Age-related macular degeneration and the RPE secretome

Imagine trying to focus on an object, but always seeing a large dark spot blocking your view. You look up or down, but the spot is still there, in the center of your visual field. You can’t read; you can’t drive; you can’t even see faces. The spot is always in the way.

That dark spot is something that patients with age-related macular degeneration (AMD) struggle with every day. AMD gradually robs its victims of their central vision as the years go by. The disease is the leading cause of blindness in people >60 years old, and no treatment exists. Large deposits called drusen form at the back of the eye between the retinal pigment epithelium (RPE) and a fibrous structure known as Bruch’s membrane. Drusen accumulation interferes with the normal functions of the RPE, which transports nutrients from the blood to the photoreceptors and cleans the rod outer segment by phagocytosing debris. The most severe form of the disease is the wet version in which abnormal blood vessels form behind the retina and invade the photoreceptors; these fragile vessels can then hemorrhage and damage the eye.

According to Yetrib Hathout, researchers have long suspected that RPE cells are responsible for drusen formation because these cells “play a crucial role in maintaining homeostasis at the barrier between the blood and photoreceptors.” However, this hypothesis has not been widely tested. So, Hathout and colleagues at Children’s National Medical Center, George Washington University, the Medical College of Wisconsin, and the National Eye Institute analyzed the proteins secreted by RPE cells for evidence that these cells play a role in drusen accumulation. In this issue of JPR (pp 2599–2610), the researchers report that the RPE secreome consists of many proteins found in drusen deposits of AMD patients.

Once donor eyes arrived at the laboratory, the first step was to biopsy them for signs of AMD. The researchers used electron microscopy to carefully examine a cross section of each retina for the appearance of drusen. Eyes with large drusen were categorized as AMD, whereas those without drusen were categorized as controls.

But while the researchers were sorting the donor eyes by appearance, a series of scientific papers that described a gene involved in AMD was published. The gene codes for complement factor H (CFH), and a mutation at amino acid position 402 was found to increase one’s risk of developing the disease. “When this gene was discovered, we genotyped our collection to make sure that we could classify our cells based on both the phenotype and the genotype,” says Hathout. Donors who were homozygous for the CFH mutation and whose eyes contained large drusen were determined to have AMD. However, donors who had two copies of the wild-type gene and whose eyes lacked drusen were treated as controls. In addition, “heterozygous donors with drusen were studied as an indeterminate case—we didn’t know what to expect,” Hathout explains.

To compare the secretemes of the 3 samples, the researchers isolated the RPE cells from each eye and conducted stable-isotope labeling by amino acids in cell culture (known as SILAC) experiments. Although RPE cells are not derived from immortal cell lines, they can divide up to 12× in bovine serum media, says Hathout. Control cells were labeled with media containing heavy amino acids, and AMD and heterozygous cells were grown in regular media. The spent media from control cells were mixed with media from AMD or heterozygous cells. Proteins that were up- or down-regulated by at least 2-fold were considered to be significant.

Several proteins involved in angiogenesis, tissue development, and immunological responses were secreted at higher levels by AMD and heterozygous cells than by control cells, and most of the identified proteins are known drusen components. For example, clusterin is present in drusen and has been implicated in the aggregation of β-amyloid in a mouse model of Alzheimer’s disease.

Surprisingly, CFH was found in the spent media from RPE cells. “One of the most interesting things we found, and I think the AMD researcher will be excited about this, is that RPE cells make and secrete their own CFH,” says Hathout. “This finding is crucial because most of the studies say that CFH is made by the liver, and it circulates in the blood to the retina.” He hypothesizes that locally secreted CFH helps the RPE cells to clean up deposited antigen–antibody complexes. If CFH is defective, then debris would accumulate beneath the RPE, where drusen are found.

With this preliminary study behind them, Hathout and colleagues now are planning to expand the investigation and increase the number of samples. They also plan to validate the findings from this work. “We are going to follow up on some key proteins, such as clusterin and CFH, and look at their role and function,” says Hathout. He adds that several questions still are unanswered: “Why are [those proteins] secreted by RPE cells? Do RPE cells in their physiological environment secrete the same proteins?” The answers to these questions ultimately may help researchers find treatments or even a cure for AMD.

—Katie Cottingham