

D. L. Pomerleau, Class of 2005
Department of Medicine

Gamma-synuclein is a marker for retinal ganglion cell loss in glaucoma

D. L. Pomerleau, M. S. Pezda, M. Tapia, A. S. Hackam, R. K. Lee;
Bascom Palmer Eye Institute, U. of Miami School of Medicine, Miami, FL.

Purpose: Identification of molecules specifically down-regulated in the retina of glaucomatous mice. DBA2/J mice develop chronic, progressive glaucoma. In order to determine molecular pathways involved in the development of glaucoma, differential gene expression analysis was used to identify genetic markers associated with the pathogenesis of glaucoma in a mouse model.

Methods: Pooled whole retinas from young, non-glaucomatous (younger than 3 months of age) and from aged, glaucomatous (12 months and older) DBA2/J mice were homogenized and total RNA was extracted using Trizol and purified with Qiagen RNeasy separation columns. First strand cDNA was synthesized and hybridized to Affymetrix GeneChip Mouse Genome 430 2.0 gene expression microarrays. Retina cDNA was also used for real-time PCR with alpha-, beta-, and gamma-synuclein and thy-1 primers to quantitatively confirm the microarray gene expression data. In addition, immunohistochemistry using gamma-synuclein antibodies was used to determine protein expression levels.

Results: Gene expression microarray data was analyzed with Affymetrix Microarray Suite 5.0 software and gamma-synuclein was identified as one of the top fifteen most highly down-regulated genes expressed in aged, glaucomatous DBA2/J retinas compared to young, non-glaucomatous DBA2/J retinas. This finding correlated with the histological loss of retinal ganglion cells (RGC) in the glaucomatous retinas. Gamma-synuclein down-regulation was confirmed on the transcriptional level using real-time PCR and on the translational level by immunohistochemistry, which also localized expression of gamma-synuclein in the retina to RGC. A direct relationship was found between gamma-synuclein and thy-1 expression levels by real-time PCR.

Conclusions: In a genome wide screen of genes expressed in the mouse retina, we found aged DBA2/J retinas from 12 to 17 month old mice with glaucomatous loss of RGC have significantly decreased levels of gamma-synuclein expression that correlate with age and severity of RGC loss. The progressive loss of RGC in glaucoma also correlated with loss of RGC marker thy-1 expression. The finding that thy-1 levels are directly correlated to gamma-synuclein levels suggest that gamma-synuclein can be used a marker for RGC loss, since both molecules are selectively expressed in RGC. Gamma-synuclein has been suggested to be an anti-apoptotic molecule. The association of decreased gamma-synuclein expression levels with decreased numbers of RGC suggest expression of gamma-synuclein may be neuroprotective.