Targeting neuronal mitochondria for neuroprotection in glaucoma

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ISER 2018 Metabolic dysfunction / bioenergetics in glaucoma
Neurodegenerative hypothesis

Normal aging
- Decreasing metabolic function
- Increasing mitochondrial stress
- Increasing likelihood of mitochondrial and cellular failure

Outcome: Age-dependent decline in neuronal health

Neurodegeneration
- Decreasing metabolic function
- Increasing mitochondrial stress
- Increasing vulnerability to disease-related stresses

Outcome: Increased instance of cell death and neuronal dysfunction – neurodegenerative disease

Conspiring factors:
- e.g. Genetics
- Other diseases
- Environment

Are neurodegenerative diseases an age- and metabolism-related phenomena?
Can we target these pathways to develop broad neuroprotective strategies?
Glaucoma:
A common neurodegeneration affecting ~80 million patients for which there are no neuroprotective strategies
Aim:
Identify age- and IOP-dependent molecular changes within RGCs that precede glaucomatous neurodegeneration

Goal:
Develop clinically translatable neuroprotective strategies for glaucoma
Work undertaken at The Jackson Laboratory, Simon John lab using the DBA/2J mouse model of glaucoma
DBA/2J glaucoma

- Gpnmb<sup>R150X</sup>
- Tyrp1<sup>b</sup>
- Iris disease
- Increased IOP
- RGC and optic nerve degeneration
Defining cell specific molecular processes in glaucoma

- RNA-sequencing of RGCs from DBA/2J mice (D2), plus age and sex matched controls (D2-Gpnmb+)
- Computational analysis and pathway analysis
- Identify target pathways and molecules
- Test target molecules in DBA/2J glaucoma

**Aim:** identify age- and IOP- dependent molecular changes within RGCs that precede glaucomatous neurodegeneration
Unsupervised HC of RCGs

- 82 samples total (~25000 RGCs/sample)
- 4 mo (young controls)
- 9 mo (high IOP but lacking neurodegeneration)
- Samples all came from mice with no neurodegeneration (indistinguishable from D2-Gpnmb+ controls)
- Hierarchical clustering (HC) allowed molecular definition of early glaucoma stages

Williams et al, Science, 2017
Dysregulation of mitochondrial transcripts

- Mitochondrial : nuclear read total ratio
- Oxidative phosphorylation

Williams et al, Science, 2017
Early mitochondrial perturbations

- RNA-seq identifies early mitochondrial perturbations
- This is especially evident in genes encoding OXPHOS proteins and NAD+ synthesis enzymes
- EM analysis identifies mitochondrial abnormalities in RGC dendrites and synapses that precede detectable measures of glaucoma (*i.e.* NFL thinning, axon loss, optic nerve cupping)

*Williams et al, Science, 2017*
Age- and IOP-dependent declines in NAD synthesis and NAD synthesis transcripts

**A**
- Preiss-Handler pathway
- De novo
- Tryptophan (Trp)
- NAMN → NAAD
- NAD/S

**Salvage**
- NAM → NAMPT
- NMNAT1

**Retina**
- pmol NAD(t) / mg protein

**B**
- Age-dependent changes
- 4 month vs. 9 month D2-Gpnmb
- NAD depletion is age-related (occurs in D2 and D2-Gpnmb+ controls)
- Corresponds with decreases in glutathione levels, increased DNA damage, and PARP activation in D2 glaucoma. All NAD depleting processes.

**C**
- IOP-dependent changes
- D2 vs. D2-Gpnmb+ (9 month)

Hypothesis: declining NAD is a critical insult that renders aging RGCs susceptible to insults from high IOP

- Williams et al, Communicative & Integrative Biology, 2017
- Williams et al, Science, 2017
Can repleting retinal NAD levels prevent glaucoma?

Nicotinamide (amide of vitamin B₃)
Repleting NAD using NAM *in vivo*

- Nicotinamide (NAM, the amide of vitamin B₃)
- Early start (6 mo, pre-IOP elevation, prophylactic)
- Late start (9 mo, high IOP in majority of eyes, interventional)

**Figure:**

- Graph showing changes in Retina pmol NAD(t) / mg protein over time with NAM treatment.

**References:**

Williams et al, Science, 2017
NAM supplementation prevents optic nerve degeneration in glaucoma

- Nicotinamide (NAM) protects from human-relevant glaucomatous damage; RGC loss, ON degeneration, ON cupping
- Protects as intervention and prophylactically
  - 550 mg/kg/d NAM in water
  - 2000 mg/kg/d NAM in food and water
  - ~2 - 10 g/d for 60 kg human

Williams et al, Science, 2017
Williams et al, Communicative & Integrative Biology, 2017
NAM prevents formation of abnormal mitochondria

- Abnormal mitochondria accumulate in RGC dendrites and soma following periods of elevated IOP
- NAM prevents these changes and likely promotes healthy mitochondrial function, mitochondrial motility, and mitophagy

Williams et al, Science, 2017
NAM prevents glaucomatous changes at a transcriptomic level

- RNA-seq provides a very sensitive measure of early molecular changes
- Nicotinamide prevents IOP-dependent transcriptomic changes
- Nicotinamide also potently prevents many age-related transcriptomic changes (98%)

NAM prevents even the most sensitive transcriptomic changes

Williams et al, Science, 2017
PERG decreases in an IOP-dependent manner and is recovered by NAM treatment.

Timing corresponds with early transcriptomic changes, mitochondrial abnormalities, and synapse and dendritic loss.

Williams et al. Science, 2017
Further testing of declining NAD+ hypothesis
WLDS raises NAD in DBA/2J mice

Williams et al, Frontiers in Neuroscience, 2017
WLD$^S$ and nicotinamide in combination robustly protect from glaucoma

Williams et al, Frontiers in Neuroscience, 2017
Targeting dendrites and synapses with nicotinamide and WLDs

Williams et al, Frontiers in Neuroscience, 2017
Harder et al, PNAS, 2017
Can we establish a long-term, retinal ganglion cell specific, NAD-repleting therapy?
Retinal gene therapy

- Gene therapy has been proven successful in a handful of rare, Mendelian eye diseases

- Gene therapy has the potential to target specific cell populations and deliver a specific gene candidate

- Gene therapy may be life-long (or at least long-term)
Gene therapy in glaucoma

- 5.5 months of age
- AAV2.2 murine Nmnat1 under a CMV promoter, ~1.5μl (3.1x10^{10} gc/ml)
- >80% of RGCs still transduced at 12 months (>95% within first month)
- Included a NAM^{lo} (550 mg/kg/d) group

Williams et al, Science, 2017
**Nmnat1** gene therapy in glaucoma

First successful gene therapy in complex age-related disease

NAD Modulates Vulnerability to Glaucoma

Glaucoma Susceptible (without treatment)
- Early mitochondrial changes
- Early gene expression changes
- Early DNA damage
- HIF-1α and PARP activation
- Synapse loss
- Dendritic atrophy
- Soma loss
- Axon degeneration
- Loss of axoplasmic transport
- Terminal connections lost

Glaucoma Resistant (with treatment)
- Prevents mitochondrial abnormalities
- Prevents gene expression changes
- Prevents DNA damage
- Prevents HIF-1α and PARP activation
- Prevents synapse loss
- Prevents dendritic atrophy
- Prevents soma loss
- Prevents axon degeneration
- Preserves axoplasmic transport
- Preserves terminal connections

Williams et al, Journal of Glaucoma, 2017
Further activation of Nmnat1 and Nmnat2

- Identifying new neuro-protective molecules following severe optic nerve insult (axotomy explant culture)
- Rapid, reproducible loss of retinal ganglion cells that allows facile and quick testing of candidate drugs and genes

Dr James Tribble
Further activation of Nmnat1 and Nmnat2

- Decrease in *Nampt, Nmnat2* transcript following axotomy
- Mirrors changes we see in DBA/2J glaucoma

*P* = 0.0017  
*P* = 0.042

Tribble & Williams, *unpublished*
Further activation of Nmnat1 and Nmnat2

- EGCG (a major, ~30%, component of green tea polyphenols) (known activator of Nmnat1, Nmnat2; NAD+ producing enzymes)

Subcellular Compartmentation and Differential Catalytic Properties of the Three Human Nicotinamide Mononucleotide Adenylyltransferase Isoforms

TABLE 1: Influence of various nucleotides and known poly-ADP-ribose polymerase and poly-ADP-ribose glycohydrolase inhibitors on the activity of the Nmnat1 and Nmnat2 isoforms.

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Tribble & Williams, unpublished
Further activation of Nmnat1 and Nmnat2

• EGCG (a major, ~30%, component of green tea polyphenols) (known activator of Nmnat1, Nmnat2; NAD+ producing enzymes)

• EGCG and whole green tea polyphenols robustly prevent RGC loss following axotomy in the μM range

Tribble & Williams, unpublished
Hypothesis:
Metabolic decline is a more general process for RGC vulnerability and degeneration in glaucoma
Decreasing retinal pyruvate in glaucoma

- Enrichment of genes / pathways – pyruvate metabolism, gluconeogenesis
- NAM corrects most of these changes except 2 genes (Mpc1, Gpnmb)
- MPCs are well expressed in rodent, monkey, human RGCs
- Oral pyruvate leads to increased retinal pyruvate levels

Williams et al, unpublished in collaboration with Casson et al
Repleting pyruvate prevents glaucoma

DBA/2J
- 500 mg/kg\([\text{bw}]\)/d
- + NAM 550 mg/kg\([\text{bw}]\)/d group

Axotomy explant culture

Rat OHT

Williams et al, unpublished in collaboration with Casson et al
Preventing formation of abnormal mitochondria

- Thy1.2 CFP (blue RGCs)
- MitoY (Eno2 promoter, Cox8a gene-targeting sequence fused to N terminus) (yellow mitochondria)
- B6J, D2, D2-Gpnmb<sup>+</sup>, and D2.Wld<sup>S</sup> backgrounds, plus treatment conditions (NAM, pyruvate (PYR))

Williams et al, unpublished
Glaucoma as a metabolic optic neuropathy

- RGCs are particularly vulnerable to metabolic and physical stressors.
- Metabolism and mitochondrial health decline with age and are exacerbated by periods of elevated IOP.
- NAD decline makes RGCs susceptible to elevated IOP.
- Restoring NAD (nicotinamide, Nmnat1 gene therapy, or WLDs) improves mitochondrial health and protects RGCs.
- Targeting Nmnats prevents RGC degeneration (EGCG, GTP, NAM) following axotomy.
- Further targeting metabolic decline (pyruvate) prevents RGC degeneration in multiple models of glaucoma.
- Targeting neuronal metabolic decline and mitochondria may offer safe, neuroprotective treatments for glaucoma and other age-related neurodegeneration and ophthalmic degenerations.
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