The Retina: It’s More than What Meets the Eye

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Take Home Points

- 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

- Molecular Rheostats are small molecules that modulate multiple cellular pathways
- Many of them are found in Nature
- These 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
- Nearly every disease has a retina manifestation
Importantly Components of Retinal and Macular Disease

- Immune Dysregulation
- Oxidative Stress
- Microglial Activation
- Toxic RNA
- Inflammation

RPE photoreceptors → Retinal Cell Death → Retinal/Macular Degeneration
Retinal Disease Inciting Factors

- **INFLAMMATION**
- Oxidative stress
- Immune system imbalance
- Mitochondrial dysfunction
- Dysfunction/Death of photoreceptors and retinal tissue
Chronic Diseases Inciting Factors

- **INFLAMMATION**
- Oxidative stress
- Immune System imbalance
- Mitochondrial dysfunction
- 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
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.
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 microperimeter
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 40 60 100

Early AMD

AVERAGE THRESHOLD
36 25 23 0

FIXATION STABILITY
Unstable
P1=6% - P2=27%

PERCENT REDUCED THRESHOLDS
0 40 60 100

Severe AMD

AVERAGE THRESHOLD
36 25 23 0

FIXATION STABILITY
Unstable
P1=6% - P2=27%

PERCENT REDUCED THRESHOLDS
0 40 60 100
How can a cell or tissue return to homeostasis?
Molecular Rheostats

Have the potential to affect retinal disease independent of mutant gene/protein
Cellular Protein Homeostasis Network (PHN) is Comprised of Multiple Networks

- 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 Disease

Gain of Function
- Alzheimer’s Disease
- Huntington’s Disease
- Parkinson’s Disease
- Type-2 Diabetes
- ALS
- Liver disease
- Retinal diseases
- >20 amyloid diseases

Loss of Function
- Cystic Fibrosis
- Gaucher’s Disease
- Tay Sachs Disease
- Pompe Disease
- Emphysema
- Long QT Syndrome
- >100 genetic diseases
- Retinal disease

Environmental Insult
- Genetic Aberration
- Cellular Stress

Normal PHN

Diseased PHN
Visualizing PHN Changes During Disease Progression

Gene Up-regulation

Gene Down-regulation
The assessment of PHN changes across pathways and functional sub-networks provides more information than single target changes (which may have limited incidence rates across patient populations).

Functional pathway analyses further elucidate underlying pathogenic mechanisms, potential therapeutic targets, biomarkers and patient stratification strategies.
Characterizing the PHN: Developing PHN Signatures

Transcriptional Profiling + Proteomic Profiling + Functional Profiling → PHN Analysis

PHN Signatures
- Disease-specific
- Stage-specific
- Tissue-specific

human tissue samples, animal and cell-based disease models allows the creation of PHN models that define disease progression
Traditional target-based discovery is focused on efficacy and selectivity.

In contrast, we’re developing therapeutic drugs that target key pathways and sub-networks that regulate the disease-related protein or stimulate a cellular response.

We have developed a platform to enable small molecule identification based on modulating the function of the PHN.
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

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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**

- **Left Panel:**
  - Time of Hydroquinone Incubation: 0 hrs, 4 hrs, 8 hrs, 12 hrs, 16 hrs, 24 hrs.
  - Cell Viability (%): 100, 80, 60, 40, 20, 0.
  - Statistical Significance: ***

- **Right Panel:**
  - Time of Hydroquinone Incubation: 0 hrs, 4 hrs, 8 hrs, 12 hrs, 16 hrs, 24 hrs.
  - Cell Viability (%): 100, 80, 60, 40, 20, 0.
  - Statistical Significance: ***

**Time Dependence of HQ-mediated ARPE-19 cell death**

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  - Cell Viability (%): 100, 80, 60, 40, 20, 0.
  - Statistical Significance: ***

**16hr. Resveratrol Pre-treatment**

- **Right Panel:**
  - Fold Change: 1, 2, 3.
  - Resveratrol (μM): 0, 10, 25, 50.
  - Fold Change: 1, 2, 3.
  - Statistical Significance: ***

+ 200μM HQ (24hrs.)
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**
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

<table>
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<tr>
<th>Gene</th>
<th>p-value</th>
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<td>GRIN2A</td>
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<td>SCN4A</td>
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<td>CACNA1D</td>
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<td>ABAT</td>
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</table>
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

IL-1β, IFN-γ, TNF-α (ICM) +/- VPA

cytokines

24 hrs

hRPE cell monolayer

SFM

24 hrs

IL-1β, IFN-γ, TNF-α (ICM) +/- VPA

cytokines
# Effect of VPA on Cytokine Release

<table>
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<tr>
<th>Time (hrs)</th>
<th>Sample</th>
<th>hIL6 pg/ml</th>
<th>hIL8 pg/ml</th>
<th>hRANTES pg/ml</th>
<th>hMCP1 pg/ml</th>
<th>hVEGF pg/ml</th>
<th>hSDF1b pg/ml</th>
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<td>ICM + VPA</td>
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Note: The data is presented in pg/ml units.
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
## Average change in VF area

<table>
<thead>
<tr>
<th>Mean change (no treatment)</th>
<th>All eyes combined</th>
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<tr>
<td>0 log&lt;sub&gt;e&lt;/sub&gt; units</td>
<td>p = 0.09</td>
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<tr>
<td>-0.011 log&lt;sub&gt;e&lt;/sub&gt; units</td>
<td>p = 0.02</td>
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<tr>
<td>Berson et al. 2002</td>
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<tr>
<td>-0.033 log&lt;sub&gt;e&lt;/sub&gt; units</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>Massoff et al. 1990</td>
<td></td>
</tr>
</tbody>
</table>
Visual Acuity Testing Results

• At baseline:
  – OD = 0.457 logMAR (~20/57)
  – OS = 0.300 logMAR (~20/40)

• At follow-up:
  – OD = 0.260 logMAR (~20/36)
  – OS = 0.153 logMAR (~20/28)
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

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
VPA Dry ARMD

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

- 750 mg/d: change (log MAR) range: -1.2 to 0.0, n = 9
- 500 mg/d: change (log MAR) range: -0.8 to 0.0, n = 4

**B.** Change in BCVA dry ARMD eyes (better of OD & OS, or available)

- 750 mg/d: change (log MAR) range: -1.2 to 0.0, n = 9
- 500 mg/d: change (log MAR) range: -0.8 to 0.0, n = 4
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
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