The Linking of Cardiolipin Remodeling to Mitochondrial β-oxidation and Cardiolipin to T-cell function

Grant M. Hatch
Department of Pharmacology & Therapeutics
Manitoba Institute of Child Health (MICH)
Faculty of Medicine, University of Manitoba
Winnipeg, Manitoba, Canada
Synopsis

• Brief Introduction to cardiolipin

• Role of Stomatin-like protein 2 in altering cardiolipin and mitochondrial function in T-cells

• Role of α subunit of Human Trifunctional Protein in cardiolipin remodeling
Cardiolipin – a unique phospholipid!

- Cardiolipin is a unique phospholipid.
- It has a unique structure with two glycerol molecules connected by phosphodiester bridges.
- It contains four fatty acid chains.
- Cardiolipin is important in the structure of the inner mitochondrial membrane.
Cardiolipin

1. Major mitochondrial membrane phospholipid

   - comprises 7-16% of the entire phospholipid mass of the cell depending upon the tissue

   - 21% of phospholipid mass of the inner mitochondrial membrane

   - synthesized on mitochondrial inner membrane
Cardiolipin

1. Major mitochondrial membrane phospholipid

2. Required for activation of enzymes of electron transport chain/respiratory supercomplex assembly

- both content and fatty acid composition are important

- e.g. delipidated cytochrome oxidase is reconstituted by addition of cardiolipin

- cardiolipin is the “glue” that holds the respiratory chain supercomplexes together
Cardiolipin

1. Major mitochondrial membrane phospholipid

2. Required for activation of enzymes of electron transport chain

3. Role in protein and lipid import into mitochondria

- CDP-DG, phosphatidylserine, malate dehydrogenase, ornithine carbamyltransferase precursor proteins
Cardiolipin

1. Major mitochondrial membrane phospholipid

2. Required for activation of enzymes of electron transport chain

3. Role in protein and lipid import into mitochondria

4. Regulator of apoptosis
   - required for caspase-8 activation
   - CL/MLCL binds to Bid/t-Bid, regulated by PLS3
   > cytochrome c release from mitochondria
Mammalian Cardiolipin *de novo* Biosynthesis: “The CDP-DG Pathway”

Phosphatidic acid

- CTP
- PP$_i$

CDP-DG

Phosphatidylglycerolphosphate

- G-3-P
- CMP

PGPS

Phosphatidylglycerol

- P$_i$

PTPMT1

Phosphatidylglycerol

CDP-DG

Cardiolipin
Human and Mammalian Cardiac Cardiolipin is Highly Enriched with 18:2 (Tetralinoleoyl-CL or L₄-CL)

<table>
<thead>
<tr>
<th>Major Cardiolipin Species</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>sn-1-sn-2</strong></td>
<td><strong>sn-2-sn-1</strong></td>
<td><strong>%</strong></td>
</tr>
<tr>
<td>18:2-18:2</td>
<td>18:2-18:2</td>
<td>80</td>
</tr>
<tr>
<td>18:2-18:1</td>
<td>18:2-18:2</td>
<td>12</td>
</tr>
<tr>
<td>18:1-18:2</td>
<td>18:2-18:2</td>
<td></td>
</tr>
</tbody>
</table>

Cardiolipin Remodeling Pathways

1. ALCAT1
2. Tafazzin (TAZ)
3. MLCL AT-1
4. Them5?

Synopsis

• Brief Introduction to cardiolipin

Role of Stomatin-like protein 2 in altering cardiolipin and mitochondrial function in T-cells

Role of α subunit of Human Trifunctional Protein in cardiolipin remodeling
**Stomatin-like protein 2 (SLP-2)**

- stomatin – prohibitin – flotillin – HflC/K (SPFH) superfamily

- highly conserved family of proteins that mediate interactions with cell membranes

- upregulated in many cancers

- modulates MMP and ATP production
SLP-2 is Upregulated Upon Peripheral Blood Mononuclear Cell Activation
SLP-2 Localizes to the Immunological Synapse Upon Jurkat T-Cell Activation

SLP-2 expression increases effector responses whereas down-regulation of SLP-2 correlates with loss of TCR signaling and activation (Kirchhof et al. 2008 J. Immunol.)
SLP-2 is Localized Primarily to Mitochondria in Jurkat T-Cells
Expression of SLP-2 in T-cells Increases Number of Metabolically Active Mitochondria
SLP-2 Expression in T-Cells Stimulates Cardiolipin and Mitochondrial Biogenesis
SLP-2 Binds to Cardiolipin

<table>
<thead>
<tr>
<th>Total Protein</th>
<th>CL</th>
<th>PC</th>
<th>PE</th>
<th>PI</th>
<th>PS</th>
<th>PG</th>
<th>PC+PE</th>
<th>CL+PC+PE</th>
<th>No Lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
</tr>
</tbody>
</table>

hrSLP-2
SLP-2 Interacts with Prohibitins

SLP-2 IP

<table>
<thead>
<tr>
<th>Beads</th>
<th>WCL</th>
<th>SLP-2</th>
<th>Pre-imm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PHB1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PHB2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>SLP-2</td>
<td></td>
</tr>
</tbody>
</table>

SLP-2 Immunoprecipitation

Whole Cell Lysate

Doxycycline (μg/mL): 0, 0.05, 0.1, 0.25, pre-imm

<table>
<thead>
<tr>
<th>37 -</th>
<th>25 -</th>
<th>37 -</th>
<th>25 -</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PHB1</td>
<td>PHB2</td>
<td>SLP-2-GFP</td>
<td>SLP-2</td>
</tr>
</tbody>
</table>
SLP-2 Expression in T-Cells Stimulates Mitochondrial Activity and Interleukin-2 Secretion in Response to T-Cell Stimulation
T-Cell Specific SLP-2 Knock Out Mice Exhibit Near Normal T-cell Number

**Graphs and Biological Data:**

- **Graph 1:** Comparison of cell count across different cell types (Thymus, Spleen, Lymph Node) between WT (solid bars) and KO (open bars).
- **Graph 2:** Expression levels of SLP-2 and GAPDH in different cell types (Naive T Cells, Blast T Cells, non T Cells) for WT (solid bars) and KO (open bars).

**Legend:**
- Wt – solid bars
- KO – open bars
T-Cells Deficient in SLP-2 Exhibit Reduced Mitochondrial Complex Protein/Activities, insoluble Prohibitin-1 and Cardiolipin
T-Cell Specific SLP-2 Knock Out Reduces *in vivo* Interleukin-2 Secretion in Response to T-Cell Stimulation and Delays Cardiac Allograft rejection
Summary I:

SLP-2 expression in T-cells stimulates cardiolipin and mitochondria biogenesis/activity and T-cell ability to respond to stimuli


SLP-2 KO in T-cells reduces cardiolipin in PHB complex fractions, mitochondrial metabolism and T-cell ability to respond to stimuli linking cardiolipin to T-cell function

*Christie et al., J. Immunol. 2012 (in revision)*

SLP-2 functions to recruit prohibitins to cardiolipin-enriched microdomains in which ETC complexes are optimally assembled
Synopsis

• Brief Introduction to cardiolipin

• Role of Stomatin-like protein 2 in altering cardiolipin and mitochondrial function in T-cells

• Role of α subunit of Human Trifunctional Protein in cardiolipin remodeling
Expression of human MLCL AT-1 in Barth Syndrome lymphoblasts (patient ΔTAZ1) increases cardiolipin and mitochondrial Complex II + III activity.

* p<0.05

Human MLCL AT-1 is a shortened version (59 kDa) of the α subunit of Human Trifunctional Protein (74 kDa)
Human MLCL AT-1 is a shortened version (59 kDa) of the α subunit of Human Trifunctional Protein (74 kDa)

Trifunctional protein

- multifunctional, membrane-bound beta-oxidation enzyme protein catalyzing three enzyme activities:
  - long-chain enoyl-Coenzyme A hydratase
  - long-chain 3-hydroxyacyl-Coenzyme A dehydrogenase
  - long-chain 3-oxoacyl-Coenzyme A thiolase

- heterocomplex of two subunits, 4 alpha and 4 beta
Human recombinent α subunit of Trifunctional Protein exhibits MLCL AT \textit{in vitro} activity and stimulates [1-^{14}\text{C}]fatty acid incorporation into cardiolipin in Hela cells.
MLCL AT-1 is likely a splice variant of the α subunit of Trifunctional Protein

\[ \alpha \text{TFP} \quad \beta \text{-actin} \]

\[ \text{ON-1 OFF-1 ON-2 OFF-2} \]

\[ \text{ON-1 OFF-1 ON-2 OFF-2} \]

\[ \text{ON-1 OFF-1 ON-2 OFF-2} \]

\[ *p<0.05 \]
Expression of MLCL AT-1 or α subunit of Trifunctional Protein in Normal or BTHS Lymphoblasts increases L₄-cardiolipin

Lysophospholipid | Enzyme activity (pmol/min/mg protein)
--- | ---
Monolysocardiolipin | 56.3 ± 1.3
Lysophosphatic acid | ND
Lysophosphatidylglycerol | ND
Lysophosphatidylcholine | ND
Lysophosphatidylethanolamine | ND

CTRL | αTFP | MLCL AT-1
--- | --- | ---
Relative Gene Expression (2^-ΔACT)

BTHS | αTFP | MLCL AT-1
--- | --- | ---
Relative Gene Expression (2^-ΔACT)

LLLL | N | N59 | N74 | B | B59 | B74
--- | --- | --- | --- | --- | --- | ---
CL (nmol/mg)

* p<.05
* p<.001
MLCL AT enzyme activity is not increased by knock down of TAZ in normal human lymphoblasts but MLCL AT-1 expression restores [1-\(^{14}\)C]linoleate into cardiolipin after TAZ knock down

\[ \ast p < .001 \]
Summary II:

αTFP exhibits MLCL AT activity and expression of αTFP stimulates cardiolipin remodeling with linoleate and increases L₄-cardiolipin levels in normal and BTHS lymphoblasts linking an enzyme of β-oxidation to cardiolipin remodeling

*Taylor et al. Biochem. J. 2012 (submitted)*

MLCL AT-1 activity is not increased by TAZ knock down indicating that TAZ and MLCL AT-1 may not complement each other in cardiolipin remodeling but MLCL AT-1 expression may compensate for loss of TAZ
Current Studies:

1. Will expression of MLCL AT-1 or αTFP in Taz knock down mice attenuate development of the cardiac defects?

2. Role of SLP-2 in mitochondrial dysfunction in BTHS
Acknowledgements

Hatch lab:

William Taylor, Fred Xu, Edgard Mejia, Laura Cole

Darah Christie, Quim Madrenas – Robarts Research Institute, McGill University
Genevieve Sparagna, Robert Murphy – University of Colorado at Boulder
Zaza Khuchua – Childrens’ Hospital Medical Center, Cincinnati

Funding: