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Review

Barrier properties of cultured retinal pigment epithelium

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ABSTRACT

The principal function of an epithelium is to form a dynamic barrier that regulates movement between body compartments. Each epithelium is specialized with barrier functions that are specific for the tissues it serves. The apical surface commonly faces a lumen, but the retinal pigment epithelium (RPE) appears to be unique by a facing solid tissue, the sensory retina. Nonetheless, there exists a thin (subretinal) space that can become fluid filled during pathology. RPE separates the subretinal space from the blood supply of the outer retina, thereby forming the outer blood–retinal barrier. The intricate interaction between the RPE and sensory retina presents challenges for learning how accurately culture models reflect native behavior. The challenge is heightened by findings that detail the variation of RPE barrier proteins both among species and at different stages of the life cycle. Among the striking differences is the expression of claudin family members. Claudins are the tight junction proteins that regulate ion diffusion across the spaces that lie between the cells of a monolayer. Claudin expression by RPE varies with species and life-stage, which implies functional differences among commonly used animal models. Investigators have turned to transcriptomics to supplement functional studies when comparing native and cultured tissue. The most detailed studies of the outer blood–retinal barrier have focused on human RPE with transcriptome and functional studies reported for human fetal, adult, and stem-cell derived RPE.

1. Introduction

One of the essential functions of the retinal pigment epithelium (RPE) is to regulate the ionic composition of the subretinal space, thereby providing photoreceptors the environment they need to function properly. RPE also provides nutrients, removes waste and water from the subretinal space, and participates in the visual cycle. As the concept of a blood–brain barrier evolved, each of these functions has fallen under the rubric of barrier function (Bradbury, 1979). Historically, electrophysiologists led the study of the RPE barrier. Many express a preference for fresh tissues as their experimental model rather than cultured cells (Wimmers et al., 2007). However, culture offers greater flexibility for detailed study of mechanisms in a controlled environment. The growing interest in using human stem cells for RPE transplantation, disease modeling, and drug testing further highlights the need for reliable culture models.

There are two blood–retinal barriers: an inner barrier, formed by retinal endothelial cells, and an outer barrier, formed by RPE in collaboration with Bruch's membrane and the choriocapillaris. The RPE's role as a tissue barrier was recently reviewed (Strauss, 2005; Rizzolo, 2007; Wimmers et al., 2007; Rizzolo et al., 2011). Briefly, the underlying choriocapillaris is fenestrated, which means windows (fenestrae) in the wall of the epithelium allow solutes to readily diffuse between the capillary lumen and basal surface of RPE (Korte et al., 1989). Accordingly, RPE separates the overlying subretinal space and photoreceptors from serum components and regulates the barrier functions described below.

Tissue barriers have five overall functions (Fig. 1): *1) Regulated diffusion through the paracellular space:* Diffusion is regulated by a band of anastomosing strands, known as the tight junction, that encircle each cell to bind neighboring cells with a semi-permeable, semi-selective seal. The selectivity of the junctions depends on the claudin family of proteins; each epithelium expresses a unique subset of claudins, allowing for epithelium-specific selectivity. Other proteins, such as occludin, affect permeability. The details of tight junction composition and function, in general and in respect to RPE, are beyond the scope of this discussion, but have been recently reviewed (Rizzolo et al., 2011). *2) Transcellular facilitated diffusion:* Solutes can enter the cell passively through a membrane channel and exit by a channel on the opposite side of the monolayer. *3) Transcellular active transport:* In the first two pathways, solutes move passively down a concentration or electrical gradient. For vectorial transport against a gradient, energy is required. A

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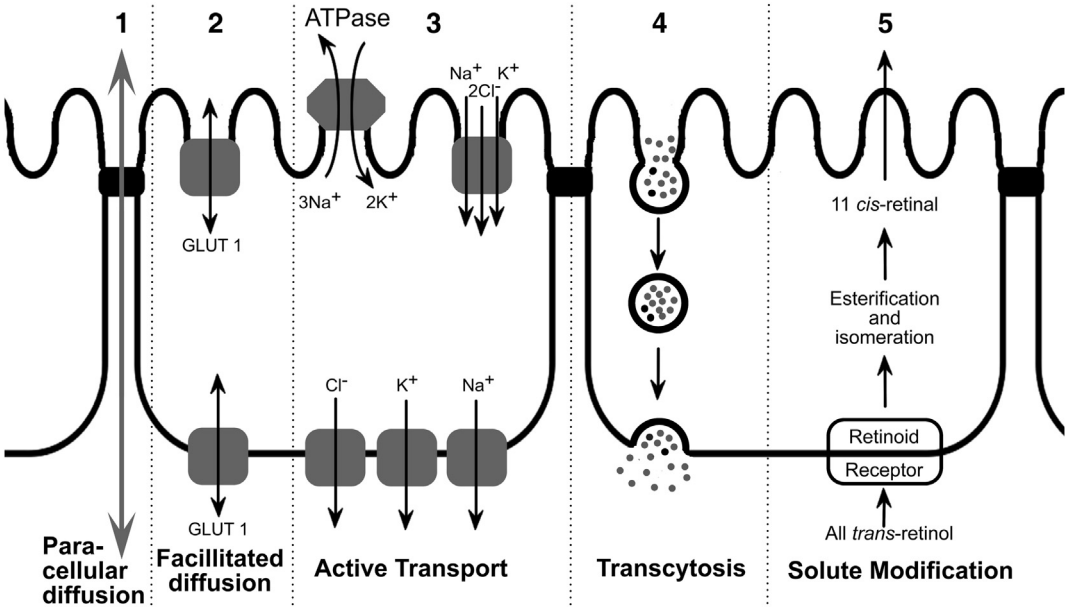

Fig. 1. Five Mechanisms for the transepithelial movement of solutes. The apical membrane appears at the top of Fig. 1) Paracellular diffusion: Small solutes can diffuse across tight junctions, but the junctions allow some solutes to diffuse more readily than others. 2) Facilitated diffusion: Channels allow diffusion in either direction, but are specific for certain solutes. Example, glucose does not readily cross tight junctions, but a variety of glucose transporters allow glucose to diffuse through the cells. Because the retina consumes high levels of glucose, diffusion is favored in the basal to apical direction. 3) Active transport: Pumps, such as the Na^+/K^+-ATPase, use the energy of ATP to pump ions vectorially across the plasma membrane. The electrochemical gradients that the pumps establish drive other solutes vectorially through channels, cotransporters, and antiporters. The example shown is for the absorption of NaCl or KCl. 4) Transcytosis: Bulk phase transcytosis is illustrated. Receptor-mediated transcytosis may also occur. 5) Solute modification: All trans-retinol (carried by retinol binding protein) enters the cell by receptor-mediated endocytosis. Though a series of metabolic reactions, briefly outlined, retinol is isomerized and exported to the retina. Reprinted from Rizzolo et al. (2011) with permission.

collection of transmembrane pumps, transporters, and channels are expressed on either the apical (subretinal) or basal (choroidal) plasma membrane. The polarized distribution of these proteins allows substrates to be transported across the RPE in either the apical-to-basal or basal-to-apical direction. *4) Transcytosis:* Solutes captured by receptor-mediated or bulk-phase endocytosis can be translocated within a vesicle to the opposite side of the cell. *5) Metabolic processing:* Substrates enter the cell from one side, are metabolically processed, and then secreted from the opposite side of the cell. An ideal culture model of barrier function would faithfully reproduce each component.

The conundrum that presents itself is that some of these components are difficult to study *in vivo* or tissue explants, which makes it difficult to evaluate culture models. To supplement assays of function, several laboratories have developed molecular signatures to define an RPE cell. These approaches try to identify not only unique gene expression, but genes required for specialized RPE functions, and therefore, expressed at higher levels than other cells. In this analysis, a gene must not only be expressed, but expressed in the proper amount relative to other genes. This research suggests there are life-stage and species-specific definitions of RPE.

Transcriptomics and assays of function often examine the bulk properties of an epithelial monolayer, but it should be noted that cells within the RPE monolayer are heterogeneous (Burke and Hjelmeland, 2005). For example, in bovine RPE, the Na,K-ATPase (Fig. 1, pathway 3) was expressed at higher levels in the periphery than in the posterior pole (Burke et al., 1991). E-cadherin (Fig. 1, pathway 1) was found in patches of ~20 cells in which the distribution of the Na,K-ATPase was less polarized (Burke et al., 2000). Microheterogeneity persisted when the cells were placed in culture (McKay et al., 1997; Burke et al., 1999). In human fetal RPE (hfRPE), there is heterogeneous expression of claudins and response to proinflammatory cytokines (Peng et al., 2012). RPE cultures described in the literature are derived from different species and at different stages of the life cycle. Because the monolayer is heterogeneous, Subsets of RPE cells are conceivably over-represented in one protocol relative to another. Accordingly, caution should be used when combining data from different models into a uniform theory. Instead, it would be valuable to explore differences related to species and life-stage.

2. How closely does cultured RPE model native cells?

It is difficult to compare *in vivo* and *in vitro* measures of barrier function. Some of the simpler methods are discussed here. With the ease of microarray and RNA-sequencing technology, functional assays are being supplemented with molecular definitions of RPE.

2.1. Methods of measuring barrier function

Barrier function is often assessed using the transepithelial electrical resistance (TER) due to the ease of measurement. Table 1 summarizes data obtained for RPE in explants and in culture. TER is an amalgam of the resistances of tight junctions, the apical plasma membrane, and the basolateral plasma membrane. It is often assumed to represent the resistance of the ion current across the tight junction, but this is true only for relatively leaky junctions (Reuss, 1997). TER approximates the junction resistance only when the apical and basolateral membrane resistances are substantially higher than that of the paracellular pathway [pathway (1) in Fig. 1]. EVOM^2 (World Precision Instruments, Sarasota) is a convenient ohmmeter that can measure the resistance of cultured cells without breaking sterility. Although it allows cultures to be followed over weeks to months, it is subject to several artifacts. 1) Measurements are often made near ambient temperature, which yields an overestimate of the resistance at body temperature. 2) The stick