Aging Eye and Vision

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Turning Back Time: Breakthrough research in reversing glaucoma and age-related vision loss

Bruce R. Ksander, Ph.D.
Glaucoma

- Chronic neurodegenerative disease causing irreversible blindness
- A leading cause of blindness in the world (>75 million people worldwide)
- Multiple forms of glaucoma (open angle, closed angle, secondary glaucoma)

All share a common endpoint: death of Retinal Ganglion Cells (RGCs)
Elevated Intraocular Pressure (IOP)

A significant risk factor for glaucoma

Healthy

Flow of Aqueous humor

Drainage Canal

Elevated IOP

Drainage canal becomes blocked and leads build up of AqH and increased IOP

Glaucoma

The increased IOP causes damage to the optic nerve and RGCs

Only treatment for glaucoma

Lowering IOP through surgical and pharmacological approaches.

Lowering IOP **does not** halt progression of disease

IOP-independent therapies are needed for the treatment of glaucoma
Glaucoma is a multifactorial disease

Inherited retinal degeneration
Retinitis Pigmentosa

Correct the gene mutation
Prevent disease

Elevated IOP
Dysfunctional drainage

Oxidative stress

Mitochondrial dysfunction

Inflammation

127 risk genes linked to glaucoma

How do we treat a multifactorial disease?
Age is the **single most significant** risk factor for developing neurodegenerative diseases such as glaucoma.

One of the root-causes of glaucoma is aging.

How can we target aging?

Developed a treatment for glaucoma that reverses the age of retinal neurons (RGCs)

What is aging?
• Slow gradual decline of tissue and organ function over time

Widely believed that aging moves in one direction only and cannot be reversed
• Research has shown this is not the case
Department of Genetics, Harvard Medical School
Paul F. Glen Center for the Biology of Aging

David Sinclair, Ph.D.
Professor

Yuancheng Lu
Graduate Student
Epigenetic “marks” on your DNA drive the aging process
As you age, “Epigenetic Marks” accumulate on DNA

Epigenetic Marks

1-year-old DNA

This is a biomarker of aging
Called the **epigenetic clock**

80-year-old DNA
Epigenetic marks are not merely “markers” of aging

Accumulating markers *cause aging* by changing how the cell functions.

Can you reverse aging by removing these epigenetic markers?
Epigenetic reprogramming reverses the clock by removing the epigenetic markers.

80-year-old DNA functions like 40-year-old DNA.

Cells regain youthful functions.

Epigenetic reprogramming reverses the clock by removing the epigenetic markers.
Epigenetic reprogramming reverses the clock by removing the epigenetic markers

80-year-old DNA

Epigenetic reprogramming

Enzymes
Target Aging

Target Multifactorial Diseases
How can epigenetic reprogramming” be used as a treatment for glaucoma?
Gene therapy to trigger epigenetic reprogramming

**Vector**

**Plasmid**

*genes enzymes*
Gene therapy to trigger epigenetic reprogramming
Gene therapy to trigger epigenetic reprogramming

Vector + Plasmid
Gene therapy to trigger epigenetic reprogramming

Target of glaucoma
Retinal Ganglion Cell

Vector
Gene therapy to trigger epigenetic reprogramming

Target of glaucoma
Retinal Ganglion Cell

Vector
Plasmid
Gene therapy to trigger epigenetic reprogramming

Target of glaucoma
Retinal Ganglion Cell
Plasmid
Gene therapy to trigger epigenetic reprogramming

Target of glaucoma
Retinal Ganglion Cell

Enzymes
Gene therapy to trigger epigenetic reprogramming

Target of glaucoma
Retinal Ganglion Cell

Enzymes
Epigenetic markers

Reserves the epigenetic clock
Gene therapy to trigger epigenetic reprogramming

Old cells are reprogrammed to restore youthful function
Using this new type of gene therapy, we restored vision in mice lost due to glaucoma
Measuring Visual Acuity in Mice

Snellen visual acuity eye chart

High visual acuity

Stripped pattern rotation

Low visual acuity

Left eye response

Right eye response
Measuring Visual Acuity in Mice

Snellen visual acuity eye chart

- **Low visual acuity**
  - E 1 20/200
  - F 2 20/100
  - T 3 20/70
  - L 4 20/50
  - P 5 20/40
  - E 6 20/30
  - D 7 20/25
  - F 8 20/20

- **High visual acuity**
  - L 9
  - E 10
  - F 11

- **Loss of vision**
  - Indicated by red circles on images of mice.
Measuring Visual Acuity in Mice

Snellen visual acuity eye chart

High visual acuity

Low visual acuity

High visual acuity
Inducible mouse model of glaucoma

Microbead injection

Elevated IOP
4 wks

Saline
- Microbeads

****

Elevated IOP
4 wks

IOP (mmHg)

Days post microbead injection
Inducible mouse model of glaucoma

**Microbead injection**

- Elevated IOP 4 wks

**Graph:**
- **X-axis:** Days post microbead injection
- **Y-axis:** IOP (mmHg)
- Comparison of Saline and Microbeads

**Bar graph:**
- Comparison of Normal and Glaucoma visual acuity
- Significance indicated with ****
Inducible mouse model of glaucoma

Elevated IOP 4 wks

Treat w/ Gene Therapy

Visual Acuity

Days post microbead injection

IOP (mmHg)

Baseline

Microbead injection

Elevated IOP 4 wks

Saline

Microbeads

****
Inducible mouse model of glaucoma

- Elevated IOP after 4 wks

- Treat with Gene Therapy

- Microbead injection

- Visual Acuity

- IOP (mm Hg) over time

- Days post microbead injection

- Graph showing visual acuity and IOP levels in normal and glaucoma conditions.
Conclusions / future implications

- Demonstrated that we can reverse age and restore function in mouse retina
- Proof-of-principal experiments / pre-clinical experiments

- Working hard to translate this approach to the clinic
  - Safety testing
  - Efficacy in human cells “ex vivo”

- Implications for treating other age-related diseases in the eye
  - Age-related macular degeneration (AMD)

- Age-related diseases in general
  - Alzheimer’s
  - Cardiovascular disease
  - Diabetes
Epigenetic Regulation of Retinal Aging

Anand Swaroop, PhD
Senior Investigator & Chief,
Neurobiology, Neurodegeneration & Repair Laboratory
Progressive functional decline
Reduced adaptive response to stress
Increased susceptibility to disease
Dysfunction or death of photoreceptors is a major cause of (currently) incurable vision impairment.

Rods – dim light vision, 95%
Cones – day light vision, color, 5%
High energy demands
Mitochondria-rich

Yang et al. *Prog Retin Eye Res* 2015
Aging of the Retina/Rod Photoreceptors

- Rate of peripheral rod loss is highest between 2nd and 4th decades (~50%)
- Central rods have a slower progressive loss, 30% are lost by the 9th decade. Cone density does not change
- Rod changes with aging in both humans and mice
- Rod loss precedes cones, in normal aging and AMD
- Rods are primary drivers of aging-related synaptic remodeling
Aging of The Retina/Photoreceptors

Genetics

The Aging Retina

Environment

Stochasticity

Campello et al. Annu Rev Vis Sci. 2021
Integrative Epigenomic Analysis of rod aging

Flow-sorted photoreceptors (98% purity)

Nrlp-EGFP

3 12 18 24 months

Whole-genome-bisulfite sequencing
ATAC-seq
RNA-seq
CUT&RUN

Akimoto et al., PNAS 2006

Corso-Díaz et al., Cell Reports 31:107525, 2020
DNA methylation changes in aging rods

Corso-Díaz et al., *Cell Reports* 31:107525, 2020
Changes in methylated regions are associated with Rod-specific and Universal Aging Pathways

A subnetwork showing association with Metabolic Genes

Decrease in oxygen consumption and enhanced FA dependency

Corso-Díaz et al., *Cell Reports* 31:107525, 2020
Epigenome and Metabolic Dysregulation in Retinal Aging

Features of our genome
- DNA methylation
- Chromatin architecture
- Gene/Protein expression

Cellular changes
- Mitochondria & metabolic stress
- Oxidative stress
- Advanced glycation end products
- Metabolic signaling

Campello et al. *Annu Rev Vis Sci.* 2021
Summary and Hypothesis

- DNA methylation (epigenome) changes occur with age at gene regulatory regions in rod photoreceptors.
- Age-related changes in methylation are associated with gene expression changes in cell type-specific and shared aging pathways.
- Mitochondria and metabolic dysregulation is observed in aging rods.

Can manipulating the epigenome impact mitochondrial function and reduce the impact of aging on retinal/rod function?
Adherence to a Mediterranean diet and cognitive function in the Age-Related Eye Disease Studies 1 & 2

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Adherence to the Mediterranean Diet and Progression to Late Age-Related Macular Degeneration in the Age-Related Eye Disease Studies 1 and 2

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Can Healthy Diet Slow Down the Impact of AGING?

**EXPERIMENTAL DIETS**

- Mediterranean Diet
- High Fat Diet
- AREDS(2) Supplements
- B-Vitamin Supplements

**FUNCTIONAL AND MOLECULAR ASSESSMENTS**

- Structure & Function
  - Electroretinogram
  - Histology
- Biochemical Assays
  - Mitochondrial function
  - Lipid and FA profiling
- Genomic Analyses
  - Genome-wide methylation
  - Chromatin accessibility
  - Transcriptome

**“Young” Epigenome**
- Maintenance of youthful epigenetic patterns
- Intact genomic integrity
- Youthful appearance
- Maintenance of mitochondrial metabolism
- Minimized retinal degeneration
- Increased electroretinogram light response

1 year old

**“Old” Epigenome**
- Epigenetic and transcriptional noise
- Reduced genomic integrity
- Visible signs of aging
- Altered metabolism
- Increased retinal degeneration
- Reduced electroretinogram light response

2 years old
NNRL represents Diversity in every way
Dec 2020