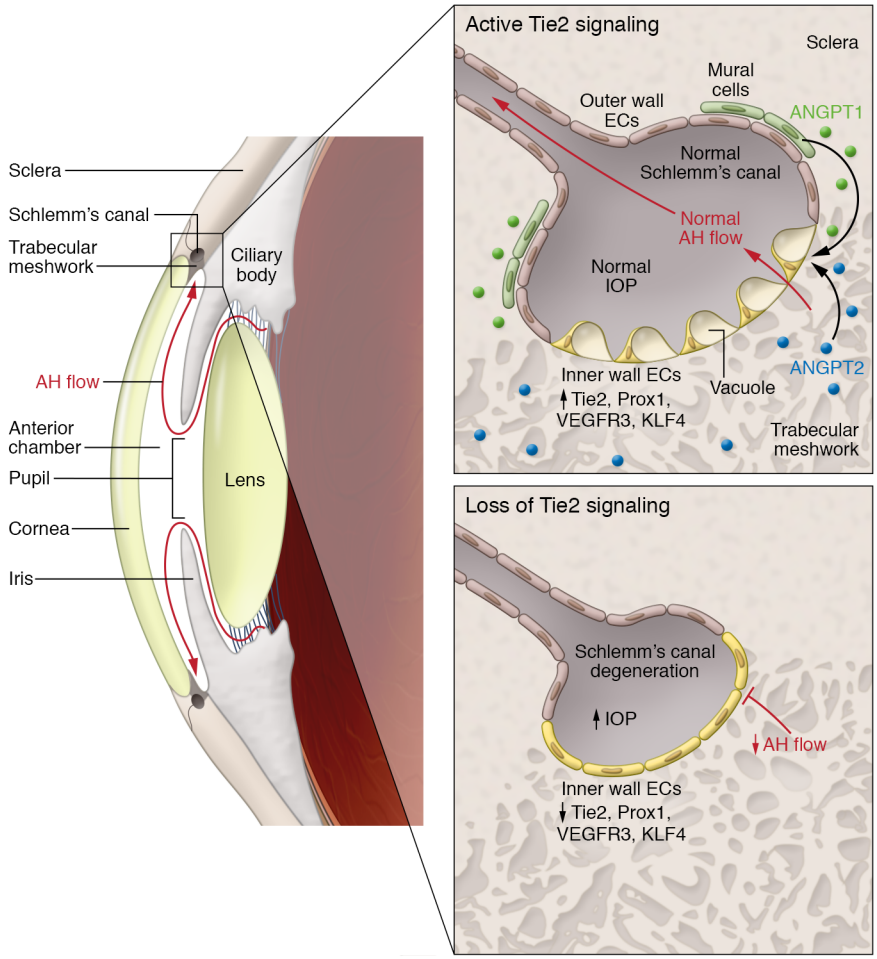

Figure 1. Role of the ANGPT/TIE2 pathway in maintenance of SC. Aqueous humor (AH) produced by ciliary body epithelium is drained primarily via TM and SC situated at the iridocorneal angle of the eye. Equilibrium between AH production and drainage is essential for maintenance of normal IOP. Under normal conditions in which Tie2 can actively signal, ECs of the inner wall of SC, juxtaposed to TM, are subjected to the gradients of pressure and AH flow. They are characterized by high expression of PROX1, TIE2, VEGFR3, and KLF4 and the presence of giant vacuoles, pressure-dependent EC outpouches that provide a passageway for AH flow across SC wall. SC mural cells and the TM are sources of ANGPT1 and -2, respectively. ANGPT/TIE2 signaling maintains SC integrity and function; its inactivation leads to loss of SC EC specialization, including decreased expression of PROX1, VEGFR3, and KLF4, and ultimately results in SC degeneration and increased IOP.

and was considered to be a blood vessel for a long time (6). However, some functional characteristics, such as the absence of pericyte coverage, a discontinuous basement membrane, an absence of blood filling, and the known role of SC in AH and immune cell draining from the eye to the blood circulation, led to suggestions that SC shares traits with the lymphatic vasculature (9). This mystery was solved definitively in 2014, when several research teams independently described the developmental origins and molecular mechanisms of SC formation (10–14). SC was shown to develop postnatally from the choroidal vein and to present a hybrid molecular phenotype, characterized by expression of some LEC markers, such as PROX1, VEGFR3, integrin α9, and CCL21, as well as blood endothelial markers, including TIE2, endomucin, CD34, and plasmalemma vesicle–associated protein (PLVAP) (10–14). Uniquely, SC ECs do not express two typical LEC markers, podoplanin and LYVE-1, that are widely used to identify peripheral lymphatic vessels.

Development of SC by EC sprouting from vein and further coalescence into a primordial vessel is reminiscent of formation of major embryonic lymphatic vessels, such as the thoracic duct (2, 10, 12). However, molecularly, the two processes appear to be distinct. During thoracic duct formation, ECs acquire lymphatic identity and expression of PROX1 before budding from embryonic veins, whereas in SC development, ECs first form the primordial blood vessel and then, only after this is complete, is expression of lymphatic markers PROX1 and VEGFR3 initiated (10, 12, 13). In line with these findings, ablation of VEGFC/VEGFR3 signaling completely prevents embryonic lymphatic vasculature development (1); however, SC is still formed in mice lacking both *Vegfc* and *Vegfd* and in *Vegfr3*-knockout mice, but is not able to grow and expand (12).

Angiopoietin/TIE pathway in SC development and maintenance

The specialized nature of the SC endothelium was further brought to light after the identification of a key role for the angiopoietin/TIE (ANGPT/TIE) pathway in SC development (11) and, as shown in this issue by Kim et al., in SC maintenance (15). Signaling of ANGPT growth factors via TIE receptors is important for regulating vascular stability and remodeling during development and in numerous pathological situations in both blood and lymphatic vessels (16). In blood vessels, ANGPT1 acts as a TIE2 agonist and stabilizing factor, whereas ANGPT2 can act both as a context-dependent TIE2 antagonist and a relatively weak agonist (16). Rescue of lymphatic vascular remodeling defects in *Angpt2*-deficient mice by knockin of *Angpt1* indicates that, unlike in blood vessels, in lymphatic vasculature, ANGPT2 has a primarily agonistic function (17). Intriguingly, SC demonstrates a unique dependence on agonistic ANGPT/TIE2 signaling for its development, as mice with either double inactivation of *Angpt1* and *Angpt2* or with *Tie2* deletion completely lack SC and develop glaucoma (11). The importance of TIE2 signaling in SC development was further underscored by the discovery in 2016 of TIE2 heterozygous loss-of-function mutations in patients with primary congenital glaucoma (PCG) (18). The work from Kim et al. (15) now provides further fascinating insights into the mechanisms of SC maintenance. Through the detailed analysis of mice with inducible inactivation of *Angpt1*, *Angpt2*, and *Tie2*, Kim and colleagues show that ANGPT1 and ANGPT2 are produced in distinct

locations in the vicinity of SC and that the combination of both ligands needs to be delivered continuously to adult SC ECs. Absence of continuous TIE2 signaling results in increased EC apoptosis, reduced levels of PROX1, and shear stress–responsive KLF4 as well as decreased formation of giant vacuoles, indicative of diminished AHO (Figure 1). Functionally, both *Angpt1*/*Angpt2* double-knockout and *Tie2*-knockout animals rapidly developed elevated IOP, which resulted in neuronal damage and impaired vision. As aging is one of the key risk factors in the development of POAG (8), Kim et al. also took the SC research one step further by showing that aged SC is characterized by decreased TIE2 expression and phosphorylation, low expression of Angpt2 and Angpt1, poor EC cell junction organization, and reduced formation of giant vacuoles (15). Taken together with previous studies, which have documented decreased AHO and reduced expression of PROX1 and VEGFR3 in aged SC (12, 13), these data suggest that loss of TIE2 signaling underlies age-dependent deterioration of SC function. In line with these observations, intraocular injections of TIE2-activating ABTAA-ANGPT2 antibody complexes (19) promoted SC EC proliferation and PROX1 and KLF4 expression and decreased IOP in aged mice, indicating a potentially important therapeutic avenue for prevention and treatment of age-related SC degeneration.

Future directions

The work of Kim et al. raises a number of interesting questions for future studies. As a mechanistic framework, Kim et al. propose a model in which ANGPT1/ANGPT2 signaling via TIE2 activates downstream ERK signaling and induces expression of PROX1, which then acts as a gatekeeper of SC maturation and function. Such a model is compatible with observations that TIE2 is present and activated before PROX1 is expressed in SC endothelium and that PROX1 expression is reduced in SC ECs upon loss of TIE2 signaling (15). Furthermore, earlier studies showed that ectopic elevation of ERK signaling in vivo, through overexpression of constitutive active RAF1, was sufficient to induce PROX1 expression in embryonic veins and arteries, normally negative for PROX1 (20). However, data from genetic mouse models show that TIE2 and ANGPT1/ANGPT2 signaling are dispensable for PROX1 expression and LEC identity establishment and that the main consequence of ANG1/TIE2 signaling in blood vessels is vascular maturation and stabilization (16). Therefore, it will be interesting to investigate the mechanism of the unique response of SC ECs to ANGPT/TIE2, which triggers partial lymphatic endothelial reprogramming of this vascular bed, and the crosstalk of the ANGPT/TIE2 pathway with VEGFC/VEGFR3 signaling, which is important for SC maturation (12). Similarly, given that PROX1 is important for the development and maintenance of both peripheral lymphatics and SC (15, 21), another open mechanistic question is whether this transcription factor defines similar or distinct transcriptional programs in LECs versus SC ECs.

One interesting aspect of SC biology uncovered by Kim et al. and previous studies is a positional heterogeneity of SC ECs. Indeed, the highest TIE2, PROX1, and VEGFR3 expression and an overall LEC-like phenotype are observed in inner wall SC ECs, which, juxtaposed to TM and subjected to a basal-apical pressure gradient and transmural flow, whereas outer wall ECs have more blood EC–like features (6, 10, 15). A better understanding of how biomechanical stimuli, TIE2 signaling, and perhaps other TM-derived factors contribute to SC EC specialization will undoubtedly be a fruitful area for further research. Conversely, another relevant line of investigation is whether SC regulates the development and function of the TM. Intriguingly, although ANGPT2 is largely reported to be an endothelial-specific gene that is induced in response to hypoxia, inflammation, and growth factor signaling (16), Kim et al. documented high levels of ANGPT2 in TM cells, raising the mechanistic question of how these highly specialized cells activate ANGPT2 expression. Finally, in addition to TIE2, several other causative genes responsible for PGC, such as CYP1B1 and LTBP2, have been identified, but their mechanism of action is so far unclear (22). It will be therefore important to investigate how TIE2/ERK/PROX1 signaling intersects with other PGC genes in the regulation and maintenance of SC function.

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