Genetic and Environmental Factors in AMD

Stephen Tsang, Columbia University Medical Center
Jin Yang, Columbia University

Purpose: To explore the mechanisms of AMD with iPSC-derived RPE cells carrying an ARMS2/HTRA1 allele mutation.

Methods: The ARMS2/HTRA1 mutation was confirmed through gene sequencing. Patient-specific iPS was derived from fibroblasts and differentiated into RPE. Immunostaining and immuno-blots confirmed RPE-specific marker expression. A high-risk group with mutant alleles and low-risk wild-type group were fed 5 times with 10uM A2E over 10 days. Fluorescent microscopy examined autofluorescence. After 10 minutes of blue light treatment, suspended fluorescent cells were compared between groups. Transmission electron microscopy (TEM) was used to compare morphology of iPSC-derived RPE cells, both pre- and post-treatment and with native cells from two monkeys (aged 1 and 24). Waters synapt G2 QTOF Mass Spectrometry analyzed label-free shotgun proteomics with identityE quantitation software (MS). Western blots further assayed proteins.

Results: Neither high-risk nor low-risk cells displayed changes in RPE-specific markers. HTRA1 was elevated among high risk groups. Pre-treatment iPSC-RPE cells resembled 1-year-old monkey RPE morphologically. After treatment, a mixture of phacosomes and lipofuscin appeared as in aged monkey cells. Higher SOD2 protein concentrations were pronounced in low risk cells. In low risk cells, SOD activity kit showed a combination of A2E with blue light stimulated SOD; there was almost no response in the high risk group.

Conclusions: iPSC-RPE cells treated with A2E and blue light appeared similar to AMD patient RPE cells, suggesting utility as a disease model. Changes in activity of SOD2 may reflect the disease mechanisms of ARMS2/HTRA1-related AMD.