

Genetic Induction of Autophagy and Lysosomal Function in the ATP Binding Cassette Subfamily A Member 4 (ABCA4) knockout Retinal Pigment Epithelium

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Abstract:

Purpose: Abnormal functioning of the autophagy and lysosomal pathways is implicated in the pathogenesis of age-related macular degeneration (AMD). Therefore, restoration of both these pathways has the potential to enhance cellular clearance and prevent RPE degeneration. Transcription factor EB (TFEB) is a known master regulator of autophagy and lysosomal pathways. We tested the hypothesis that genetic induction of TFEB in the RPE of the ABCA4 KO mouse, a model of Stargardt's disease, can enhance both autophagy and lysosomal pathways and prevent cellular dysfunction in the RPE.

Methods: ABCA4 KO mice were subretinally injected with AAV-TFEB-GFP into one eye of the 6 week and 6-month-old mouse, the contralateral eye was injected with AAV-GFP as control. The expression of the transgene was evaluated by immunostaining with the GFP antibody. The expression of TFEB and downstream target genes was evaluated by qRT-PCR analysis. Immunoblotting was used to measure the changes in the expression of autophagy pathway proteins. Retinal function and thickness were evaluated by Electroretinogram (ERG) and OCT analysis, respectively.

Results: Our studies show the expression of the TFEB transgene upon subretinal injection in the ABCA4 KO RPE. The expression of the TFEB and TFEB downstream target genes involved in both the autophagy and lysosomal pathways was elevated in the RPE of the injected eye compared to the contralateral control eye. Our results also showed an increase in the thickness of the retinal layers and an increase in the retinal function.

Conclusions: Subretinal injection of TFEB enhanced the expression of TFEB pathway genes in the RPE. Enhanced LCII and decreased SQSTM1 expression indicated an induction of the autophagy pathway. Our results, taken together indicate that induction of TFEB enhances autophagic responses, thereby causing an increase in cellular clearance in the ABCA4 KO RPE.