Development of novel drugs for glaucoma

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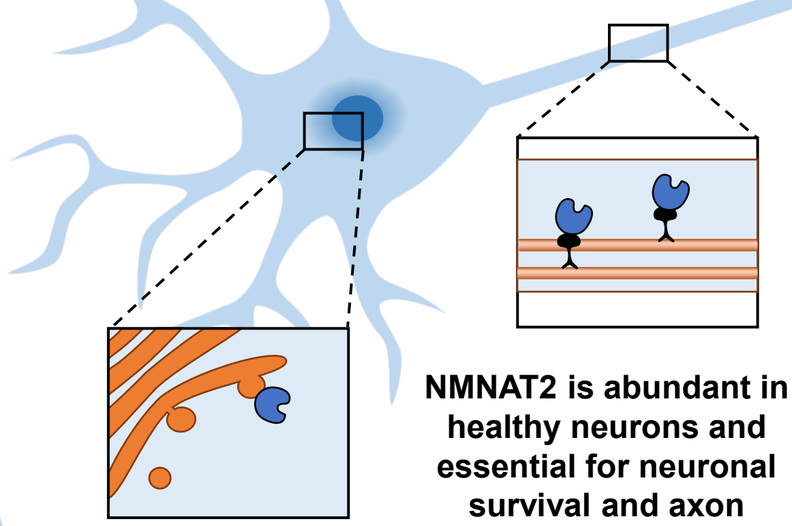
Introduction

There is a great need for treatments that target neurodegenerative processes. Glaucoma is a common neurodegenerative disease. Our group has previously discovered that the capacity to maintain nicotinamide adenine dinucleotide (NAD; an essential redox cofactor) declines in an age-dependent manner in the retina and the optic nerve[1]. Preventing NAD depletion by administering NAD precursors, such as nicotinamide (NAM; the amide form of vitamin B_3), has been demonstrated to be robustly protective against neurodegeneration in multiple animal models of glaucoma[2]. **Administering high-dose NAM to existing glaucoma patients improves visual function[3].**

NMNAT2 is a cytoplasmic axon maintenance factor, essential for NAD synthesis in neurons[4]. Our group has generated evidence demonstrating that the green tea polyphenol, epigallocatechin gallate (EGCG), is strongly neuroprotective in glaucoma models (*unpublished*). EGCG is also increases the activity of NMNAT2[5]. Based on the structure of EGCG, we have initiated a drug development process, identifying the structure-activity relationship between EGCG and NMNAT2 and we are actively developing novel compounds that show increased stability and activity.



Preventing NAD depletion is neuroprotective

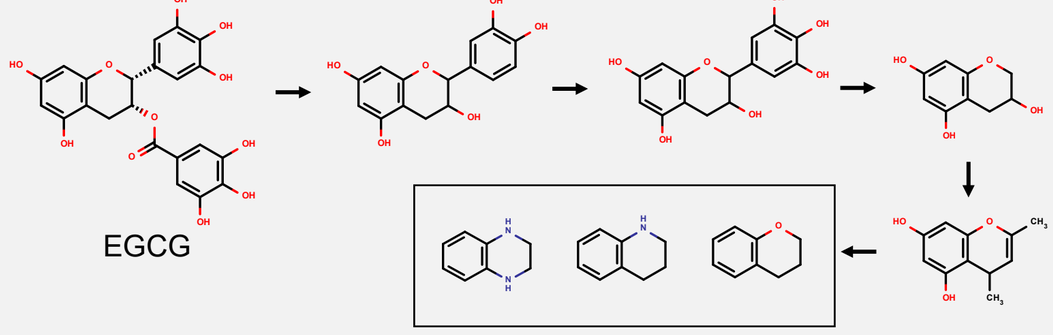
NMNAT2 is abundant in healthy neurons and essential for neuronal survival and axon maintenance

Activating NMNAT2 with modulators increase NAD levels

Methods

Structure refinement

EGCG is an unstable molecule and metabolizes rapidly in the body. The structure was refined by first removing reactive groups and studying which moieties are essential for an NAD boosting effect, and then continuing with exploring various chemical cores, identifying the most potent NAD booster(s).



Assess biological activity

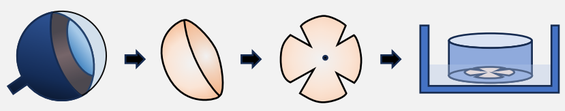
Luminometry-based NAD assays measuring NAD content in mouse primary cortical neurons after incubation with compounds for 2 hours. Compounds were measured at 5 and 50 µM.

Specificity to the salvage pathway

To test specificity to the salvage pathway, the upstream enzyme in the salvage pathway, NAMPT, was blocked with a specific inhibitor, FK866. FK866 (100 µM) was incubated with cells for 1 h and then co-incubated with the compounds for 2 hours.

Neuroprotection in retinal explant model

Assessing neuroprotection of the compounds was done in the retinal explant model. Retinas from mice were dissected and cultured with the compounds at 50 µM for three days. Retinal ganglion cells were stained and counted.

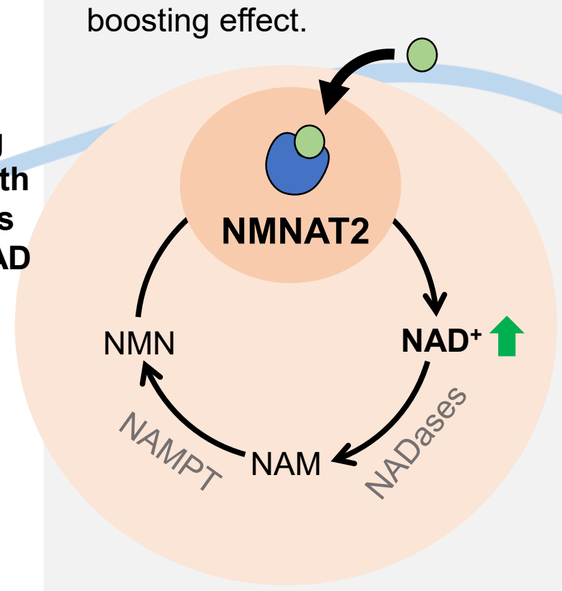


Conclusions

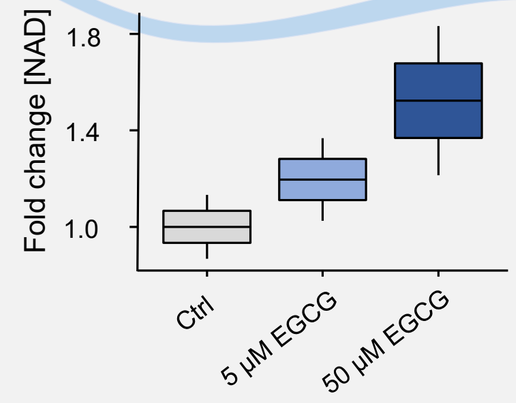
- There is a pressing need for neuroprotective treatments for glaucoma and other neurodegenerative diseases
- Increasing NAD is a promising neuroprotective strategy
- EGCG activates NMNAT2, an axonal maintenance factor essential for neuronal NAD levels
- EGCG is an unstable compound and based on the structure of EGCG, over 80 compounds have been tested for their NAD boosting effects

Results

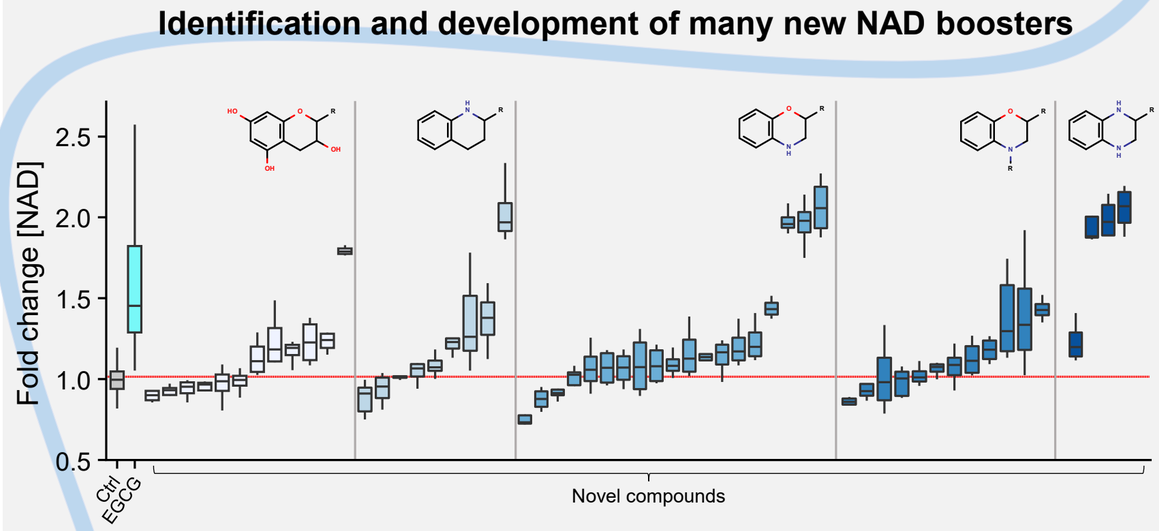
EGCG demonstrates an increase of NAD in primary cortical neurons. 56 compounds were initially tested, and multiple compounds showed a greater NAD-boosting effect than EGCG alone. By inhibiting the upstream enzyme in the salvage pathway, NAMPT, via the addition of FK866 to the media, we could identify compounds that were dependent on NMN for their NAD-boosting effect.

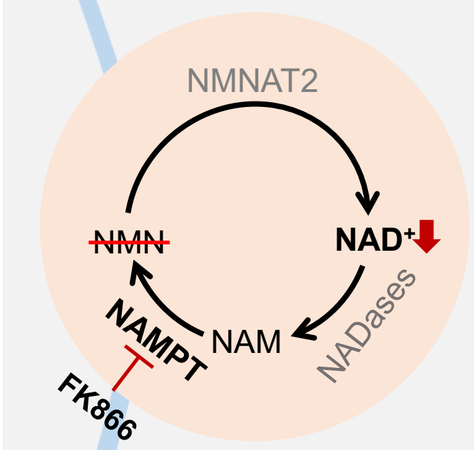


EGCG has an NAD boosting effect in cortical cells



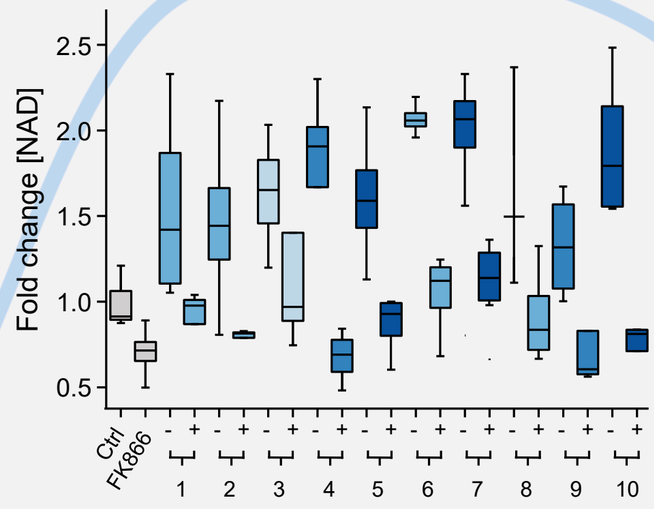
Identification and development of many new NAD boosters

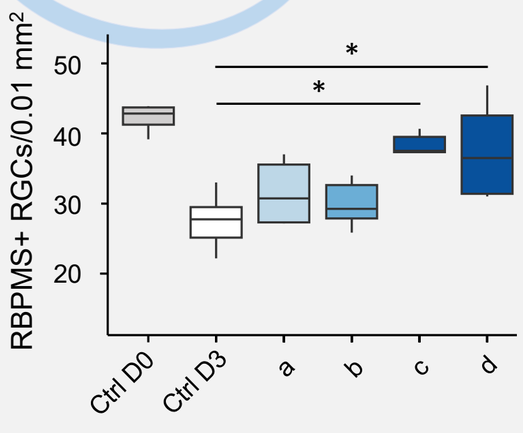




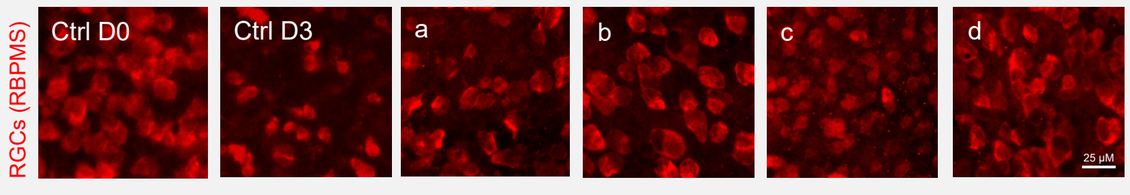
Blocking NAMPT with the inhibitor FK866 demonstrates specificity for the salvage pathway

Multiple compounds are dependent on NMN for their NAD boosting effect





Novel NAD-boosters are neuroprotective



References

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