Editorial

Stem Cell INVESTIGATION

Human induced pluripotent stem cells illuminate pathways and novel treatment targets for age-related macular degeneration

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Age-related macular degeneration (AMD) is the most common cause of legal blindness in the United States and is the leading cause of visual impairment in the aging population, especially in those over 55 years of age. By 2020, an anticipated 196 million individuals will be affected with AMD (1). Geographic atrophy is characterized by a slow progressive degeneration of the retinal pigment epithelium (RPE), resulting in the gradual loss of photoreceptors. The wet, or neovascular, form is characterized by the growth of abnormal new blood vessels from beneath the retina that can cause severe and rapid vision loss due to hemorrhage and exudation. Most current treatments are directed against neovascular AMD and are focused against stimulators of angiogenesis (such as vascular endothelial growth factor). These treatments are limited in their applicability, require invasive intravitreal injections, which are burdensome for both patient and physician, and are not capable of preventing or reversing vision loss over the long term. Currently, there are no effective treatments for atrophic AMD.

Significant work in AMD genetics has established *CFH* and *ARMS2/HTRA1* as having large influences on AMD risk in populations of various ethnicities (2-8). Although more than 40 additional AMD-associated risk variants at other loci have been found through case-control association studies with candidate genes (9-12), genome-wide association studies of large cohorts (13,14), systems biology (15,16), and studies of pathways shared by multiple disorders (17,18), *CFH* and *ARMS2/HTRA1* genotypes, as well as advanced age, account for most of the known attributable risk for the disorder. Robust AMD associations with common and/or rare variants in *CFH* and other complement pathway genes including C2/CFB (19), *C3* (9,10), *C9* (20), *CFI* (13,21), and *VTN* (14) suggested that complement inhibition might be a good therapeutic option. However, to date, results of clinical trials using complement inhibitors have not been promising. For example, eculizumab did not improve vision very effectively (22) and lampalizumab had a limited effect in reducing AMD progression (23,24). Recent results reported for the Spectri phase III clinical trial for patients treated with lampalizumab, to reduce lesion their size for geographic atrophy proved to be disappointing (https://www.roche.com/media/store/releases/med-cor-2017-09-08b.htm). Clearly, there is a need to develop more appropriate and effective therapies for AMD, possibly considering the genotype(s) at specific risk loci of the individual when designing a clinical trial toward the goal of personalized medicine (25).

Progress in development of novel effective therapies for AMD based on genetic targets and specific variants could be expedited using rodent model systems, but these models have multiple limitations, most notably the fact that rodents

don't have maculae to be able to measure pathophysiological endpoints. In a recent article, Saini and colleagues report findings from a series of experiments conducted in human RPE cells, a monolayer in the macula that is essential for photoreceptor support, survival and adversely affected by AMD. These cells were differentiated from human-induced pluripotent stem cells (hiPSCs) derived from eye tissue obtained from patients with documented AMD including two individuals homozygous for AMD risk alleles at the *ARMS2/HTRA1* locus (*ARMS2/HTRA1+*) and from donors without evidence of AMD. Although expression of nine AMD and drusen associated transcripts was not significantly different in the hiPSCs from AMD and healthy control subjects, the authors observed significantly increased levels of transcripts from a set of complement and inflammatory proteins (including *C3*, *CFI*, and *CFH*) in hiPSCs from AMD subjects. These differences were greatest in comparisons of controls with AMD *ARMS2/HTRA1+* subjects.

These experiments alone are noteworthy for at least two reasons. First, although several years have passed since retinal and RPE cells were first derived from human and mouse iPSCs (26,27) and successful submacular transplantation of RPE cells derived in this manner has been performed recently in two patients with neovascular AMD (28), use of transplanted of autologous iPSC-derived RPE cells as a treatment for AMD has many challenges and ultimately may not be effective (28,29). Second, Saini and co-workers provided evidence for the first time that the *ARMS2/HTRA1* AMD risk genotype impacts expression of AMD-related proteins, particularly those in the complement system, and thereby provides some clues about how the role of this pathway may function in the disease. However, selection of risk variants from both *ARMS2* and *HTRA1* as the basis for the *ARMS2/HTRA1+* genotype does not allow for the determination of which of these genes actually effects expression of the AMD-related proteins. Nonetheless, this is a solid starting point for future studies with a larger sample size.

The most remarkable aspect of this study was the use of the hiPSC lines to test the therapeutic potential of nicotinamide (NAM), a vitamin B3 derivative with anti-inflammatory properties (30) and discover its connections to AMD pathways. Selection of NAM as a therapeutic agent was reasonable in light of evidence showing that elevated serum levels of factors in the vitamin B pathway (vitamin B12, homocysteine, and folate) reduce susceptibility to early and advanced AMD (31). In the current study, it was observed that expression of two AMD- and drusen-associated proteins, clusterin and vascular endothelial growth factor A, was inhibited in hiPSC-RPE cultured cells treated with NAM regardless of the donor group, and this effect was most pronounced in *ARMS2/HTRA1+* cells. Secretion of the drusen- and Alzheimer disease-related protein $A\beta_{42}$ was inhibited in the NAM-treated AMD but not control hiPSC lines. Next, Saina and co-workers performed bioinformatics analysis to examine the global effects of NAM on the RPE transcriptome. Pathway enrichment analysis suggested that NAM primarily affects the PI3K-Akt signaling and pathway followed by six other pathways including complement and coagulation cascades. Perhaps not surprisingly (and proof of principle), the most significant disease associated gene ontology (GO) terms were macular degeneration and cone-rod dystrophy. In-depth analysis of genes showing at least nominally significant changes revealed that NAM increased expression of ribosomal synthesis genes in the nucleus and mitochondria, DNA/RNA polymerase, histones, and the gene encoding the sirtuin 1 (SIRT1) protein. Decreased expression of these genes has been shown to be associated with aging (32,33). Further experiments in the hiPSC-RPE lines showed that NAM can effectively decrease inflammatory cytokine production and repress the complement pathway, including C3.

These experiments underscore the importance of targeting more than one disease pathway or mechanism at a time, while taking into consideration genotype risk. This may be a way forward to obtain more effective AMD therapies as many biochemical pathways overlap and have more than one function.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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