Experimental Molecular Therapeutic Strategies for Treating Barth Syndrome: Elucidation of the Functional Role of the Mitochondrial Lipidome

Barth Syndrome Conference 2012

Michael Kiebish
All experiments and analysis were performed while at Washington University School of Medicine. The data and conclusions presented do not necessarily reflect the opinions of my current employer.
Cardiac lipidomic, metabolic, and adaptive mechanisms

Effects of cardiac specific upregulation of cardiolipin synthase and the subsequent lipidomic and bioenergetic effects

Targeting phospholipases by transgenically expressing/ablating iPLA$_{2\gamma}$ and its subsequent lipidomic effects

Novel interpretation of the role of the mitochondrial lipidome in health and disease as it relates to Barth syndrome
Multi-Omic Integrated Strategy to Elucidate Functional Changes in Pathophysiology

Lipidomic Analysis
Integrated Function
Transcriptomic Analysis

Signaling Lipidomic Analysis

Bioenergetic Analysis
Barth Syndrome Mouse Model

Cardiac Anionic Lipidomic Spectrum

Wildtype Mice

Tafazzin Knockdown Mice

DLCL

MLCL

CL

MLCL

Tafazzin Knockdown Mice

***

Major DLCL cluster 463.4

462.4

592.5

582.5

592.5

PS, IS 678.5

PG, IS 693.5

PS, IS 693.5

PG, IS 693.5

T14:0 CL, IS 619.5

Major CL cluster 723.5

744.6

747.5

747.5

744.6

747.5

747.5

PS, IS 678.5
Values represent the mean ± S.E. cardiolipin molecular species content (nmol/mg protein) in 2 month old WT and Tafazzin knockdown mice (N = 4)
Barth Syndrome Mouse Model

Respiratory and Enzymatic Characterization

State 3 Respiration

Adenine Nucleotide Translocase Activity

<table>
<thead>
<tr>
<th>Substrate Control Ratio</th>
<th>WT</th>
<th>TAZ KD</th>
</tr>
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<tbody>
<tr>
<td>Succinate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palm-Carn</td>
<td></td>
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<tr>
<td>Pyruvate</td>
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<tr>
<td>Glutamate</td>
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<table>
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<tr>
<th>Functional ANT Activity</th>
<th>WT</th>
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<tr>
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Barth Syndrome Mouse Model

Electron Transport Chain Activities

**Enzyme Activity**
(nmol/min/mg protein)

<table>
<thead>
<tr>
<th>ETC Complex</th>
<th>WT</th>
<th>TAZ KD</th>
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<tbody>
<tr>
<td>I</td>
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<td>II</td>
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<td>III</td>
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<td>IV</td>
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<tr>
<td>V</td>
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</tbody>
</table>

**Barth Syndrome Mouse Model**
Oxidized Lipidomics

Linoleic

DiHOMEs
HODEs
oxoODE
EpOME

Calcium Influx
Immune Response

Arachidonic

HETEs
EETs
Prostanoids

Vasodilation
Vasoconstriction
Inflammation

DHA

RVD
DiHDoHE
DiHDPA
DHoHE

Anti-Inflammatory
Barth Syndrome Mouse Model

Cardiac Oxidized Lipid Analysis

Oxidized Content (pg/mg tissue)

Linoleic Acid

Docosahexaenoic Acid

Arachidonic Acid

WT  TAZ KD
Barth Syndrome Mouse Model

Transcriptomics/GSEA Analysis

Aminoacyl tRNA Biosynthesis
Nucleotide Metabolism
Gene Expression
Valine, Leucine, and Isoleucine Biosynthesis
Hypoxia and p53 in Cardiovascular System
GTP Hydrolysis
One Carbon pool by Folate

Protein Translation
Peptide Chain elongation
Purine Metabolism
Amino Acid Synthesis and Interconversion
Metabolism of Proteins

Branched chain amino acid catabolism
IL12 and STAT4 Dependent Signaling Pathway in Th1 Development
Bioactive Peptide Induced Signaling Pathway
Valine, Leucine, and Isoleucine Degradation
Transcription factor CREB and its extracellular signals
TAK1/p38 MAPK Activation
Barth Syndrome Mouse Model

- Demonstrates the lipidomic abnormalities discovered in boys with Barth syndrome

- Displays altered substrate utilization (decreased fatty acid and increased glutamate stimulated respiration)

- Demonstrates altered mediator lipidomic signature

- Compensatory enzyme kinetics (Adenine nucleotide translocase and Electron transport chain) and gene expression (microarray analysis)
The CLS Model Provides a Molecular Therapeutic Tool to Investigate the Role of Cardiolipin in Attenuating Mitochondrial Dysfunction in Disease
Cardiolipin Synthase Mouse Model

**Cardiolipin Synthase Activity**

- **WT** vs **CLS**
- Activity measured in pmol/hour/mg protein

**Cardiolipin**

- Molecular species content (nmol/mg protein)
- Age (Days)

**Tetra18:2 Cardiolipin**

- Age (Days)
- Black Diamonds (Wildtype) and White Squares (CLS)

Kiebish *et al* 2012 (in press JBC)
Cardiolipin Synthase Mouse Model

Kiebish et al. 2012 (in press JBC)
Cardiolipin Synthase Mouse Model

Oxidized Lipid Species

18:2 → 20:4 → 22:6

[Graph showing the content of oxidized metabolites for LA, DHA, and AA with WT and CLS comparisons]
Cardiolipin Synthase Mouse Model

Mitochondrial Enzyme Activities

**Adenine Nucleotide Translocase Activity**

**Electron Transport Chain Enzyme Activity (nmol/min/mg mitochondrial protein)**

ETC Complex

I  II  III  IV  V

**Functional ANT Activity (nmol cATP/mg protein)**

Pyruvate  Palmitoyl-L Carnitine  Glutamate  Succinate

WT  CLS

*  **
Pyruvate = Normal
Glutamate =
Palm-Carnitine =

State 3 Respiration

Respiratory Substrate

OMM

IMM

Amino Acids

Fatty Acids

Sugars

TCA Cycle

Reductive Substrate

Palm-Carnitine =

Respiratory Substrate
Cardiolipin Synthase Mouse Model

Transcriptomics/GSEA Analysis

Aminoacyl tRNA Biosynthesis
P53 Signaling Pathway
Glycine, Serine, Threonine Metabolism
Amino Acid Synthesis and Interconversion

AHSP Pathway
Goal was to use cardiolipin synthase over expression in the heart to attenuate altered cardiolipin molecular species in Barth Syndrome, thus restoring the homeostatic balance of mitochondrial substrate utilization.
Values represent the mean ± S.E. cardioliopin molecular species content (nmol/mg protein) in WT, Taz, CLS, and Taz x CLS treated with doxycycline for 2 months (N = 4)
Values represent the mean ± S.E. cardiolipin molecular species content (nmol/mg protein) in WT, Taz, CLS, and Taz x CLS treated with doxycyline for 2 months (N = 4)
Values represent the mean ± S.E. choline glycerophospholipid molecular species content (nmol/mg protein) in WT, Taz, CLS, and Taz x CLS treated on doxycycline for 2 months (N = 4)
**Substrate Control Ratio**

**State 3 Ratio**

**Complex IV**

**Cytochrome Oxidase Activity (nmol oxygen/min/mg protein)**

- **WT WT**
- **WT CLS**
- **TAZ WT**
- **TAZ CLS**

- **Succ (M) Rot**
- **Glut (M)**
- **Palm-Carn (M)**
- **Pyr (M)**

* and ** symbols indicate statistical significance.
Although cardiolipin molecular species were less remodeled in the Taz X CLS mouse model, the overall bioenergetic phenotype was attenuated, possibly suggesting that alternative factors may be influencing bioenergetics.
Does iPLA$_{2\gamma}$ expression attenuate dysfunctional cardiolipin remodeling as well as effect survival of the cross?
Values represent the mean ± S.E. cardiolipin molecular species content (nmol/mg protein) in WT, Taz, Taz x iPLA$_{\gamma}$KO, and Taz x iPLA$_{\gamma}$-TG treated on doxycycline for 2 months (N = 4)
Conclusion

- Through the utilization of various transgenic models as well as investigating dynamic flux of the lipidome, we can elucidate the lipidomic/bioenergetic connection that initiates pathological sequelae or that can be identified for therapeutic efficacy.

- Regulation of the mitochondrial lipidome may hold unknown mechanism that go well beyond just structure and enzyme kinetics.

- The inducible Tafazzin shRNA knockdown mouse model of Barth Syndrome is an invaluable tools to discover unknown mechanism that embody the Barth Syndrome phenotype which can be therapeutically targeted.
Acknowledgements

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Washington University School of Medicine

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Dr. Chris Jenkins

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Mr. Harold Sims
Mrs. Zhongdan Zhao
Mrs. Hua Cheng
Mrs. Shaoping Guan
Values represent the mean ± S.E. ethanolamine glycerophospholipid molecular species content (nmol/mg protein) in WT, Taz, CLS, and Taz x CLS treated on doxycycline for 2 months (N = 4)
Cardiolipin Synthase Mouse Model

Cardiolipin Molecular Species

A. WT

B. CLS

Kiebish et al 2012 (in press JBC)