Purpose: The loss of retinal ganglion cells (RGC) and their axons is a leading cause of blindness and includes traumatic (optic neuropathy) and degenerative (glaucoma) eye diseases. Mesenchymal stem cells (MSC) have demonstrated significant neuroprotective and axogenic effects on RGC in both of the aforementioned models. The present study aimed to isolate exosomes from bone marrow-derived MSC (BMSC) and test them in a rat optic nerve crush (ONC) and glaucoma model.

Methods: Exosomes were isolated from human BMSC and characterized by electron microscopy, flow cytometry and CD63 ExoELISA. Using an in vitro axotomized rat RGC model and in vivo rat models of ONC and glaucoma, we treated/injected 3x10⁵ exosomes into the cell culture well/vitreous. To measure their neuroprotective and axogenic capacity, we used immunohistochemistry, optical computed tomography (OCT) and electrophysiology (ERG). The composition of miRNA in exosomes from human BMSC and control human fibroblasts was investigated by RNA sequencing and used to identify candidate target mRNA in RGC.

Results: Both BMSC and fibroblasts secrete similar numbers of exosome (1.03x10⁹ and 1.17x10⁹ cells, respectively). Treatment of RGC cultures with exosomes led to significant RGC survival (299 ± 24.1 RGC/well) compared to both fibroblast exosome treated (72.3 ± 6.4RGC/well) and untreated (121.3 ± 6.2) cultures. Following intravitreal transplantation, exosomes successfully integrated into the inner retinal layers, including RGC. After ONC (21d), BMSC exosomes provided significant therapeutic effects as compared with fibroblast exosomes or untreated eyes. For the three measured outputs, the thickness of the retinal nerve fibre layer was 33.8 ± 4.8 μm, 21.6 ± 1.5 μm, and 18.0 ± 2.1 μm, respectively; RGC density was 73.3 ± 7.8/mm² of retina, 20 ± 2.2/mm² of retina, and 23.6 ± 7.7/mm² of retina, respectively; and positive scotopic threshold response was 28.6 ± 8.1 μv, 13.2 ± 3.4 μv, and 13.7 ± 1.1 μv, respectively. The significant therapeutic benefits were also seen in the treatment of glaucoma models. The therapeutic benefit of BMSC exosomes was reduced significantly if isolated following knockdown of Argonaute 2, a protein that complexes with miRNA and is integral to their function.

Conclusions: We demonstrate for the first time that BMSC-derived exosomes offer significant therapeutic benefit to the protection of RGC, an effect mediated at least partially by their miRNA.

Commercial Relationships: Ben Mead, None; Stanislav I. Tomarev, None
Support: This work was supported by the Intramural Research Programs of the National Eye Institute.

Program Number: 2954
Presentation Time: 11:30 AM–11:45 AM
QTA020V, a novel rAAV2 vector, delays retinal ganglion cell loss following optic nerve crush in the mouse

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Purpose: AAV-mediated BDNF expression has previously been shown to protect RGC in animal models of glaucoma, but long-term efficacy is compromised by TrkB receptor down-regulation. We designed a novel rAAV2 vector, QTA020V, capable of secreting mature BDNF (mBDNF) and enhancing TrkB receptor expression in RGCs. The efficacy of QTA020V in delaying RGC loss was assessed

**Program Number: 2953**
**Presentation Time: 11:15 AM–11:30 AM**
BMSC-derived exosomes promote retinal ganglion cell survival

Ben Mead, Stanislav I. Tomarev, National Eye Institute, National Institutes of Health, Bethesda, MD.
in a mouse model of optic nerve crush (ONC). Transgene expression and effects on visual function were also examined.

Methods: Adult mice were injected intravitreally with QTA020V (TrkB-viral-2A-mBDNF, CAG promoter), QTA024V (TrkB-viral-2A-mBDNF, human synapsin 1 promoter), QTA001V (proBDNF only, CAG promoter) or control (eGFP only, CAG promoter) (1-2μL, 1x10^10 viral particles per eye). Unilateral ONC was performed 3 weeks later. Transgene expression and intraocular pressure (IOP) were assessed at multiple time points. Electrotetrognetography (ERG) was used to measure the positive scotopic threshold response (pSTR), A-and B-wave in dark adapted mice. Surviving Bm3A-labelled RGCs were counted in retinal flat-mounts 7 days post-injury.

Results: Seven days after ONC, QTA020V transjected retinas, expressing mBDNF and TrkB, had 67% more surviving RGC than control eGFP injected eyes (1453±60 vs 868±34 cells/mm²; P<0.0001, n=7). RGC survival with QTA020V was greater than with a vector expressing BDNF alone (1135±53 cells/mm²; P<0.05 vs controls). Replacing the CAG promoter of QTA020V with the neuron-specific, but weaker, human synapsin 1 promoter reduced efficacy (surviving RGCs = 1223±89 cells/mm²; P<0.01 versus controls, n=9). Serial ERG recording did not show any significant changes between QTA020V-treated and either saline injected or untreated eyes. The pSTR component of the ERG, attributed to RGC, was not different in QTA020V-treated and naive eyes (19.67±4.59 vs 17.81±2.69 vs 23.03±2.66μV; P=0.56, n=8). IOP was similar in all groups (QTA020V: 19.32±1.04mmHg; vehicle: 19.45±0.58mmHg; naïve: 19.98±0.98 mmHg; n=5).

Conclusions: A novel, dual vector construct expressing TrkB and mBDNF under the regulation of the CAG promoter (QTA020V) had significantly greater efficacy in delaying RGC death than control and reference vectors expressing eGFP or proBDNF. Studies are ongoing to assess long term retinal transgene expression by QTA020V and its efficacy in reducing RGC death in experimental glaucoma.

Commercial Relationships: Keith R. Martin, QUETHERA Ltd (I), QUETHERA Ltd (C), QUETHERA Ltd (F), QUETHERA Ltd (P); Andrew Osborne, QUETHERA Ltd (F); Tasneem Khatib, None; Amanda Barber, None; George Kong, None; Peter S. Widdowson, QUETHERA Ltd (I), QUETHERA Ltd (P), QUETHERA Ltd (E)

Support: QUETHERA Ltd

Program Number: 2955
Presentation Time: 11:45 AM–12:00 PM
Regeneration of retinal ganglion cell dendrites and synapses after axonal injury: the role of insulin on regrowth and reconnection

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Purpose: Evidence indicates that axonal injury triggers early alterations in retinal ganglion cell (RGC) dendrites leading to substantial deficits. In this study, we asked whether dendrites can be stimulated to regenerate and reconnect with pre-synaptic targets once they have retracted. Specifically, we investigated the role of insulin, a potent activator of the mammalian target of rapamycin (mTOR), on dendritic and synaptic regeneration.

Methods: Optic nerve axotomy was performed in mice expressing yellow fluorescent protein in RGCs. Insulin was administered daily starting at three days after injury, when dendrites have substantially retracted. To investigate the mechanisms of insulin action, the following compounds were used: i) rapamycin, an inhibitor of mTOR complex 1 (TORC1), ii) siRNA against rictor, an essential component of mTOR complex 2 (TORC2). Gold particles coated with CMV:tdTomato and CMV:PSD95-YFP, which has been previously used as a synaptic marker, were biolistically delivered to visualize RGC glutamatergic postsynaptic sites. Seven days post-lesion, RGC dendritic trees and synapses were 3D-reconstructed using Imaris (Bitplane) and analyzed. RGC survival was assessed by quantification of RBPMS-labeled cells.

Results: Our data show that insulin promotes remarkable dendrite regeneration, restoring dendritic length, field area, and complexity to values found in naïve retinas (N=5/group, 40-50 RGCs/group). Importantly, insulin induced robust regeneration of excitatory synapses in OFF-‐transient, OFF- and aON-‐sustained RGCs. Inhibition of only TORC1 resulted in loss of dendritic tree complexity, while length and field area were preserved. In contrast, blockade of TORC2 resulted in reduced dendritic length and field area but did not alter complexity. Insulin also stimulated RGC survival which was dependent on both TORC1 and TORC2 activity.

Conclusions: Our data support several important conclusions: 1) insulin promotes substantial RGC dendrites and likely synapse regeneration after axonal injury, 2) both mTOR complexes are required for successful dendritic regeneration, with TORC1 controlling tree complexity and TORC2 governing dendrite length and arbor area, 3) insulin stimulates robust RGC survival through TORC1 and TORC2 activation. Strategies to regenerate dendrites and synaptic connections in injured RGCs may have implications to restore vision in glaucoma.

Commercial Relationships: Jessica Agostinone, None; Wan-Qing Yu, None; Rachel O. Wong, None; Adriana Di Polo, None

Support: CIHR
to saline-injected controls. These pressure-induced decreases were not observed in ccl5-/− mice, compared to saline-injected controls (p<0.05). The number of degenerating axon profiles increased by nearly 10-fold in optic nerve from WT mice versus only 3-fold in ccl5-/− mice (p<0.05). Anterograde transport of CTB to the SC decreased by almost 40% in WT mice (p<0.01), but not in ccl5-/− mice. Retina from microbead-injected WT mice exhibited reorganization of beta-tubulin+ processes in the inner plexiform layer. This reorganization was significantly less noticeable in ccl5-/− mice than WT. IOP-dependent pathology noted in WT mice was accompanied by decreases in amplitudes of b-wave and oscillatory potentials, particularly for the combined cone and rod response (p<0.05). This decrease was not observed in ccl5-/− mice.

Conclusions: Our data suggests that Ccl5 deficiency improves structural and functional outcomes for RGCs in a mouse model of inducible glaucoma.

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Program Number: 2957
Presentation Time: 12:15 PM–12:30 PM
Inhibiting complement C3 activation by gene therapy reduces glaucoma progression
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Purpose: Complement activation is associated with glaucoma, and precedes neurodegeneration in animal models. Knockout of the classic pathway initiator C1q delays disease in DBA/2J (D2) mice. All three complement activation pathways converge at C3 cleavage. Here, we test the therapeutic effect of limiting C3 activation during glaucoma progression in D2 mice using ocular gene therapy.

Methods: We utilized CR2-Crry, which is the soluble rodent-specific complement inhibitor (sCrry) linked to a complement receptor 2 (CR2) targeting moiety that directs sCrry to sites of C3b-fragment (iC3b/C3dg/C3d) deposition. CR2-sCrry was packaged using quadruple YF mutant capsid AA V2 vector with CBA promoter. AAV2-CR2-Crry or control AAV2-GFP was delivered by bilateral intravitreal injection in 7-month old (mo) D2 female mice. At 10 and 12mo, RGC and axonal density were quantified in confocal images of retinal wholemounts immunostained for RGC and axonal markers (Brn3b, pNF), and optic nerve damage was scored as mild, moderate or severe by proportion of degenerative/lost axons (< 10%, 10-50% or > 50%, respectively). Naive 5-7mo D2 retinal wholemounts were immunostained for C3d, Brn3 and pNF. We conducted one-way ANOVA for Brn3b/pNF counts by group/age, followed by Student’s t-test.

Results: Preceding therapy, 5-7mo D2 showed C3d-stained RGC somata, dendrites and axons. At 10 and 12mo, AAV-CR2-Crry-treated D2 mice maintained 38% more healthy optic nerves, and had 37% and 52% less severe nerves at each age, compared with naive D2 (FIG. 1). AAV-GFP had a minimal effect, with 8% more healthy and 17% less severe nerves relative to 10mo naive D2. AAV-CR2-Crry-treated retinas showed uniformly intact and bundled intraretinal axons at 750-μm eccentricity, with 3.3 axons/fascicle at 10mo (p=0.014) and 2.6 at 12mo (p=0.001), compared to variably dystrophic and depleted fascicles in age-matched naive mice (2.6 and 1.5 axons), and in 10mo AAV-GFP controls (2.4 axons). AAV2-CR2-Crry suppressed RGCs loss, showing 84% higher Brn3b-nuclei density at 10mo (p=0.001) and 168% at 12mo (p=0.001), whereas 10mo AAV-GFP-treated eyes only increased RGC density by 27% (FIG. 2).

Conclusions: Viral gene therapy targeted to block C3 activation provides a high-precision and potent neuroprotective strategy to reduce the advance of chronic glaucoma. Ongoing studies test this in acute models, and define molecular changes in retina, nerve and their innate immune cells.
Metabolic stress in glaucoma engages early activation of the energy biosensor AMPK leading to neuronal dysfunction
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Department of Neuroscience, University of Montreal Hospital Research Center, Montreal, QC, Canada.

**Purpose:** Metabolic stress has been proposed to contribute to neuronal damage in glaucoma, but the mechanism driving this response is not understood. The adenosine monophosphate-activated protein kinase (AMPK) is a master regulator of energy homeostasis that becomes active at the onset of energy stress. AMPK is a potent inhibitor of the mammalian target of rapamycin complex 1 (mTORC1), which we showed is essential for the maintenance of retinal ganglion cell (RGC) dendrites, synapses, and survival. Here, we tested the hypothesis that AMPK is an early mediator of metabolic stress in glaucoma.

**Methods:** Unilateral elevation of intraocular pressure was induced by injection of magnetic microbeads into the anterior chamber of mice expressing yellow fluorescent protein in RGCs. Inhibition of AMPK was achieved by administration of siRNA or compound C. RGC dendritic trees were 3D-reconstructed and analyzed with Imaris (Bitplane), and survival was assessed by counting Brn3a or RBPMS-labeled soma and axons in the optic nerve. RGC function was examined by quantification of anterograde axonal transport after intraocular administration of cholera toxin β-subunit. Retinas from glaucoma patients were analyzed for expression of active AMPK.

**Results:** Ocular hypertension triggered rapid upregulation of AMPK activity in RGCs concomitant with loss of mTORC1 function. AMPK inhibition with compound C or siRNA effectively restored mTORC1 activity and promoted an increase in total dendritic length, surface and complexity relative to control retinas. Attenuation of AMPK activity led to robust RGC soma and axon survival. For example, 95% of RGCs (2983 ± 258 RGCs/mm², mean ± S.E.M.) survived with compound C compared to 77% in vehicle-treated eyes (2430 ± 233 RGCs/ mm²) (ANOVA, p<0.001) at three weeks after glaucoma induction (n=8-10/group). Importantly, blockade of AMPK activity effectively restored anterograde axonal transport. Lastly, RGC-specific upregulation of AMPK activity was detected in human glaucomatous retinas relative to age-matched controls (n=10/group).

**Conclusions:** Metabolic stress in glaucoma involves AMPK activation and mTORC1 inhibition promoting early RGC dendritic pathology, dysfunction and neurodegeneration.

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