Removal of early senescent cells to protect retinal ganglion cells in glaucoma

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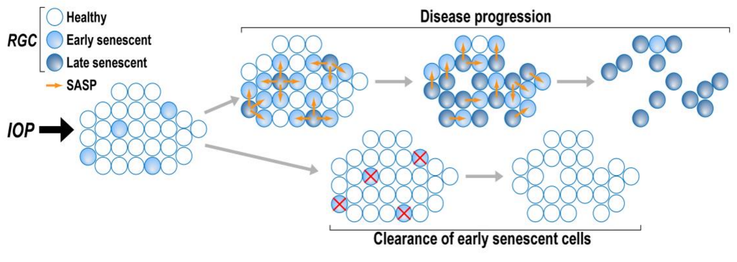
Abstract

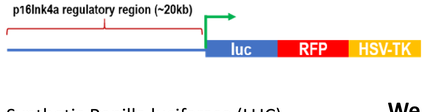
Glaucoma is a group of diseases with diverse molecular mechanisms of pathogenesis, all of which converge on a common pathway leading to typical optic nerve damage and consequent characteristic patterns of visual field loss, what may ultimately progress to blindness. Of all the glaucoma-associated risk factors, patient's age is by far the strongest and consistently reported. Concurrent with the age-related increase in the prevalence of glaucoma is the age-related decreased population of retinal ganglion cells (RGCs) in the retina. Pathological studies have shown a steady decrease of RGC number during normal aging, starting at a young age and continuing at a rate of approximately 5000 cells per year. Glaucomatous loss of RGCs can be therefore viewed as a premature aging effect. In our recent work, we observed that the expression of p16Ink4a, a gene whose expression levels increase during normal aging, is strongly up-regulated upon increased IOP, leading to enhanced senescence in the RGCs, and, most likely as a direct consequence, to RGC death. Importantly, senescent cells contribute to aging and age-related diseases by altering tissue microenvironments via their senescence-associated secretory phenotype (SASP) molecules, which are largely composed of inflammatory chemokines and cytokines, matrix-remodeling proteases and growth factors. We thus theorize that glaucoma progression is accelerated due to prolonged exposure to microenvironment alterations caused by naturally aging senescent cells.

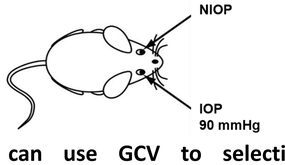
Hypothesis



Materials and Methods

p16-3MR transgenic mice

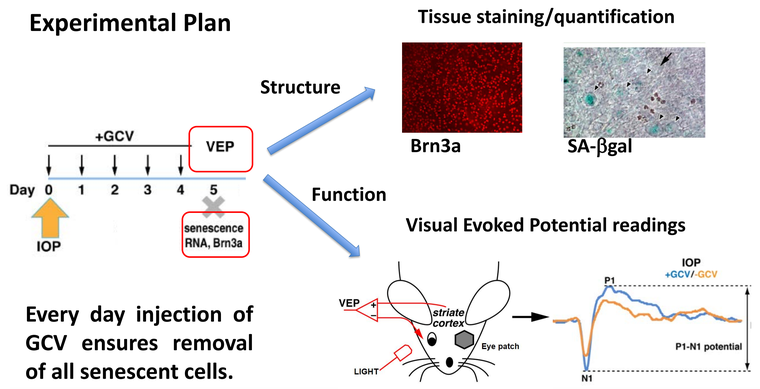




- Synthetic Renilla luciferase (LUC)
- Monomeric RFP (mRFP)
- Truncated herpex simplex virus thymidine kinase (HSV-TK)

We can use GCV to selectively remove p16Ink4a⁺ cells in p16-3MR mice and study its effect on RGC survival and function.

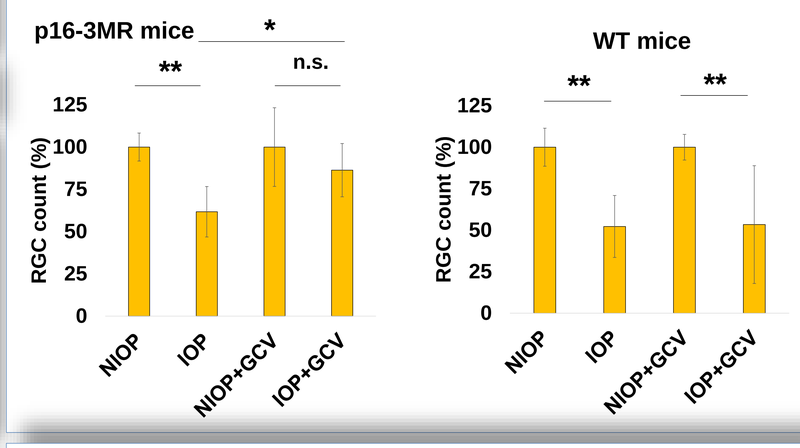
Experimental Plan



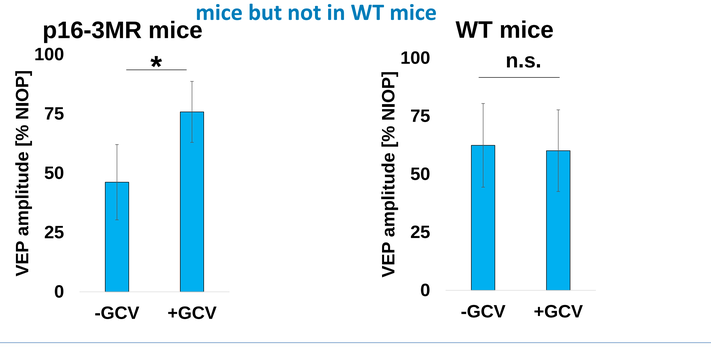
Every day injection of GCV ensures removal of all senescent cells.

Results

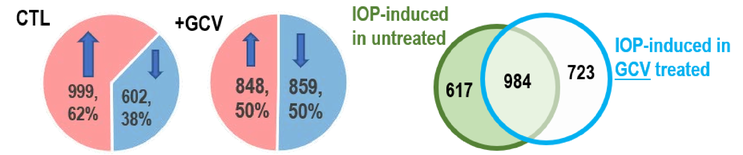
GCV-dependent removal of senescent cells improves RGC Survival in p16-3MR mice but not in wild type (WT) mice.



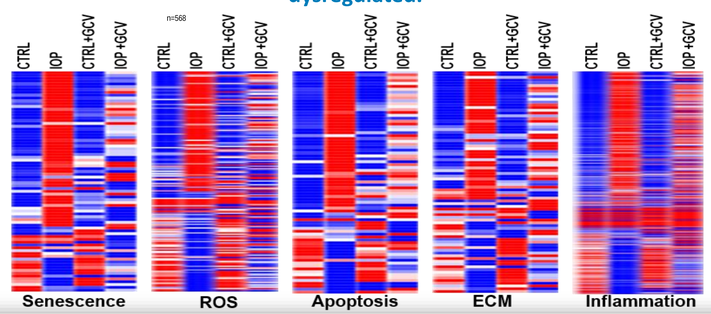
GCV treatment significantly improves P1-N1 visual potential in in p16-3MR mice but not in WT mice



IOP treatment changes transcriptional program in the retina. GCV treatment modifies the change.



Removal of senescent cells by GCV downregulates many genes activated in response to IOP elevation. Several pathways characteristic to senescence are dysregulated.



Conclusions

We concluded that the GCV treatment of transgenic animals has a protective effect on structure and function of the retina. This exploratory project provided preliminary data for future investigations aimed at screening senolytic drugs that can be used to treat glaucoma patients as well as to understand the process of neuroprotection.

Next steps

Our future efforts will be concentrated on two points: i) testing several senolytic drugs that will have similar effect on RGC survival upon IOP elevation; ii) find out which RGC cells are affected by the IOP elevation and whether all RGC subtypes are protected by the senolytic treatment.

Acknowledgements

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DSK laboratory website
http://dsklab.ucsd.edu