Glucoma is characterized by progressive dysfunction and loss of retinal ganglion cells (RGCs). RGCs are on a metabolic knife-edge during times of stress that may be exacerbated by aging and genetic impairment. During these periods the viability of RGCs is reliant on mitochondrial regulation to maintain cellular homeostasis and bienergetic status. Emerging research suggests that a spectrum of mitochondrial DNA mutations in glaucoma patients 1. These abnormalities are also present in leukocytes, suggesting a systemic susceptibility to metabolic defects (as opposed to mitochondrial changes in the eye as a consequence of high intracellular pressure). Such systemic susceptibility is expected to increase glaucoma susceptibility with age. However, the role of mitochondria and metabolic health in glaucoma is yet to be fully elucidated.

We have previously discovered mitochondrial dysfunction and mitochondrial abnormalities occurring prior to neuronal dysfunction in glaucoma (in glaucoma patients and animal models) 1,2. These studies identified that NAD (nicotinamide adenine dinucleotide), an essential co-factor for many enzymes in the trinuclear complex, was depleted via administration of nicotinamide (NAM; the amide of vitamin B3, an essential stress, driving glaucomatous neurodegeneration. Preventing NAD production) robustly protects from age-related neuronal metabolic decline and pathogenically low NAD leads to glaucoma susceptibility, glaucoma patients have abnormalities are also present in leukocytes, suggesting a systemic susceptibility to metabolic defects (as opposed to mitochondrial changes in the eye as a consequence of high intracellular pressure). Such systemic susceptibility is expected to increase glaucoma susceptibility with age. However, the role of mitochondria and metabolic health in glaucoma is yet to be fully elucidated.

Methods

Mice: MitoY (TgEno3-YpcDnt/Tbub, founder line 1919), was generated as one of a number of uncouplers of oxidative phosphorylation. These mice carry YFP fused in-frame with the mitochondrial targeting sequence of COXVI under the control of a mEF1α (neuron specific). We rederived new founders from cryopreservation and backcrossed onto a clean C57BL/6J background. Axotomy model: Adult, male, C57BL/6J mice were euthanized, eyes enucleated, and retinas prepared as tissue culture in retinal explant media 1. In this system, RGCs degenerate over 0-7 days; with 3 days ex vivo (DEV) representing ~50% RGC loss from control. Rats: Ocular hypertension was induced in adult, male Brown Norway rats by intracranial injection of paramagnetic beads bilaterally and tramcul meshwork and Schlemm’s canal. This resulted in significant and robust IOP elevation. IOP was recorded by hand-held rebound tonometry. 14 days postinjection was used as an endpoint for this study.

Microscopy: For mitochondrial image analysis in the MitoY mouse, retinas were flatmounted and imaged following 1 week of oralNAM administration (500 mg/kg/d). For mitochondrial image analysis in the MitoY mouse, retinas were flatmounted and imaged following 1 week of oralNAM administration (500 mg/kg/d). For mitochondrial image analysis in the MitoY mouse, retinas were flatmounted and imaged following 1 week of oralNAM administration (500 mg/kg/d). For mitochondrial image analysis in the MitoY mouse, retinas were flatmounted and imaged following 1 week of oralNAM administration (500 mg/kg/d).

Figure 1: Establishing MitoY imaging protocols. Left: Retinas from naive mice were flattened, fixed, and counterstained with DAPI. Retinas were imaged with an 0.4x optical zoom on a Zeiss LSM800 Airyscan confocal microscope. Right: An inset around a selected YFP channel is shown, and volume reconstructed in Imaris. This allows the detailed analysis of volumes metrics (volume, surface area, ellipticity).

Figure 2: MitoY in retinal ganglion cell specific. MitoY+ retinas were stained with antibodies against RBPM3 (RGCs) and P0 (amacrine cells). In the inner retina, MitoY+ is specific to RGCs. There are MitoY+ rods and rod bipolar cells in the outer retina (data not shown).

Figure 3: Chronic nicotinamide administration changes RGC mitochondrial metabolism. MitoY+ retinas were flattened and imaged following 1 week of oral NAM administration (300 mg/kg). From this following NAM administration metabolism, mitochondrial volume increases (represented by the rightward shift in the cumulative frequency plot), and mitochondrial become larger and more oblate (see schematic shape below plot). Rainclouds plot represent >15,000 reconstructed mitochondria per group.

Figure 4: Nicotinamide is robustly protective against axotomy induced insult. (A) Schematic of axotomy explant model. (B) Example of retinas at control (day 0), 3 days post-axotomy, or 3 days post-axotomy + NAM treatment. (C) Dopplers showing RGC density (left) and at ganglion cell layer (right).

Figure 5: Mitochondrial morphology changes follow axotomy. Astylosis results in the rapid degeneration of RGCs. Within 12 hours of axotomy (DEV 0.5) mitochondrial morphology changes and this is halted by NAM administration to the media. Rainclouds plot represent >15,000 reconstructed mitochondria per group.

Figure 6: Retinal and optic nerve NAD is depleted following ocular hypertensive insults. Ocular hypertension was induced in rats (see Figure 7), and 14 days post-ocul hypertensive shocks retinas and optic nerves were processed for NAD assays. Total NAD (NAD+); NAD+/NADH was reduced in both retina and optic nerve.

Figure 7: Nicotinamide protects from ocular hypertensive glaucoma. (A) Brown Norway rats underwent intracameral injections of paramagnetic beads bilaterally and were pulled into the drainage structures of the eye via a rare earth magnet (5 mm tips). Histology demonstrate that beads reached within the drainage structures of the trabecular meshwork and Schlemm’s canal. This resulted in significant and robust IOP increase that was sustained until euthanasia (B). 14 days of NAM results in significant RGC atrophy which is robustly protected by nicotinamide treatment. AUC = area under the curve. NT = normotensive, OHT = ocular hypertensive. Blue line in B represents 2 standard deviations above the NT IOPs.

Figure 8: Inner plexiform layer (IPL) mitochondrial content declines following ocular hypertensive insults. Retinas were sectioned and labeled with antibodies against RBPM3 (RGCs) and P0 (amacrine cells). In the inner retina, MitoY+ is specific to RGCs. There are MitoY+ rods and rod bipolar cells in the outer retina (data not shown).

Conclusions

• Retinal ganglion cells are particularly vulnerable to metabolic and physical stressors.

• Metabolomic and mitochondrial health decline with age and is exacerbated by periods of elevated intracranial pressure.

• Retina and optic nerve NAD+ declines following ocular hypertensive stimuli.

• Increasing NAD+ by nicotinamide treatment prevents retinal ganglion cell degeneration following two glaucoma-related insults; axotomy and ocular hypertensive stimuli.

• Nicotinamide alters mitochondrial size and morphology in normal and stressed retinal ganglion cells.

• Targeting NAD+ decline via nicotinamide has amazing potential as a cost effective, potent neuroprotective for glaucoma with limited side effects.

• These treatments may be even more efficacious in combination with intracranial pressure lowering strategies.

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Additional references


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