The eye is an extension of the brain and a highly sophisticated sensory organ. The intricacy of the eye is well appreciated on examination of the exquisite posterior pole, composed of numerous interconnected cell and tissue types. These types broadly include the multicellular neural retina; a single layer of epithelial cells providing support to the retina, called the retinal pigment epithelium; a multilaminar extracellular matrix called Bruch’s membrane; and the choroid, a thin, trilaminar connective tissue adjacent to Bruch’s membrane, composed of a dense web of vessels, pigments, and immune cells. The posterior pole can further be partitioned into two regions, the macula and periphery, adding another tier of complexity to the eye. The anatomic macula, when evaluated histologically, is an oval-shaped region approximately 5 mm in diameter, centrally located in the posterior pole of eye. Though covering approximately 4% of the retina, the macula is densely composed of cone photoreceptors, facilitating high-resolution color vision. The rest of the retina is rich in rod photoreceptors, responsible for side, or peripheral, and night vision. Other regional differences include, but are not limited to, variances in the structural architecture of the retinal layers, cellular distribution, and blood flow. Thus, it may come as no surprise that human retinal diseases demonstrate regional selectivity, in which a cell or tissue type is particularly vulnerable in one area but mostly unaffected in adjacent zones. In this issue of The American Journal of Pathology, Mullin et al explore several essential questions in this realm: Is there region-associated heterogeneity in retinal pigment epithelial cells, whose dysfunction is central to the development of several vision-debilitating diseases? What are the differences in the expression profiles of retinal pigment epithelial cells in the macula versus the periphery? Could regional differences in the levels of gene expression in retinal pigment epithelial cells be linked to susceptibility to disease?

Why Focus on the Retinal Pigment Epithelium?

Mullin et al emphasized retinal pigment epithelial cells. These cells present as a single-cell layer in the posterior pole, are highly specialized, are multifunctional, and sit at the intersection of the neural retina and the outer retinal vasculature and therefore are crucial facilitators of retinal function and structure. Dysfunction and/or degeneration of retinal pigment epithelial cells has been coupled to aging as well as to a number of retinal diseases, including, but not limited to, Stargardt disease, Best disease, retinitis pigmentosa, Sorsby fundus dystrophy, malattia leventinese, central serous retinopathy, and age-related macular degeneration (AMD). Though compromised retinal pigment epithelial cell function is a common denominator among these retinal diseases, the location in which these cells are dysfunctional and/or degenerated is an essential pathologic characteristic, often driving the severity of the disease, as seen in patients with AMD, which by standard definition occurs within the macular region.

Technological Advances in the Approach to Identifying Retinal Pigment Epithelial Subpopulations

Several prior studies have attempted to distinguish heterogeneity in the retinal pigment epithelium. Using...
single-cell RNA sequencing or in vitro culturing of cells isolated from human donor eyes, two studies have reported on populations of retinal pigment epithelial stem-like cells with a potential to proliferate and express progenitor markers.\textsuperscript{7,8} Another study evaluated cellular morphometry in flat mounts of human retinal pigment epithelial monolayers from cadaveric tissues and identified five different concentric epithelial subpopulations that varied in size, shape, and degree of hexagonality, suggesting potential physiological differences in these groups.\textsuperscript{9} Certainly, the results from Mullin et al\textsuperscript{5} support heterogeneity among retinal pigment epithelial cells, as have results from other studies that have examined gene expression in epithelial cells using DNA microarray and RNA sequencing.\textsuperscript{10–13}

Mullin et al used cutting-edge sequencing technology, namely multimodal, single-nucleus profiling of chromatin accessibility and transcript abundance in retinal pigment epithelial cells isolated from the macula and periphery of human donor eyes with relatively short postmortem times.\textsuperscript{5} This approach not only circumvented some of the limitations of previous studies that performed transcriptomics and proteomics, but also confirmed some previous observations.\textsuperscript{10–13} Several genes associated with cell homeostasis and signaling were found to be enriched in the macula including SULF1, a modulator of cell–cell signaling involved in heparan sulfate proteoglycan processing, and WFDC1 or WAP four-disulfide core domain protein 1, involved in tissue remodeling through interactions with matrix metalloproteinase 9. In contrast, SLC4A5, the gene that encodes sodium bicarbonate cotransporter 4, associated with potential retinal detachment and retinal pigment epithelial cell dysfunction, and ELN, the gene encoding elastin, were found to be more highly expressed in the periphery than in the macula. These findings were also cross-compared to a previously published single-cell RNA sequencing database from this group.\textsuperscript{14} Next, the study by Mullin et al\textsuperscript{5} examined regional differences in chromatin accessibility, pinpointing differentially accessible peaks found within gene bodies and within noncoding regions. Peaks within both exons and introns of aldehyde dehydrogenase 1 family member A3 were found to be enriched in the peripheral retinal pigment epithelium. It is noteworthy that the filtered and processed data from this study are available on the Spectacle website (http://singlecell-eye.org, last accessed April 19, 2023).

**Looking Beyond Location**

The identification of regional differences in gene expression and chromatin accessibility in retinal pigment epithelial cells leads to the question of the potential impact on cell physiology and function. Given the multifunctional nature of retinal pigment epithelial cells, the Mullin team\textsuperscript{5} began by determining whether subpopulations of retinal pigment epithelial cells were to emerge by focusing on the distribution of genes related to the vision cycle. They discovered four populations of epithelial cells with differential expression of RLPBP1, LRAT, and RPE65, the genes encoding retinaldehyde binding protein 1, lecithin retinol acyltransferase, and retinoid isomerohydrolase, respectively. Future additional studies are needed to determine whether regional and/or subpopulations of cells express other functional gene family members related to retinal pigment epithelial cells, including, but not limited to, those involved in phagocytosis, lysosomal function, and autophagy.

**Implications for Disease**

AMD is a leading cause of vision impairment in the elderly population in the Western world. Though distinctive pathology, such as the accumulation of lipid- and protein-rich focal deposits, called drusen, located between the retinal pigment epithelium and the inner collagenous layer of Bruch’s membrane, may manifest in the periphery, it is the presence of drusen within the macular region that is a characteristic of the early stages of AMD.\textsuperscript{1} One intriguing utilization of the vast amount of sequencing data collected in this study was the additional probing for 33 loci known to be associated with the risk for AMD previously reported by Fritsche et al.\textsuperscript{15} The team identified three single-nucleotide polymorphisms related to AMD that fell within three retinal pigment epithelial peaks, from which they determined that polymorphisms in the "noncoding chromatin may exert a direct pathogenic effect through the regulation of nearby gene expression."\textsuperscript{15,1758} Importantly, they also concluded that, given that many of the loci related to AMD were not found in open chromatin in non-AMD human epithelial cells, their effects in disease may be mediated by other cell types.\textsuperscript{16} This hypothesis aligns well with the complexity that surrounds the development and progression of AMD, in which multiple cell types are involved and/or affected. There is room, however, for more exploration in this area, including a cross-comparison of the current data with future assessments of retinal pigment epithelial cell single-nucleus RNA and ATAC sequencing profiles from donor eyes with early, dry AMD. Furthermore, single-cell nucleus omics has the potential to provide unparalleled resolution into the molecular foundations driving cellular heterogeneity and to guide and refine the understanding of potential differences beyond macula versus periphery. Specifically, it may provide important insight into variations within the macular region itself, which can be divided into the ubmo, foveola, foveal avascular zone, fovea, parafovea, and perifovea—essential information given that the fovea/parafoveal zone is associated with rod-mediated dark adaptation, which is delayed in patients with increasing AMD severity.\textsuperscript{17}
Perspectives

The comprehensive study by Mullin et al.\(^5\) provides further emphasis on abandoning the rudimentary concept that all retinal pigment epithelial cells are the same. Still many questions remain at the functional level, including: Do retinal pigment epithelial cells behave differently on a mechanistic level in the macula versus periphery?; What are some differences in their basic cellular metabolic processes, phagocytosis, capacity to clear toxins, and response to oxidative stress?; Does age, an established risk factor in the development and progression of some retinal diseases such as AMD impact the function and gene expression profile of the retinal pigment epithelial cells in a region-specific manner?; and Do other populations of cells in the retina and choroid, both affected in AMD, exhibit regional differences at the functional and gene expression levels? The effort invested in answering questions such as these, using advanced spatial transcriptomic and proteomic technologies, promises to broaden molecular-related insight into spatial dynamics, the interplay between heterogenous cell types, and the pathobiology of macular disease.

References


