Mitochondria in Aging, Diabetes, and Atherosclerosis - Introducing Humanin

National Lipid Association
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DISCLOSURES:

• Dr. Cohen owns stock in CohBar Inc.
• Consultant to Novo Nordisk & Teva
• SAB member of Amgen
• SAB member Antisense Therapeutics
Outline

- The Mitochondria in atherosclerosis
- What is Humanin
- Metabolo-Protection by Humanin
- Cardio-Protection by Humanin
- Athero-Protection by Humanin
- Humanin Levels in Disease States
- Mitochondrial Ethnic Disparity
- What’s Next for Mitochondrial Peptides
A Primer on Mitochondrial Biology

- Originally prokaryotic; Maternally inherited
  - regulates:
    - Cellular respiration
    - Integrator of apoptotic signals
    - ROS production/oxidative stress
- Mitochondrial function declines with age as a result of accumulated mutations in the mitochondrial DNA
- Certain mtDNA haplo-groups are longevity-associated
- Mitochondrial dysfunction is common in multiple diseases of aging including:
  - Diabetes
  - Neurodegeneration
  - Cardiac Disease
- The MT-DNA encodes for 13 proteins, 2 rRNA and 22 tRNAs
- Multiple signals and hundreds of proteins are sent to the mitochondria – BUT –
- Mitochondrial-Cellular communication remains elusive
The Discovery of Humanin

- Cloned nearly simultaneously by three different groups.
  - 1) From a cDNA library using a neuronal death trap and showed it to be a potent protective factor (PNAS 2001)
  - 2) by Y2HS it as a BAX partner/antagonist (Nature 2003)
  - 3) We cloned it with a Y2HS as a BP3 partner/antagonist with Diabetes relevance (PNAS 2003)
- All three groups cloned its mRNA and identified it to contain 60-80% of the mitochondrial 16S rRNA sequence
- Shown to have wide ranging cytoprotective effects

HN protects from AD-related neurotoxicity

Healthy Neurons    Neurons + Aβ    Neurons + Aβ + Humanin
Humanin: The First Mitochondrial Peptide


- Produced from the mitochondria
- Gene within a gene. Highly conserved
- Present in Brain, Testes, Prostate, Seminal Plasma, CSF, and Plasma
- Cytoprotective / metaboloprotective
- Produced as a polyadenylated mRNA smaller and distinct from the rRNA
- Translated in the cytoplasm and secreted
Mechanisms of Action of Humanin

1. CNTFR
   - gp130
   - P
   - STAT3
   - Neuro-protection
   - Metabolic Effects

2. WSX-1
   - FPRL1
   - P
   - ERK1/2
   - Calcium Mobilization
   - Anti-inflammatory

3. Bax/BP3
   - Intracellular
   - Apoptosis prevention
The life-cycle of Humanin

Transcription of MDP ORFs in the mitochondria

Rho-0 Cells

Translation of MDP mRNA in the cytoplasm

mRNA binding protein/transporter?

MDP mRNA

HN
CO-1
Beta-actin

Cells Parent

Rho-0 Cells

Humanin

CO-I

PBS CHX
Protective Actions of Humanin (Diseases of Aging)

*in vivo* studies in rodents

**Neuroprotective/Cytoprotective**
- Prolongs Survival and function in ALS and Alzheimer mouse models
  - Protects against experimental stroke
  - Prevents chemotherapy-induced toxicity

**Metaboloprotective/Anti-inflammatory**
- Treats and prevents type 1 diabetes in NOD mice
  - Improves blood sugar in Zucker diabetic rats
- Prevents the development of hepatic steatosis in high fat diet

**Cardioprotective/Vasoprotective**
- Decreases myocardial infarct size
  - Protects the Kidney from various insults
- Delays atherosclerosis in an Apo-E KO mice
Potential Roles for a Mitochondrial Peptide in Atherosclerosis

**Possible Mechanistic Pathways:**

- **Lipid Metabolism Modulation**
  - No evidence

- **Anti-inflammatory Activity**
  - Shown in Several Systems
  - HN is Expressed in Vessel Walls

- **Cytoprotective Effects**
  - Protects from Oxidative Stress
  - Reduces ROS Production

- **Mitochondrial Dysfunction**
  - Recognized Feature of Atherosclerosis
  - Mitochondrial Genetic Alterations Linked
MDPs inhibits ROS generation induced by oxidized LDL in endothelial cells

(Bachar et al, Cardiovascular Research 2010)
Humanin analogues do not change lipids or cytokines in ApoE-KO mice (Oh 2011)

### Experimental Design
APO-E KO mice
N=40 per group
16 week treatment
Low or High Cholesterol Diet
Saline or Humanin Analogue (0.4 mg/kg/day HNGF6A)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>APO-E</th>
<th>APO-E+ HN</th>
<th>APO-E High Chol</th>
<th>APO-E High Chol +HN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bwt (g)</td>
<td>21.7 ± 0.5</td>
<td>21.0 ± 0.3</td>
<td>24.3 ± 0.5*</td>
<td>24.9 ± 0.5*</td>
</tr>
<tr>
<td>T-Chol (mg/dL)</td>
<td>64.8 ± 3.1</td>
<td>68.4 ± 3.1</td>
<td>1150.4 ± 95.4*</td>
<td>1120.0 ± 87.7*</td>
</tr>
<tr>
<td>HDL-Chol (mg/dL)</td>
<td>45.4 ± 2.4</td>
<td>46.4 ± 3.0</td>
<td>201.6 ± 19.4*</td>
<td>208.6 ± 19.7*</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>65.2 ± 8.0</td>
<td>65.0 ± 5.5</td>
<td>88 ± 0.0</td>
<td>96.8 ± 17.7</td>
</tr>
</tbody>
</table>

*Indicates significant difference compared to control group.
Humanin analogues protects against atherosclerosis (and improves endothelial function) in ApoE-KO mice

0.4 mg/kg/day HNGF6A for 16 weeks

Oh et al; Atherosclerosis 2011
Endothelial function is normalized by humanin treatment.
Humanin prevents intra-renal microvascular remodeling and inflammation in APO-E KO mice

3-dimensional micro-CT images of the kidney

Humanin prevents intra-renal microvascular remodeling and inflammation in APO-E KO mice

3-dimensional micro-CT images of the kidney

Humanin

--- High Cholesterol ---
Humanin Reduces Tissue Inflammation

**MCP1**

**TNFa**

**OPN**
Acute Humanin Therapy Attenuates Myocardial Ischemia and Reperfusion Injury in Mice

Muzumdar 2010
HN Metabolic actions *in vivo*

- Improves blood sugar in Zucker rats (*Metab.* 2008)
- Prevents diabetes in NOD mice (*PLoS-ONE* 2009)
- Acts via STAT-3 in the hypothalamus
- The Atherosclerosis–Diabetes connection

![Graph](image-url)
Development of a Humanin Assay

- Found in plasma, CSF, seminal plasma
- Levels fall in older mice and humans (to about 30% of young levels)
- Higher levels in patients without family history of heart disease
- Related to Insulin Sensitivity, Obesity, Diet, and Exercise
- Low in Alzheimer’s CSF

Humanin levels are reduced in AD

* P<0.005

PloS One. 2009 Jul 22;4(7):e6334
Humanin & Longevity

GH transgenic
Low Humanin
High GH
Bigger
Short-lived
GHD (Ames) mice
Absent GH
Smaller
Long-lived

Humanin levels are higher in familial exceptional longevity

Humanin (ng/ml)

Control  GH-Tg

HN (pg/ml)

Control  Offspring

P<0.03

(Olfenarians)
Humanin levels are reduced in patients with endothelial dysfunction (Widemar 2012)

Plasma HN levels (pg/ml)

Endothelial function

Related to human endothelial function

A

%Change in Coronary Blood Flow (in response to max ACh)

Humanin (ng/ml)
Ethnic Disparity in the prevalence of heart disease
African-American SNPs within the mtDNA associated with disease

- G10398A - Breast Cancer
- T5655C - Deafness
- T921C - Possibly LVNC
- T7389C - Prostate Cancer
- T15942C - Possibly LVNC
- G5046A - Dementia
- T6221C - Prostate Cancer
- G6150A - Prostate Cancer
- A6663G - Prostate Cancer
- T7389C - Prostate Cancer
- G10398A - Breast Cancer
Ethnic Disparity and Humanin

- African Americans are at higher risk for heart disease & diabetes
- AA have distinct mitochondrial haplotypes
- 300 subjects (half AA and Half Whites) were studied from BVAIT
Humanin levels are regulated by B-vitamins

Levels of Humanin were measured 6-months after initiation of B-Vitamin Randomization

B-Vitamins supplementation resulted in improved outcomes in a subpopulation of patients with elevated homocysteine.
The human Mitochondrial Transcriptome
Mattick and colleagues.
Cell. 2011

Used deep sequencing to provide evidence of multiple, tissue specific, short to medium mito-mRNA species that do not conform to the 13 mitochondrial genes.

In our analysis, the 16S and other regions are enriched with polyA-mRNA

16S rRNA deep-seq

Presence of A vs. G at the rs2854128 SNP affects levels
Subjects with alternative SNP have significant decreases in humanin levels and Changed Coronary Calcium

Effect of SNPs at locus rs2854128

- **Coronary Calcium Present**
- **No Coronary Calcium**

**Genotype**
- Reference (G)
- Alternative (A)

**Number of Patients**
- ALT
- REF

*p<0.05*
Humanin a Mitochondrially-Derived Peptide (MDP): a new target for Atherosclerosis

- Metabolism & Survival
- Energetics & Protection
- Retrograde Communication Signals
- Apoptotic signals
MDPs: >70 Novel potential regulatory peptides

Small Humanin-Like Peptides
SHLPs

Mutations described in Various Diseases

(16,569 NUCLEOTIDES)
Translational Implications

• Humanin and other MDPs are clearly expressed and have functional significance
  – Regulate vascular function in vitro and in vivo
  – Regulate gene expression and metabolism
  – Do not regulate plasma lipids

• Developmentally regulated and reduced in aging and high Risk Groups

• May be important in multiple disease states (particularly atherosclerosis)
  – As novel therapeutic targets
  – As Diagnostic markers

• Multiple MDPs appear to be produced…

• Serve as retrograde signals from the mitochondria
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