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Effects of diabetic retinopathy on the barrier functions of the retinal pigment epithelium

Tina Xia, Lawrence J. Rizzolo *

Departments of Surgery and Ophthalmology and Visual Science, Yale University School of Medicine, PO Box 208062, New Haven, CT 06520-8062, USA

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ABSTRACT

Diabetic retinopathy is a debilitating microvascular complication of diabetes mellitus. A rich literature describes the breakdown of retinal endothelial cells and the inner blood-retinal barrier, but the effects of diabetes on the retinal pigment epithelium (RPE) has received much less attention. RPE lies between the choroid and neurosensory retina to form the outer blood-retinal barrier. RPE's specialized and dynamic barrier functions are crucial for maintaining retinal health. RPE barrier functions include a collection of interrelated structures and activities that regulate the transepithelial movement of solutes, including: diffusion through the paracellular spaces, facilitated diffusion through the cells, active transport, receptor-mediated and bulk phase transcytosis, and metabolic processing of solutes in transit. In the later stages of diabetic retinopathy, the tight junctions that regulate the paracellular space begin to disassemble, but there are earlier effects on the other aspects of RPE barrier function, particularly active transport and metabolic processing. With advanced understanding of RPE-specific barrier functions, and more in vivo-like culture models, the time is ripe for revisiting experiments in the literature to resolve controversies and extend our understanding of how diabetes affects the outer blood-retinal barrier.

1. Introduction

Diabetic retinopathy is a debilitating complication of diabetes. The vascular leakage associated with the disease leads to macular edema, neovascularization, hemorrhage, and consequently, severe vision loss. Growing evidence implicates the breakdown of both the inner and outer blood-retinal barrier (BRB) in the development of disease. These two barriers correspond to the retina's two circulations: the fenestrated choriocapillaris and the inner retinal vascular beds (Fig. 1). The retinal vessels of the inner BRB originate from the central retinal artery and course through the neurosensory retina, nourishing the inner retinal layers (Bill, 1975; Bill & Sperber, 1990; Chen, 2016). Unlike the leaky endothelium of the systemic circulation, the retinal endothelial cells are joined by the low-permeability tight junctions that typify the tight junctions of the central nervous system (Butt, Jones, & Abbott, 1990; Rubin & Staddon, 1999). This selective barrier prevents potentially toxic molecules from entering the inner retina. The outer BRB lies in the choroid and retinal pigment epithelium (RPE) on the opposite side of the neurosensory retina. The capillaries of the choriocapillaris are fenestrated (Korte, Burns, & Bellhorn, 1989). Although they nourish the outer retinal layers, they are incapable of forming a barrier on their own (Bill & Sperber, 1990; Foulds, 1990). The RPE lies between the choriocapillaris and neurosensory retina and collaborates with the choriocapillaris and Bruch's membrane to establish the outer BRB. Tissue-specific tight junctions and transporter proteins regulate the high selectivity of the RPE and help to maintain the health of the retina (Reichhart & Strauss, 2014; Rizzolo, Peng, Luo, & Xiao, 2011).

Study of the inner BRB has dominated research on diabetic retinopathy, and will be the subject of other reviews in this volume. This article will focus on the outer BRB. RPE defines the selectivity of the outer BRB, and RPE cellular functions are critical for maintaining the health and integrity of photoreceptor cells. Alterations of the RPE are observed in diabetic retinopathy, even in early disease. Given the essential functions of RPE, it is important to understand injuries to specific barrier functions of this epithelium

Abbreviations: BRB, blood brain barrier; ERG, electroretinogram; hfRPE, human fetal RPE; Na/K-ATPase, $3Na^+/2K^+$-ATPase driven pump protein; OCT, optical coherence tomography; RPE, retinal pigment epithelium; TER, transepithelial electrical resistance; VEGF, vascular endothelial growth factor.

* Corresponding author.
E-mail addresses: tina.xia@yale.edu (T. Xia), lawrence.rizzolo@yale.edu (L.J. Rizzolo).

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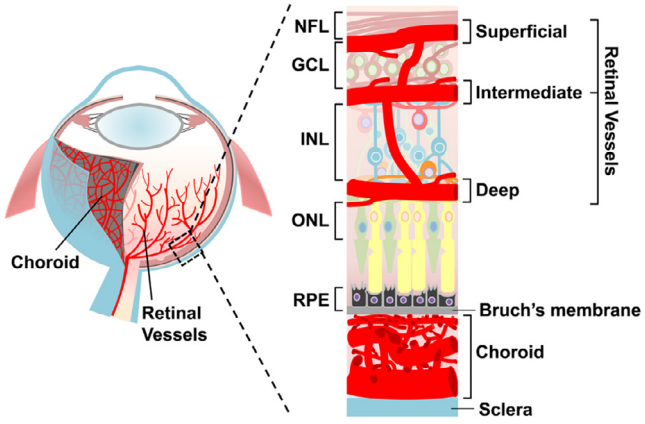

Fig. 1. Schematic drawing of the inner BRB (retinal vessels) and outer BRB (RPE plus Choroidal vessels). NFL, Nerve Fiber Layer; GCL, Ganglion Cell Layer; INL, Inner Nuclear Layer; ONL, Outer Nuclear Layer. Reprinted with permission (Chen, 2016).

and how these injuries might contribute to the development of diabetic retinopathy. The different types of barrier functions which define RPE selectivity and permeability will be categorized and discussed in detail. Subsequently, evidence on the effects of diabetes on each type of RPE barrier function will be reviewed.

2. Normal barrier functions of RPE

The RPE is essential to the survival and function of photoreceptors in the retina. Among its functions, RPE provides nutrients for the retina and removes waste, including the disc membranes shed by the outer segments of photoreceptors (Marmor & Wolfensberger, 1998). Because the retina lacks lymphatic drainage, the RPE removes the fluid that is extravasated from, but not recovered by, the inner retinal vessels. It also functions in the visual cycle by processing vitamin A and regenerating 11-*cis*-retinal for transport back to the photoreceptors (Deigner, Law, Canada, & Rando, 1989; Redmond et al., 1998). Additionally, RPE regulates the ionic environment of the photoreceptors (Reichhart & Strauss, 2014; Rizzolo et al., 2011). These RPE functions can be categorized by general types of barrier function: regulated paracellular diffusion, facilitated diffusion, active transport, transcytosis, and metabolic modification. Barrier function requires a polarized distribution of plasma membrane channels and transporters and a semi-selective, semi-permeable seal between the neighboring cells of an epithelial monolayer. The paracellular seal is created by tight junctions that encircle each cell and binds neighboring cells. Tight junctions also divide the plasma membrane into apical and basolateral membrane domains. A few RPE-specific examples of barrier function follow (Fig. 2):

Paracellular diffusion through the spaces between neighboring RPE cells is regulated by tight junctions. Tight junctions are composed of roughly 100 proteins; the ones that determine paracellular selectivity and permeability are members of the claudin family. Claudins are strand-forming proteins and are responsible for the anastomosing network of strands that join neighboring cells (Fig. 3). The subset of claudins that are expressed depends on the physiology of the epithelium and can also vary among species and during development. For example, in human RPE claudin-19 and claudin-3 predominate to create a barrier that is slightly cation-selective (Peng, Rao, Adelman, & Rizzolo, 2011; Peng et al., 2016).

Facilitated diffusion and active transport use specialized transporter proteins to move solutes across the monolayer through the cells. In *facilitated transport*, solutes diffuse down their electrochemical gradient through channel proteins. In *active transport*, energy is expended to move solutes and molecules against their electrochemical gradient. Polarized expression of transporter proteins on RPE cells therefore allow the unidirectional movement of substrates (Reichhart & Strauss, 2014; Strauss, 2005). Facilitated diffusion across one plasma membrane can be combined with a transporter on the opposite plasma membrane to effect active vectorial transport. In RPE, active transport drives Cl^- across the apical plasma membrane into the cell. Then, basolateral Cl^- channels allow Cl^- to diffuse across the basolateral membrane, resulting in active, apical-to-basal transepithelial transport.

Transcytosis, uses vesicles to move solutes across the cell. Bulk phase or receptor mediated endocytosis would bring solutes into the cell. Endocytic vesicles that move across the cell then fuse with the opposite plasma membrane and secrete their contents by exocytosis. In RPE, some of the VEGF that is secreted apically is recovered by receptor-mediated endocytosis and secreted from the basolateral membrane (Peng, Adelman, & Rizzolo, 2010).

Metabolic pathways modify a solute as it moves across the cell. In RPE, vitamin A enters the cell by receptor-mediated endocytosis of the basolateral membrane and is converted to 11-cis-retinal before it is secreted by the apical membrane. Another important metabolic function is the phagocytosis of shed photoreceptor outer segments. RPE then process these endocytosed segments for degradation. In aged individuals, accumulated, undegraded material is extruded through the basolateral membrane.

These barrier functions of RPE are sensitive to environmental changes and changes in cell signaling. The responses to oxidative stressors or thermal injury, for example, indicate some degree of RPE compensation during injury (Han, Lyu, Park, Jang, & Park, 2015; Klettner, 2012). However, prolonged stress eventually induces RPE dysfunction, which can lead to retinal disease.

Diabetes increases oxidative stress and induces hypoxia in the retina, contributing to the development of diabetic retinopathy (Arden & Sivaprasad, 2011). These stressors likely impact both the retinal and choroidal circulations supplying the retina. Although the RPE can withstand high levels of metabolic stress, eventual RPE injury in diabetic retinopathy potentially compromises each of the barrier functions mentioned above, thereby compromising the integrity of the neurosensory retina.

3. Morphological and macro-effects of diabetes on the outer BRB

Both observational and experimental studies on diabetes and the retina demonstrated vascular injury in the disease. Microaneurysms, vessel segmentations, dilations and engorgement can be seen on ophthalmic exam (Ballantyne & Loewenstein, 1944; Gardiner, Archer, Curtis, & Stitt, 2007). It is thought that these changes are accompanied by altered blood flow and defective autoregulation in the retina, facilitating the progression of disease. Increased vascular permeability is also observed in diabetic retinopathy. For instance, more fluorescein dye was detected in the vitreous of juvenile diabetic patients after systemic injections compared to control subjects (Waltman et al., 1978). In experimental models, leakage of tracer molecules is increased in diabetic rodent eyes (Grimes & Laties, 1980; Ishibashi, Tanaka, & Taniguchi, 1980). As the understanding of diabetic retinopathy increased, researchers began investigating whether effects on the outer BRB were a significant underlying contributor to diabetic retinal edema.

Even in early studies that demonstrated leakage from inner retinal vessels, alterations and leakage from the outer BRB were often also noted by the investigators. Histology of post-mortem eyes from diabetic and control patients showed leakage of albumin into

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