The Other Side Of the Cornea

The endothelium can begin to falter from disease, but there are methods in development to help restore it.

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Our efforts in this column are often devoted to the examination of new topics and treatments for ocular surface diseases. Allergy and dry eye are just two examples of disorders that impact the health of the conjunctival and corneal epithelium. There is, however, literally another side to corneal physiology and corneal disease. This month, we consider the biology of the endothelium that lines the posterior surface of the cornea, and discuss how its dysfunction can lead to corneal endothelial pathologies. We’ll touch on current therapies, and then describe the status of nonsurgical approaches to the treatment of endothelial disorders.

The endothelial layer is often considered a one-trick pony; its job is to maintain stromal deturgescence by regulating—usually limiting—flow of fluid from the aqueous humor into the corneal stroma. Composed of a single layer of flat hexagonal cells lining Descemet’s membrane, corneal endothelial cells form the interface between the cornea and the aqueous humor-filled anterior chamber. At just ~5 µm thick, the CE is the thinnest of the cellular corneal layers. The CE cells actively pump water from the stroma to the aqueous humor, maintaining a flow that balances the fluid leak through the extracellular space, and thus preserving the optical clarity of the cornea.

On average, the human cornea has an endothelial cell density in the range of 5,000 to 6,000 cells/mm² at birth; that number decreases by 40 to 50 percent by adulthood at a cell loss of about 0.6 percent per year. Stromal edema typically appears when cell density decreases to between 400 and 700 cells/mm². Why the cell loss? Under normal conditions, CE cells die at a rate comparable to other endothelial cells, however; they enter a state of apparent senescence early in life. The predominant compensatory response to cell loss is cell spreading and migration for maintenance of a uniform endothelial monolayer. If cell loss is accelerated, there comes a breaking point where the density of remaining cells cannot maintain stromal deturgescence and transparency.

Fuch’s corneal endothelial dystrophy, perhaps the most common pathology defined by loss of CE cells, can lead to corneal clouding, pain and eventual blindness. Like other ocular disorders, most CE disease occurs in older individuals so, with an aging population, its incidence is likely to rise. Surgical approaches to CE failure such as corneal transplant have improved significantly in recent years, but this procedure is invasive, expensive and depends upon the availability of a healthy supply of donor corneal tissue. While this has not been a problem in the United States, worldwide shortages do exist and are likely to worsen.

To Divide or Not to Divide

Technically, it is not accurate to say that CE cells can’t divide. They can, but they don’t; a combination of cellular roadblocks freezes them in a well-differentiated state in the G₁ phase of cell division. For many of the environmental and cellular regulators of cell division, signaling pathways converge on one or more of the kinases in the cyclin family. Cyclins function as gatekeepers in the transition between the phases of mitosis. For example, inhibition of cyclin E is thought to underlie the phenomenon of contact-mediated
growth inhibition exhibited by many cells, including CE cells. Evidence from studies in both human CE and in animal models points to one cytokine found in relatively high levels in the aqueous humor; a number of studies have shown that TGF-β2 can suppress expression of key cyclins while also acting to maintain high levels of p27Kip1. These and perhaps other inputs act to prevent CE proliferation under normal physiological conditions.

Contrary to the long-standing notion of CE senescence, many recent reports suggest that CE cell division and proliferation can occur under specific conditions. For example, one group used a rat model of bullous keratopathy to show that peripheral CE cells divide and migrate toward the central endothelium, but cells in the central region do not. Other studies aimed at releasing CE cells from mitotic arrest have met with modest success and have established that the age is an important criterion; CE cells from younger donors have a much greater proliferative capacity than those from older donors.29

Predicting Endothelial Failure

Endothelial disorders are relatively common; recent estimates suggest 5 percent of individuals over 40 will experience some degree of Fuchs’ endothelial corneal dystrophy. Perhaps more important, patients with FECD who undergo surgical procedures for cataracts or glaucoma face an increase in the potential for endothelial dysfunction resulting from surgical trauma. Recent efforts have explored approaches to minimize this risk. For example, a 2013 study comparing torsional and longitudinal phacoemulsification showed that when less ultrasonic energy is used, the risk of endothelial sequela is reduced. But despite continuous refinement of surgical techniques, a modest perturbation of the CE can initiate an apoptotic cascade, accelerating the rate of cell death.

Even with these refined protocols to reduce the potential for adverse post-surgical CE outcomes, a different strategy may be required for patients already diagnosed with FECD who are also in need of cataract surgery. These patients face a complicated treatment outlook that often includes both cataract removal and eventual corneal transplant. While there has been significant progress in techniques used for endothelial transplant (see the September 2014 issue of Review for several excellent discussions of various methods for corneal transplantation) it’s clear that there is a need for non-surgical options to prevent or reverse CE cell loss.11

A significant advance came with the genome-wide association studies of FECD that identified several single risk alleles with unusually high odds ratios, confirming the strong genetic component of the disease.12-14

The association between FECD and single-sequence variants in the transcription factor 4 gene yielded odds ratios of 5.5, and those with multiple risk alleles had odds ratios of 30. It’s thought that TF4 may be involved in the differentiation of peripheral CE cell proliferation or in a limbal stem cell’s transition to an endothelial progenitor cell. Either case means the potential for endothelial regeneration is lost or impaired in individuals that carry TF4 variants.

The GWA studies are significant for another reason; they present the potential for screening patients and identifying those at risk for FECD before they are symptomatic. With such a test, therapeutic strategies to promote CE health have the potential to reduce the need for corneal transplants and all the associated sequela. We can imagine a scenario where patients will be treated prior to surgical procedures to enhance CE proliferation or attenuate activation of apoptotic signals, providing a prophylaxis against subsequent endothelial cell loss. The search for such treatments is far from a conclusion, but it is well under way.

Future Endothelial Therapies

While there is a significant body of research on potential therapies for CE diseases, most of this work has focused on in vitro studies or trials using animal models. The ultimate goal of these efforts has been the targeted prevention of endothelial cell death following various surgical procedures. Using a number of different protocols...
and experimental designs, comparisons of cell explants or cultured cells from FECD patients with control CE cells has yielded a general consensus that FECD cells are more sensitive to oxidative stress than control cells.\textsuperscript{15-17} This means that patients with one or more of the predisposing genetic traits linked to FECD are more vulnerable to triggers that initiate generation of reactive oxygen species, such as trauma or inflammation. There are likely additional factors contributing to increases in CE cell death in those with FECD: Even without the provocation of peroxide or some other oxidative stressor, FECD explants exhibit an average of 10 times the rate of apoptosis seen in control cells.\textsuperscript{17} Oxidative stress in FECD can also lead to apoptosis via effects on mitochondrial function, further supporting the idea that the molecular defect in FECD is an inability to respond appropriately to oxidative stress.\textsuperscript{18}

As a proof of principle, a number of studies have demonstrated that classical anti-oxidants can mitigate CE cell loss under conditions of stress, even in cells from FECD subjects. A study published in 2013 followed this logic and examined the effects of sulforaphane, a naturally occurring compound, RTA-408,\textsuperscript{20} (Reata Pharmaceuticals; Irving, Texas) is currently in a Phase II trial for prevention of endothelial cell loss following cataract surgery.\textsuperscript{21} A completely different therapeutic strategy is exemplified by a new study testing Rho-associated kinase inhibitors as stimulators of endothelial cell proliferation.\textsuperscript{22} This approach is aimed at the endogenous pathways that limit CE cell growth; by altering cell adhesion, ROCK inhibitors attenuate cell-contact growth inhibition. This mechanism hasn’t been clearly established, but what is clear is that endothelial wound healing is accelerated by treatments mediated, at least in part, by new cell growth rather than by prevention of cell death.

These examples demonstrate that there are at least two distinct approaches that we may adopt in our efforts to preserve corneal endothelial cell function. Whether it’s by preventing cell death or, alternately, stimulating growth of new corneal endothelial cells, the overall function of the endothelial layer can be maintained. Ideally these efforts will lead to a reduction or elimination of the need for most corneal transplants. The benefits of these new therapies will be clear for all to see.

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