MicroRNA Expression Within The Glaucomatous Retina


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Purpose: MicroRNAs (miRNAs) are small, endogenous non-coding RNAs that modulate post-transcriptional gene expression. Their role in the pathophysiology of ocular disease is a rapidly expanding field. Although the role of miRNAs upon the pathogenesis of glaucomatous damage is not known, there is supporting evidence from CNS research that highlight their potential importance. It was therefore hypothesized that miRNAs altered in CNS injury may also be altered in experimental glaucoma.

Methods: IOP was elevated in rats by unilateral injection of hypertonic saline and the IOP response monitored for 5 weeks. After sacrifice, retrobulbar optic nerve sections were graded for nerve damage. miRNA was extracted from the retina of eyes with advanced nerve damage (n=8) and from normal fellow eyes as controls (n=8). Sampling was reverse transcribed and pre-amplified for quantitative PCR using specific Taqman probes for a panel of 20 miRNAs. Results were normalized to U6sRNA and snoRNA234 as internal controls and relative expression was performed by the Livak method. Statistical comparison of miRNA expression between glaucoma and control retinae was performed by a two-tailed t-test with significance considered for values of p<0.05.

Results: Induction of glaucomatous damage was confirmed in experimental eyes with a mean injury grade of 5.0 with mean/maximum IOPs of 39.2 ± 3.2 mmHg / 51.7 ± 1.0 mmHg compared to a mean injury grade of 1.0 with mean/maximum IOPs of 28.2 ± 0.2 mmHg / 29.0 ± 0.3 mmHg in control eyes. Among the miRNAs hypothesized as likely to be affected, miR-16, let-7a, miR-181c, miR-497, miR-29b, miR-204, miR-106b and miR-25 were significantly downregulated in glaucomatous retinae compared to controls (p<0.04) and a single miRNA, miR-27a, was significantly upregulated (p=0.01). No significant change in expression of miR-21, miR-22, miR23a, miR-145, miR-200b, miR-223 or miR-424 was observed.

Conclusions: This is the first study evaluating changes in microRNA expression between healthy and glaucomatous retinae. MicroRNAs altered within the retina of eyes with advanced glaucomatous nerve damage are also known to be altered in CNS injury, with actions serving to modulate apoptosis, ischemic responses or TGF-β signaling. Further research to evaluate the role of microRNAs in the glaucomatous optic nerve head as well as retina to dissect the dynamic mechanisms of glaucomatous optic neuropathy, may ultimately lead to the identification of novel therapeutic targets.

Commercial Relationships: Hari Jayaram, None; William O. Cepurna, None; Elaine C. Johnson, None; John C. Morrison, None

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Tau accumulation in the somato-dendritic compartment of retinal ganglion cells plays a critical role in glaucomatous neurodegeneration

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Purpose: Alzheimer’s disease and glaucoma share a number of neuropathological features including the loss of retinal ganglion cells (RGCs) and the presence of amyloid beta plaques and tau aggregates. Here, we asked whether there are changes in tau protein expression in the retina and optic nerve during ocular hypertension (OHT) and, if so, whether tau contributes to RGC neurodegeneration in experimental glaucoma.

Methods: OHT was induced in Brown Norway rats by injection of hypertonic saline solution into an episcleral vein (Morrison model). Tau protein phosphorylation was evaluated by western blot analysis using a panel of antibodies against total tau and phospho-specific tau epitopes. Tau gene expression was analyzed by quantitative real-time PCR. The cellular localization of tau was investigated by retinal and optic nerve immunohistochemistry using antibodies against tau and cell-specific markers. The functional role of tau in RGC death was investigated with short interference RNA (siRNA) to selectively knockdown tau expression and a control siRNA. RGC soma or axons were quantified by Brn3a immunostaining on flat-mounted retinas or toluidine blue-stained optic nerve cross sections, respectively.

Results: Our results demonstrate that OHT leads to a rapid increase of tau protein in the retina. Both hyperphosphorylated and hypophosphorylated forms of tau were detected in glaucomatous retinas compared to intact controls (N=6-10/group). We show that soon after induction of OHT, tau accumulated primarily in RGC somata and dendrites, while tau in RGC axons within the optic nerve markedly decreased. Surprisingly, the level of tau mRNA in retinas subjected to OHT did not change with respect to intact controls (N=6/group), suggesting that the increase in retinal tau occurs from abnormal translocation from RGC axons rather than upregulation of tau gene expression. Importantly, tau knockdown using a targeted siRNA led to striking protection of RGC soma and axons from OHT-induced damage (1946±39 RGCs/mm², mean soma ± S.E.M.) compared to retinas treated with control siRNA (1599±40 RGCs/mm²) (ANOVA, p<0.001, N=9/group).

Conclusions: Our data support the conclusion that ocular hypertension glaucoma displays cardinal features of a tauopathy including abnormal compartmental redistribution of tau, altered phosphorylation profile and neurotoxicity.

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Adaptive immune responses in glaucoma promote IOP-independent RGC loss

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Purpose: To functionally test if immune processes are both initiated and capable of causing damage in glaucoma we carried out adoptive transfers between mouse models of glaucoma and normal recipient mice.

Methods: B6/Shh+D2apo (nee), immune-deficient B6.129S7-\textsuperscript{Rag1} \textsuperscript{cko} and C57BL/6J wild-type (B6) mice served as donors. Splenocytes (5x10^6) or FACS sorted CD19 (1.5x10^6) and CD3 cells were transferred to recipient mice (rec: n=5 animals/group). Intraocular pressure (IOP) was compared to ctrl or B6 recipients (RGC/mm^2 in ctrl: 2290 ± 254, B6 rec: 2290 ± 245, n=5; ctrl vs. B6, p<0.03). Transfer of neo/\textsuperscript{Rag1} splenocytes did not induce loss of RGC (RGC/mm^2 rec: 2259 ± 266 vs. 2259 ± 267, p<0.03; \textsuperscript{Rag1} rec: 1886 ± 317, p<0.02). Transfer of neo/Rag1 spleenocytes also displayed significantly reduced RGC density when compared to B6 T-cell recipients (RGC/mm^2 in B6 T-cell rec: 2251 ± 221 vs. 1831 ± 245, n=5; ctrl vs. B6, p<0.004). Transfer of neo B-cells leads to a modest reduction of RGC (RGC/mm^2 in B6 B-cell rec: 2259 ± 266 vs. 1968 ± 317, n=5; ctrl vs. B6, p=0.07). Transferred T-cells were frequently found integrated into the recipients' spleens. DsRed neo lymphocytes were occasionally observed in the retina of recipient mice but were not observed in any recipient animals.

Conclusions: These data demonstrate that glaucomatous RGC loss elicits an adaptive immune response that is capable of promoting IOP-independent RGC loss following adoptive transfer. The immunopathology in recipients is likely due to both T-cell and NGL2-dependent mechanisms.

Commercial Relationships: Oliver W. Gramlich, None; Qiong Ding, None; Michael G. Anderson, None; Markus H. Kuehn, None

Program Number: 1694
Presentation Time: 11:45 AM–12:00 PM

Impaired Lysosomal and Mitochondrial Function in Exfoliation Glaucoma

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Purpose: In the eye, exfoliation syndrome (XFS) is characterized by the aggregation of disorganized microfibrils (exfoliation material, XFM). Deposition of XFM and pigment in the aqueous outflow pathway leads to chronic intraocular pressure elevation leading in turn to glaucoma. Similar to other age-related diseases in which protein aggregates cause disease, we hypothesize that lysosomal and mitochondrial dysfunction contributes to the formation of XFM aggregates.

Methods: Tenon fibroblasts (TFs) were explanted from tissue discards obtained from older, age-matched XFS and primary open-angle glaucoma (POAG) patients who underwent trabeculectomy surgery and from young healthy donors who underwent strabismus surgery. Experiments were performed in supplemented serum-free media on collagen or in 1% FBS-containing media. Cell size and mitochondrial membrane potential (MMPT, JC1 dye) were quantified by flow cytometry. Lysosomes and microtubules were immunodetected with Lamp-1 and β-tubulin antibody, respectively. Culturing TFs in media with stabilized vitamin C for 1 month generated self-synthesizing 3D gels.

Results: Normally, under conditions of nutrient deprivation, lysosomes become peri-nuclear, where they fuse with autophagosomes, clearing the cells of waste. In XFS TFs compared to POAG TFs and healthy TFs, lysosomes did not relocalize in response to changes in nutrient conditions, suggesting that lysosomal degradation is impaired in these cells (Figure 1). In 3D culture, XFS TFs demonstrated a disorganized morphology with elevated expression of XFM-containing proteins LOXL1 and Fibulin-5. Consistent with impaired lysosomal degradation a) the percent of cells displaying depolarized mitochondria was 10x higher in XFS than in POAG TFs (26 % vs. 2%, p<0.01) and b) the build up of intracellular organelles led to a 1.7-fold increase in XFS cell size.

Conclusions: Our findings suggest that lysosomes and mitochondria are compromised in XFS TFs, leading to a toxic environment. This may lead to reduced degradation and increased secretion of XFM aggregates.

Commercial Relationships: Andrew Want, None; Stephanie Gillespie, None; J Mario Wolosin, None; Robert Ritch, None; Audrey Bernstein, None

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Program Number: 1696
Presentation Time: 12:15 PM–12:30 PM

Repeatability of Automated OCT-based 24-2 Visual Threshold Estimation in Patients with Glaucoma

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Purpose: The University of Iowa, Iowa City, IA; 2Department of Molecular Physiology and Biophysics, The University of Iowa, Iowa City, IA. 

Purpose: To functionally test if immune processes are both initiated and capable of causing damage in glaucoma we carried out adoptive transfers between mouse models of glaucoma and normal recipient mice.

Methods: B6/Shh+D2apo (nee), immune-deficient B6.129S7-\textsuperscript{Rag1} \textsuperscript{cko} and C57BL/6J wild-type (B6) mice served as donors. Splenocytes (5x10^6) or FACS sorted CD19 (1.5x10^6) and CD3 cells were transferred to recipient mice (rec: n=5 animals/group). Intraocular pressure (IOP) was compared to ctrl or B6 recipients (RGC/mm^2 in ctrl: 2290 ± 254, B6 rec: 2290 ± 245, n=5; ctrl vs. B6, p<0.03). Transfer of neo/\textsuperscript{Rag1} splenocytes did not induce loss of RGC (RGC/mm^2 rec: 2259 ± 266 vs. 2259 ± 267, p<0.03; \textsuperscript{Rag1} rec: 1886 ± 317, p<0.02). Transfer of neo/Rag1 spleenocytes also displayed significantly reduced RGC density when compared to B6 T-cell recipients (RGC/mm^2 in B6 T-cell rec: 2251 ± 221 vs. 1831 ± 245, n=5; ctrl vs. B6, p<0.004). Transfer of neo B-cells leads to a modest reduction of RGC (RGC/mm^2 in B6 B-cell rec: 2259 ± 266 vs. 1968 ± 317 in B6 B-cell rec, p=0.07). Transferred T-cells were frequently found integrated into the recipients' spleens. DsRed neo lymphocytes were occasionally observed in the retina of recipient mice and appeared to be engaged in cell-cell interaction with microglia. Elevated IOP, disruption of retinal integrity, or significant leukocyte infiltration was not observed in any recipient animals.

Conclusions: These data demonstrate that glaucomatous RGC loss elicits an adaptive immune response that is capable of promoting IOP-independent RGC loss following adaptive transfer. The immunopathology in recipients is likely due to both T-cell and NGL2-dependent mechanisms.

Commercial Relationships: Oliver W. Gramlich, None; Qiong Ding, None; Michael G. Anderson, None; Markus H. Kuehn, None

Program Number: 1695
Presentation Time: 12:00 PM–12:15 PM
Purpose: To determine relationships between SD-OCT derived regional damage to the retinal ganglion cell-axonal complex (RGC-AC) and visual thresholds for each location of the Humphrey 24-2 visual field, in all stages of open-angle glaucoma, and determine the repeatability of our predictive method.

Methods: Subjects with early, moderate and advanced glaucoma were recruited from a tertiary glaucoma clinic. Standard automated perimetry (SAP) Humphrey SITA 24-2 and 9-field Spectralis SD-OCT, covering 60° of retina, were acquired. In the repeatability subset, patients underwent SAP and OCT twice. Individual OCT volumes were aligned, nerve fiber (NFL), ganglion cell and inner plexiform layers (GCL+IPL) co-segmented. Layers were then partitioned into 54 sectors corresponding to the 24-2 grid. A Support Vector Machine was trained independently for each sector to predict the sector threshold, using these structural properties, of the associated RGC-AC trajectory that originates at the cell of interest, only on the non-repeatability subjects. Prediction of individual sector thresholds was compared in leave-one-out fashion to corresponding SAP thresholds using correlation R and average root mean squared error (RMSE). Repeatability was evaluated on the repeatability subset using test-retest correlation (R) and coefficient of determination (R²) across the 54 sectors without retraining.

Results: 122 consecutive subjects diagnosed with glaucoma, 43 early, 39 moderate, and 40 advanced, were included (122 eyes), as well as 20 additional subjects, 6 early, 7 moderate, and 7 advanced, into the repeatability subset. R of automated OCT-based predicted thresholds to SAP thresholds on 122 subjects was 0.68 (0.47 - 0.82), into the repeatability subset. R of automated OCT-based predicted thresholds to SAP thresholds on 122 subjects was R=0.98 and R²=0.95 (Fig 2 left). OCT based prediction repeatability on 20 subjects was R=0.98 and R²=0.95 (Fig 2 left) while SAP had R=0.88 and R²=0.74 (Fig 2 right).

Conclusions: Predicting individual 24-2 visual field thresholds from structural information derived from 9-field SD-OCT local NFL and GCL+IPL thicknesses using the RGC-AC concept is feasible, showing the potential for the predictive ability of SD-OCT structural information for visual function, with better reproducibility than SAP Humphrey 24-2. Ultimately, it may be feasible to complement and reduce the burden of subjective visual field testing in glaucoma patients with predicted function derived objectively from OCT.

Commercial Relationships: Michael D. Abramoff, IDx LLC (C), IDx LLC (I), University of Iowa (P); Hrvoje Bogunovic, None; Young H. Kwon, None; Brice Critser, None; Mona K. Garvin, None; Milan Sonka, University of Iowa (P)

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Program Number: 1697
Presentation Time: 12:30 PM–12:45 PM
Visual cortex activity is impaired prior to visual field loss in glaucoma

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Purpose: Glaucoma involves transsynaptic degeneration in the visual cortex. Retinal structure and visual function relationships in glaucoma have been described by a broken-stick model, in which detectable visual field (VF) functional loss emerges after substantial retinal degeneration reaches a tipping point. This study examined relationships among visual cortex activity, retinal morphology and visual function in glaucoma using both linear and broken-stick analyses.

Methods: Blood-oxygen-level-dependent (BOLD) functional MRI imaging was collected for 26 subjects including 10 advanced glaucoma (age=65.5±7.8yrs), 9 early glaucoma (age=62.9±7.1yrs) and 7 healthy control (age=64.1±8.0yrs) on a 3 Tesla scanner. Checkerboard stimuli were presented to the superior/inferior hemifield of each eye separately. BOLD % signal changes between rest and stimulation periods within Brodmann Areas (BA) 17, 18 and 19 were compared to OCT-measured peripapillary retinal nerve fiber layer (RNFL) thickness and macular ganglion cell-inner plexiform layer (GCIPL) thickness, and to VF mean deviation using linear and broken-stick analyses based on data distribution.

Results: Using linear modeling, superior/inferior RNFL/GCIPL thicknesses and VF were most strongly correlated with BOLD activity for corresponding hemifields in BA17 (superior RNFL/GCIPL/VF p=0.006/0.003/0.001, inferior p=0.001/<0.001/0.001 respectively), less in BA18 (p=0.31/0.001/0.13, p=0.02/0.02/0.04), and not significantly correlated in BA19 (p>0.05). Our data confirmed previously reported broken-stick model relating RNFL/GCIPL thickness and VF function at a tipping point of 82μm for RNFL and 66μm for GCIPL (p<0.001). Our data also demonstrated a broken-stick model relationship between BOLD signal in BA17 and VF function for the superior hemifield at a tipping point of BOLD=0.54% (p<0.001).

Conclusions: Our results from linear modeling showed that glaucomatous degeneration of inner retinal structure and visual function were more closely associated with reduced activity in the primary visual cortex than higher-order visual areas. Current data also supported previously described broken-stick models for structural OCT vs functional VF correlations. More importantly, a tipping point also existed between regional primary visual cortex activity and VF function, suggesting substantial reduction in brain activity before detectable VF functional loss.