The Retina: A Sensitive Barometer of Health and Nutrition

Shalesh Kaushal MD, PhD
Take Home Points

- The retina is, perhaps, the best barometer of overall health and nutrition in the body.
- It is the most metabolically active tissue in the body per unit weight.
- Has the largest blood supply per unit volume in the body.
- Structure and function can be measured non-invasively and quantitatively.
Take Home Points

- Nearly all diseases have a retinal manifestation
- Molecular Rheostats are small molecules that modulate multiple cellular pathways
- Many of them are found in Nature
- They are being developed as “medicines”
Anatomy of the Eye and Retina
Structural Organization of the Retina
Retinal Architecture

Ganglion cell layer -
Inner plexiform layer -
Inner nuclear layer -
Outer plexiform layer -
Outer nuclear layer -
ELM -
Outer Segments -
RPE -
Choroid -
Retinal Architecture

- Outer nuclear layer
  Photoreceptor nuclei

- Outer Segments
  Diurnal shedding

- RPE
  Phagocytosis
  Lysosomal degradation
  Retinoid recycling

- Bruch’s membrane
  RPE attachment
  Metabolic exchange

- Choroid
  Metabolic exchange
  Oxygen exchange
Retinal Facts

- Developmental related and part of the brain
- Most metabolically active tissue in the body
- Highest consumption of oxygen/unit volume in the body
- Largest concentration of omega-3 fats/unit volume in the body - more than the brain
- Highest concentration of cholesterol/unit weight in the body
- Nearly every disease has a retina manifestation
Retinal Disease Inciting Factors

- INFLAMMATION
- Oxidative stress
- Dysregulation of insulin signaling pathway
- Immune system imbalance
- Mitochondrial dysfunction
- Catabolic state
- Dysfunction/Death of photoreceptors and retinal tissue
Chronic Diseases Inciting Factors

- INFLAMMATION
- Oxidative stress
- Dysregulation of insulin signaling pathway
- Immune system imbalance
- Mitochondrial dysfunction
- Catabolic state
- Dysfunction/Death of relevant tissues/cells
Chronic Diseases

- Slow onset
- Appear multifactorial
- May have genetic component
- Multiple clinical presentations
- Appear to reversible in early stages
- *Nearly all current medical therapies target symptoms not root cause(s)*
Chronic Diseases

- Type II Diabetes
- Hypertension
- Coronary Artery Disease
- Arthritis
- Neurodegeneration
- Cancer
- Age-Related Macular Degeneration (AMD)
- Glaucoma
Age-Related Macular Degeneration
Age-Related Macular Degeneration
Age-Related Macular Degeneration (ARMD)

Number one cause of blindness worldwide. Two forms - 1) Non-exudative, dry form, 85% of patients; 2) Exudative, angiogenic form, 15% of patients
Macular Degeneration

Dry Macular Degeneration

Wet Macular Degeneration
Retinal Camera
Retinal (Fundus) Photograph
Mild Diabetic Retinopathy
Severe Diabetic Retinopathy
Retinal (Fundus) Photograph
OCT Imaging of the Retina
High Resolution Imaging of the Retina
High Resolution Imaging of the Retina
High Resolution Imaging of the Retina
Dry AMD AF/OCT Images

drusen
Wet AMD OCT Image

fluid in the retina
OCT Image of Diabetic Macular Edema
Glaucoma RNFL OCT Image

SNR: 7/10

OS

46 61 86 77 71 58 52 57 78 79 81 59 46

Average 67 Minimum 43 Maximum 94
Structure
Autofluorescence (AF)
Imaging
Normal Retinal AF Image

DR defined: Damage to the retina caused by changes in the blood vessels supplying it secondary to hyperglycemia.
Dry AMD AF Image

DR defined: Damage to the retina caused by changes in the blood vessels supplying it secondary to hyperglycemia.
Severe Dry AMD AF Images

DR defined: Damage to the retina caused by changes in the blood vessels supplying it secondary to hyperglycemia.
Functional Testing
Microperimetry
Functional Testing of the Retina

MAIA microperimter
Microperimetry Test Results
Microperimetry Test Results

Normal Status

- Average Threshold: 36, 29, 27, 0
- Fixation Stability: Stable (P1=99%, P2=100%)
- Percent Reduced Thresholds:
  - 0% to 100%

Early AMD

- Average Threshold: 36, 25, 23, 0
- Fixation Stability: Stable (P1=98%, P2=100%)
- Percent Reduced Thresholds:
  - 0% to 100%

Severe AMD

- Average Threshold: 36, 25, 23, 0
- Fixation Stability: Unstable (P1=6%, P2=27%)
- Percent Reduced Thresholds:
  - 0% to 100%
How can a cell or tissue return to homeostasis?
Some Disorders with Retinal Findings

• Type II Diabetes
• Hypertension
• Arthritis
• Neurodegeneration
• Cancer
• Age-Related Macular Degeneration (AMD)
• Glaucoma
Molecular Rheostats

Have the potential to effect multiple Chronic Diseases
The Protein Homeostasis Network (PHN) is the subset of proteins that maintain protein homeostasis

- Regulates proper protein folding, trafficking and clearance mechanisms
- Consists of 1,000-2,000 proteins in intersecting pathways

The PHN is organized into functional ‘clusters’ and can be modulated

- These include chaperones, the unfolded protein response (UPR), sorting proteins, the proteasome, autophagy, mTOR, Ca\(^{2+}\) homeostasis network, and disaggregases
The PHN is highly adaptive and responds to intrinsic and extrinsic stresses to maintain proteostasis, but has limited reserve capacity.

Exceeding PHN capacity leads to cellular dysfunction and cell death.
The PHN is Compromised during Aging and in Chronic Disease

- Accumulated stresses can compromise the PHN’s ability to maintain homeostasis
- Chronic diseases are associated with a reduced capacity to maintain protein homeostasis
The PHN is Compromised during Aging and in Chronic Disease

- CHRONIC DISEASES
- Type II Diabetes
- Hypertension
- Coronary Artery Disease
- Arthritis/Autoimmune
- Neurodegeneration
- Cancer
- Age-Related Macular Degeneration (AMD)
- Glaucoma
- Depression
### PHN Modulators Requires a New Approach: Natural Molecular Rheostats

- **Traditional Drug Discovery**
  - Small Molecule
  - Disease related protein

- **PHN Approach**
  - Molecular Rheostat

- **Traditional target-based discovery is focused on efficacy and selectivity**
- **Nature target key pathways and sub-networks that regulate the disease-related protein or stimulate a cellular response**
- **These Molecular Rheostats modulate the function of the PHN**
PHN Modulators Requires a New Approach: Natural Molecular Rheostats

- Molecular Rheostats re-establish Cellular Homeostasis
- MRs are found abundantly in Nature
- Treat disease at Root Biochemical or Genomic Cause
Molecular Rheostats- Adjusting Cellular Homeostasis

- Small molecule drugs or nutraceuticals
- Target a pathway or pathways
- Rejigger cellular homeostasis
- Could be used broadly for many retinal diseases or other chronic diseases
- Mostly given at low dose, thus low toxicity
Model for Disease

Environmental stressor +/- Abnormal gene

Cellular Dysfunction

1. Oxidative stress
2. Inflammation
3. Mitochondrial dysfunction
4. Cell death signals

Retinal cell death

Retinal degeneration

gene therapy

SMALL MOLECULES

stem cells
Assays for Protein Homeostasis Modulators

**Cell Culture**
- High throughput oxidative apoptosis assay

**Biochemical**
1. Inflammatory cytokine secretion
2. Complement activation
3. TLR activation

**Mouse Models**
1. Rod and cone ERG
2. Fundus photography
3. OCT based ONL thickness

Increasing Clinical Potential
Increasing Throughput
Screening Methods

• Developed simple, scalable oxidative apoptosis assay of RPE cells exposed to hydroquinone (HQ)

• Used high throughput robotic based assay

• Screened libraries of FDA approved and nutraceutical small molecules

• Secondary screens for inflammatory markers
Characterization of HQ-RPE Cell Culture Model of Dry ARMD

- Time Dependence of HQ-mediated ARPE-19 cell death
- Time of Hydroquinone Incubation
- Cell Viability (%) vs. Time of Hydroquinone Incubation
- 0 hrs, 4 hrs, 8 hrs, 12 hrs, 16 hrs, 24 hrs

- 16hr. Resveratrol Pre-treatment
- Fold Change vs. Resveratrol (μM)
- 0, 10, 25, 50 μM

Bar charts showing the effect of HQ and resveratrol on cell viability and fold change.
Results of HTS and Secondary Screens

- Identification of about 40 FDA approved drugs and nutraceuticals
- At least 7-8 identifiable classes
- **REAL QUESTION IS HOW TO PRIORITIZE THEM FOR POSSIBLE CLINICAL TRIALS**
Genome Wide Association Studies

- Allow for the identification of important sequence variants and their association with disease states
- We have used the NEI-AREDS and NEI-AMD SNP databases coupled with biochemical pathways analysis
- Enrichment of pathway constituents containing genes within the PHN
Association Study on Pathway Elements

Manhattan Plot

AMD-associated SNPs in Genes Encoding Pathway Constituents

$P = 0.005$
Valproic Acid

- 2-propylpentaenoic acid
- Branched-chain fatty acid
- Derived from valeric acid

- ~90% of VPA is protein-bound
- Clearance: 6-20mL/hour/Kg
Valproate Pharmacodynamics

GABA synthesis in TCA cycle
Voltage-gated ion channels
Histone Deacetylase activity

AAMD-associated genes

- **GABRB1**: $5.00 \times 10^{-6}$
- **GABBR2**: $5.94 \times 10^{-4}$
- **GRIN2A**: $5.35 \times 10^{-4}$
- **SCN4A**: $1.41 \times 10^{-3}$
- **CACNA1D**: $2.81 \times 10^{-3}$
- **GABRP**: $7.63 \times 10^{-3}$
- **GRIN2B**: $9.79 \times 10^{-3}$
- **HDAC9**: $2.93 \times 10^{-3}$
- **ABAT**: $4.10 \times 10^{-4}$
Valproate Pharmacokinetics

Glucuronidation (50%)
Beta-oxidation in mt (40%)
CYP-mediated oxidation (10%)

AAMD-associated gene ACADSB $3.73 \times 10^{-3}$
Integrative Genomics Approach

- Couples in vitro cell culture/animal model experiments with clinically relevant genomic data
- Provides insights into biology of health and disease that can inform applied clinical research
- Systematic and efficient identification of alternative or refined indications for approved drugs or those under clinical/pre-clinical development.
VPA Downregulates Inflammatory/Proangiogenic Cytokines
Methods

hRPE cell monolayer

IL-1β, IFN-γ, TNF-α (ICM) +/- VPA

cytokines

24 hrs

SFM

hRPE cell monolayer

24 hrs

SFM

cytokines

IL-1β, IFN-γ, TNF-α (ICM) +/- VPA
# Effect of VPA on Cytokine Release

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**Legend:**
- SF: SF media
- ICM: ICM media
- ICM + VPA: ICM + VPA media
- VPA: VPA treatment
Off-Label VPA Patient Study
Visual Field Changes on VPA
Visual Field Changes on VPA

Change in VF area at follow-up and natural history

change in VF area (log_e)

OD   OS

Berson et al. 2002  Massoff et al. 1990
Visual Acuity Changes on VPA

Best corrected visual acuity change
(follow-up vs. baseline)

log MAR BCVA change

OD | OS | 500 mg/day | 750 mg/day

n = 8 | n = 6
Visual Acuity Changes on VPA

![Box plot showing best corrected visual acuity change](image)
VPA Dry ARMD

A. Change in BCVA dry ARMD eyes (average of OD & OS, or available)

B. Change in BCVA dry ARMD eyes (better of OD & OS, or available)
Integrated Approach for Chronic Diseases

• Acute Treatment of Symptoms
  • Most often need pharmaceuticals

• Chronic treatment of Root Cause(s)
  • Will need nutraceuticals, supplements and herbs
  • MOLECULAR RHEOSTATS
Fish Oil
Healthy Nuts

Almonds

Walnuts

Pecans
Green Vegetables

Kale

Broccoli
Turmeric
Astaxanthin
Testing - General

- chem-7, cbc
- vitamin D, 25-hydroxy D3
- homocysteine
- C reactive protein (hs-CRP)
- Fasting BS, insulin
- HgA1C
- IL-1, IL-6
- Genomics analysis
Treatment Protocol

• Reduce/Eliminate refined sugars
• Reduce/Eliminate refined flours
• Reduce food allergens/toxins
• Reduce environmental toxins
• Reduce trans fats
Treatment Protocol

• Dark Green Vegetables
• Dark Fruits
• Teeth Cleaning every 3-4 months
• 20 minutes in the Florida sun
• 20-30 minutes of exercise
Treatment Protocol

- Good multivitamin
- Turmeric
- Astaxanthin
- Fish/Krill or Cod Liver Oil
Treatment Protocol

- Anti-inflammatory eye drops
- CAI drops
- β-blocker drops
- Minocycline
OCT Image of Diabetic Macular Edema
Current Treatments

- Laser
- Vitrectomy
- Intravitreal steroids
- VEGF inhibitors
OCT Image of Treated Diabetic Macular Edema

Before

2 months after treatment protocol
CONCLUSIONS

• Retina is a highly metabolic tissue with profound metabolic needs

• Has a substantial blood supply

• Nearly every human disease has a retinal manifestation

• There are quantitative methods to assess retinal structure and function

• Molecular Rheostats will be the new generation of therapeutics for retinal and other diseases
Shalesh Kaushal MD, PhD
skaushal108@gmail.com