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Targeting Wallerian degeneration in glaucoma

Melissa Jöe, Pete A. Williams*

Neurodegenerative diseases account for a large and increasing health and economic burden worldwide. With an increasingly aged population, this burden is set to increase. Optic neuropathies make up a large proportion of neurodegenerative diseases with glaucoma being highly prevalent. Glaucoma is characterized by the progressive dysfunction and loss of retinal ganglion cells and their axons which make up the optic nerve. It is the leading cause of irreversible vision loss and affects an estimated 80 million people. The mammalian central nervous system is non-regenerative and, once lost or injured, retinal ganglion cells cannot regenerate an axon into the optic nerve under basal conditions. Thus, strategies that provide neuroprotection to stressed, dysfunctional, or dying retinal ganglion cells are likely to be of high therapeutic and translational value. Advancing age, genetics, and elevated intraocular pressure are all major risk factors for glaucoma, however, all clinically available glaucoma treatments focus on intraocular pressure management and do not directly address the neurodegenerative component of glaucoma.

Over the last decade, there has been a growing interest in targeting the pathways of programmed axon degeneration and nicotinamide adenine dinucleotide (NAD) metabolism for neuroprotection. NAD is an essential metabolite, functioning as a cofactor and a coenzyme in various vital cellular processes, such as energy metabolism, DNA repair, and cell signaling. Alterations in NAD levels and NAD-producing enzymes have been demonstrated in neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and glaucoma. A recent study by Petriti et al. (2024) demonstrated that mitochondrial bioenergetics are associated with glaucoma pathogenesis and that the lower mitochondrial respiratory activity was correlated with worse visual field progression and lower NAD levels in peripheral blood mononuclear cells. Normal tension glaucoma patients had the weakest mitochondria respiratory activity, compared to high tension glaucoma and controls indicating a clear metabolic component to glaucoma extraneous to intraocular pressure (Petriti et al., 2024). This could help explain why, despite intraocular pressure lowering treatments, patients continue to lose vision.

The optic nerve in glaucoma undergoes a specific type of axon degeneration: Wallerian degeneration, a feature that is shared with many other neurodegenerative diseases. It is characterized by the degeneration of axons distal to injury. In glaucoma, the site of injury of the retinal ganglion cells is at the lamina cribrosa, leading to both Wallerian and retrograde degeneration. The process is named after Augustus Waller after his seminal nerve transection experiments and it involves disintegration of the cytoskeleton, mitochondrial swelling and degeneration, and axon fragmentation.

The understanding of Wallerian degeneration was rapidly advanced by discovering the Wallerian degeneration slow (*Wld*^{S}) mutant mouse whose axons survive ten-fold longer than normal when transected. The mouse expresses the Wld^S protein, a fusion of nicotinamide mononucleotide adenylyl transferase 1 (Nmnat1; an NAD synthesizing enzyme), and the terminal sequence of ubiquitination factor E4B (Ube4b) (Coleman and Höke, 2020). The protective effects of Wld^S are understood to mimic the function of NMNAT2, the axonal isozyme of the NMNATs. Unlike the native NMNAT1 protein, which localizes at the nucleus, Wld^S has a cytosolic localization. The axon-protective effects of Wld^S are postulated to be due to maintaining NAD levels within the axons. A central paradigm of Wallerian degeneration is the loss of NMNAT2 activity that depletes the neuronal NAD pool resulting in sterile-α and TIR motif-containing protein 1 (SARM1) activation and axon destruction (**Figure 1**).

Identifying the *Wld*^{S} allele has provided significant insights into the neurodegenerative processes involved in various neurodegenerative diseases, including glaucoma. The *Wld*^{S} allele is strongly protective against retinal ganglion cell degeneration (axon, soma, and dendrites) when assessed in the DBA/2J mouse (a mouse whose glaucoma closely resembles age-related pigmentary dispersion glaucoma) (Howell et al., 2007; Harder et al., 2017). Further studies investigated Wld^S in an induced ocular hypertensive glaucoma model and an optic nerve crush model in rats. These studies demonstrated that Wld^S effectively protects axons; however, it did not confer protection to the retinal ganglion cell soma or dendrites (Beirowski et al., 2008). Collectively, these findings support that axon degeneration in glaucoma follows a Wallerian degenerative mechanism and further supports that NAD metabolism is a promising mechanism to target for neuroprotection.

NAD is synthesized through three different pathways in mammals: *de novo* (from tryptophan), the Preiss-Handler pathway (from nicotinic acid), and the NAD salvage pathway (from nicotinamide with an alternate entry from nicotinamide riboside). The NAD salvage pathway is the most important NAD synthesis pathway in most of the tissues. We have demonstrated that the neuronal retina is dependent on the NAD salvage pathway to maintain its NAD levels (Tribble et al., 2023).

Our recent work has demonstrated that increasing NAD via nicotinamide administration in *in vivo* models of ocular neurodegeneration is strongly neuroprotective. This was first evidenced in DBA/2J mice where nicotinamide provides a robust neuroprotection at clinically relevant doses, both as an interventional treatment and as a prophylactic treatment (Williams et al., 2017b). When combined with the *Wld*^{S} allele, retinal NAD levels increase (above either treatment alone) and there is an increased level of neuroprotection (Williams et al., 2017a). Further supporting this,

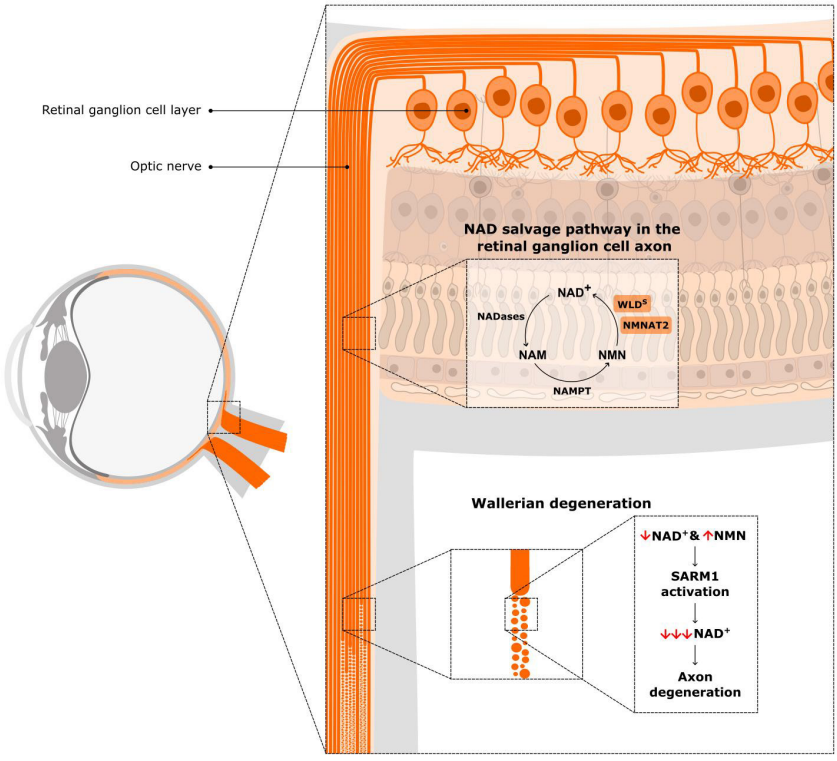

Figure 1 | **Overview of Wallerian degeneration in the optic nerve.**
The salvage pathway is the preferred and most utilized pathway for NAD synthesis in neurons. NAD is broken down into nicotinamide (NAM) by NADases. NAM is then synthesized into nicotinamide mononucleotide (NMN) by nicotinamide phosphoribosyl transferase (NAMPT) and subsequently converted back to NAD by nicotinamide mononucleotide adenylyl transferase (NMNAT), specifically NMNAT2 in the axon. WldS, a fusion protein of Nmnat1 and the terminal sequence of ubiquitination factor E4B (Ube4b) mimics the function of NMNAT2 as an axoplasmic NAD-synthesizing enzyme. Wallerian degeneration is characterized by the degeneration of axons distal to the site of injury. This process involves the disintegration of the cytoskeleton, mitochondrial swelling and degeneration, and axon fragmentation. It is triggered by a decline in NAD^+ and an increase in NMN, which activates SARM1, leading to a catastrophic depletion of NAD^+ in the axons. Created with Inkscape 1.3.2 (https://inkscape.org/). NAD: Nicotinamide adenine dinucleotide; NAM: nicotinamide; NAMPT: nicotinamide phosphoribosyl transferase; NMN: nicotinamide mononucleotide; NMNAT: nicotinamide mononucleotide adenylyl transferase; SARM1: sterile-α and TIR motif-containing protein 1; WLD^S: Wallerian degeneration slow.

nicotinamide alone protects soma, axons, and dendrites as well as providing a strong metabolic neuroprotection in an ocular hypertensive rat model and rotenone-induced retinal ganglion cell degenerative mouse model (Tribble et al., 2021; Cimaglia et al., 2024; Otmani et al., 2024). These results have translated well into the clinic, where it has been demonstrated that nicotinamide supplementation in existing glaucoma patients improves visual function (Hui et al., 2020). A Phase III clinical trial is underway. Recent studies have further demonstrated a neuroprotective role for pyruvate in glaucoma in combination with nicotinamide (Harder et al., 2020) which was also successfully translated into a Phase II clinical trial (De Moraes et al., 2022). In glaucoma patients, the site of damage is typically the lamina cribrosa - axon degeneration from the lamina cribrosa to the brain is Wallerian degeneration whereas axon degeneration from the lamina cribrosa back to the soma is retrograde (Kanamori et al., 2012). In experimental animal models, targeting NAD provides axon protection to both intra-retinal axons and axon segments post-lamina cribrosa, supporting a hypothesis that NAD can protect against Wallerian degeneration and retrograde degeneration. Long-term clinical trials will determine whether nicotinamide can protect against retinal nerve fiber layer thinning (i.e., intra-retinal axons).

Targeting proteins involved in the Wallerian degeneration pathway has also been investigated in the context of glaucoma. NMNAT2 is localized in the ER-Golgi in the cyto- and axo-plasm and has been shown to be essential for neuronal health and viability, and has been identified as one of the key players of Wallerian degeneration (Coleman and Höke, 2020). We have recently demonstrated that NMNAT2 levels are lower in human glaucoma eyes (Tribble et al., 2023). In cultured neurons, acute depletion of NMNAT2 leads to spontaneous Wallerian degeneration, and mutations in NMNAT2 have shown implication in neuronal development not viable with life and in chronic neuropathies (Coleman and Höke, 2020).

NMNAT2 is a promising target for NAD elevation and neuroprotection (Tribble et al., 2024). We have confirmed that full-length NMNAT2 gene therapy robustly protects retinal ganglion cells in microbead occlusion ocular hypertensive model (Tribble et al., 2024), which was previously demonstrated with the truncated NMNAT2Δexon6 in a silicone oil-induced ocular hypertensive model (Fang et al., 2022). Additionally, we have demonstrated the green tea polyphenol epigallocatechin gallate as neuroprotective in the microbead ocular hypertensive model and isolated its primary neuroprotective effects to the NAD salvage pathway (Tribble et al., 2024). Although epigallocatechin gallate is neuroprotective in these models, it is predicted to have very low bioavailability in humans, which may explain its lack of success in clinical trials. Based on the epigallocatechin gallate structure we synthesized analogs that lead to the development of potent NAD boosters that show neuroprotection in an ex vivo tissue model (Tribble et al., 2024). As NMNAT2 is enriched in neurons (Tribble et al., 2024), targeting NMNAT2 may be advantageous for neurodegenerative diseases to limit off-target effects.

SARM1 has been identified as the main executor of Wallerian degeneration (Coleman and Höke, 2020). SARM1 is a highly conserved enzyme with NADase activity. It is allosterically activated by high levels of NMN, leading to a rapid decline of NAD^+. As NAD^+ depletes, it results in axonal metabolic compromise and subsequent axonal degeneration. NMNAT2 inhibits this process by keeping the NMN levels low and NAD^+ high. SARM1 has also been studied in glaucoma models. In an optic nerve crush mouse model it was demonstrated that SARM1 is critical for retinal ganglion cell axon degeneration and SARM1 deficiency was as protective as *Wld*^{S} in preventing axon degeneration (Fernandes et al., 2018). These findings further confirm that Wallerian degeneration is an essential pathway in axon degeneration in glaucoma.

Targeting NAD metabolism and Wallerian degeneration is a promising therapeutic strategy for neurodegenerative diseases and glaucoma, which primarily focuses on the management of risk factors and symptoms. NMNAT2 may be a perfect target due to its neuroprotective properties and its critical role in neuronal survival and health. Given the limitations of gene therapies for polygenic diseases such as neurodegenerative disorders, the development of small molecular drugs remains the most viable approach to transition from bench to bedside in the short term. Future research should focus on optimizing these small molecules to enhance their efficacy and safety, ultimately paving the way for innovative treatments that can significantly improve patient outcomes.

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Melissa Jöe, Pete A. Williams*
Department of Clinical Neuroscience, Division of Eye and Vision, St. Erik Eye Hospital, Karolinska Institutet, Stockholm, Sweden
***Correspondence to:** Pete A. Williams, PhD, pete.williams@ki.se.
https://orcid.org/0000-0001-6194-8397 (Pete A. Williams)
https://orcid.org/0000-0001-8516-4706 (Melissa Jöe)

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靶向青光眼中的沃勒变性
文章特色分析

一、文章重要性
1. 临床需求的迫切性
青光眼是全球不可逆性视力丧失的首要原因，影响约 8000 万人。目前所有临床治疗仅针对眼压管理，未能直接干预神经退行进程，因此开发神经保护策略具有重大临床意义。
2. 机制研究的深度突破
文章系统阐述了沃勒变性作为青光眼轴突变性的核心机制，并将其与 NAD 代谢紧密联系，揭示了其在神经保护中的枢纽作用，为理解青光眼病理提供了新视角。
3. 转化医学的桥梁作用
文章不仅回顾基础研究，还涵盖了从动物模型到临床试验的转化证据，特别是烟酰胺和 NMNAT2 等靶点的临床前与临床研究进展，显示出明确的治疗潜力。

二、创新性特色
1. 聚焦沃勒变性作为治疗靶点
文章创新地将青光眼中的轴突退化机制明确为沃勒变性，并系统阐述了其与 NAD 代谢、SARM1 激活、NMNAT2 丧失等分子事件的关联，突破了传统眼压中心论的研究范式。
2. 强调 NAD 代谢的核心地位
提出并验证了 NAD 补救途径在视网膜神经节细胞中的关键作用，并通过多种模型（如 DBA/2J 小鼠、高眼压大鼠）证明烟酰胺补充可有效提升 NAD 水平，实现神经保护。
3. 提出新型治疗靶点与策略
- NMNAT2 基因治疗：首次在青光眼模型中证明其强效保护作用。
- SARM1 抑制：作为执行沃勒变性的关键酶，其缺失可模拟 WldS 的保护效果。
- 小分子 NAD 增强剂开发：基于表没食子儿茶素没食子酸酯结构合成的新型化合物，显示出良好的神经保护潜力。
4. 多模型验证与临床衔接
文章整合了遗传模型（WldS 小鼠）、诱导模型（高眼压、视神经压迫）及临床研究数据，构建了从机制到治疗的完整证据链。

三、对学科的启示
1. 范式转变：从降眼压到神经保护
文章推动青光眼治疗理念从单一眼压管理转向多靶点神经保护，强调代谢干预、轴突完整性维持等在疾病调控中的重要性。
2. 跨疾病机制的通用性
沃勒变性与 NAD 代谢机制在阿尔茨海默病、帕金森病等多种神经退行性疾病中具有共性，本文的研究思路与成果可为其他疾病提供借鉴。
3. 基因治疗与小分子药物的并进策略
文章指出，尽管基因治疗（如 NMNAT2）效果显著，但在多基因疾病中，小分子药物更具临床可行性，提示未来应聚焦于可穿透血脑屏障、高生物利用度的 NAD 增强剂开发。
4. 代谢干预作为新兴治疗方向
通过调控 NAD 代谢、线粒体功能等代谢通路，实现对神经元的保护，为神经科学和眼科疾病治疗开辟了新路径。

总结
本文是一篇具有高度前瞻性与转化价值的视角文章，不仅深化了对青光眼神经退变机制的理解，更提出了具操作性的治疗策略。其创新在于将沃勒变性机制与 NAD 代谢紧密结合，并推动从基础研究向临床应用的跨越，对神经科学、眼科学及神经保护药物开发领域均有深远影响。