Modeling Barth Syndrome using Patient-Specific, iPSC-derived Cardiomyocytes

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Harvard Stem Cell Institute

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BSF Foundation Meeting
Barth Syndrome -- a reversible cardiomyopathy?

- known single gene defect involving a metabolic pathway
- waxing and waning disease course rather than irreversibly progressive disease
- progress slowed until recently by lack of mammalian model systems
Induced Pluripotent Stem Cells

Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

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Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors

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Normal iPSC: human embryogenesis

iPSC

Renewable, patient-specific disease model, e.g. LQTS, HCM, 22q11 del
- phenotype-genotype
- drug screening
- mutation discovery?

Patient fibroblast

Replacement Therapy
Why an iPS model of BTHS?

- Human loss of function model, useful for studies of disease mechanism and interindividual variation.
- Renewable, patient-specific model of TAZ mutation for preclinical testing of BTHS treatment strategies.
- Potential for high throughput drug screening to discover novel approaches to treatment.
iPSC Model of Barth Syndrome

1. Generation and characterization of 2 iPSC lines, one from each of 2 patients with BTHS.

2. Differentiation and purification of BTHS iPSC-derived cardiomyocytes (iCMs).

3. Analysis of mitochondrial abnormalities in a neonatal rat ventricular cardiomyocyte model.

4. Analysis of mitochondrial abnormalities in BTHS iCMs.

5. Treatments to reverse BTHS iCM mitochondrial abnormalities.
## iPSC Models of Barth Syndrome

<table>
<thead>
<tr>
<th></th>
<th>BTH-H</th>
<th>BTH-C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mutation</strong></td>
<td>ATGGGGGACTG</td>
<td>ACACCTCCAC</td>
</tr>
<tr>
<td></td>
<td>ATGGGGACTG</td>
<td>ACACCCCCAC</td>
</tr>
<tr>
<td><strong>Protein</strong></td>
<td>Asp173Thr.fsX11</td>
<td>Ser110Pro</td>
</tr>
<tr>
<td><strong>Reprog.</strong></td>
<td>OSKLM retroviral</td>
<td>OSKML modRNA</td>
</tr>
</tbody>
</table>
BTHH iPSC Quality Control

ESC-like morphology in feeder free culture

Expression of pluripotency markers

Nanog

SSEA-4

Oct4

H7

BTHH
BTHH iPSC Quality Control

Karyotype

Teratoma Assay

46 XY
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# MACS CM Purification

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<th>Cardiogenesis</th>
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<td>Matrigel + RPMI-1640 + B27 - Insulin</td>
<td>no growth factors</td>
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- **Activin A** 100 ng/ml (Day 1)
- **BMP4** 10 ng/ml (Day 5)
- ~20% iCM (Day 12-19)
## MACS CM Purification

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<td>Day 12-19</td>
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**Control** vs **BTHH**

- **DAPI**
- **TNNI3**
### MACS CM Purification

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<td>Day 12-19</td>
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</table>

**Images:**
- **DAPI TNNI3**
  - Control
  - BTHH
- **Control**
  - BTHH
- **SIRPα**
  - Control
  - BTHH
- **VCAM1**
  - Control
  - BTHH
iCM Beating

Ctrl    BTHH    BTHC
MACS iCM Purification

Matrigel
Diff to iCMs

isotype (0%)
VCAMI (19%)

% Max

VCAMI
MACS iCM Purification

Matrigel Diff to iCMs → Collagenase VCAM1 MACS → Enriched iCMs

- isotype (0%)
- VCAM1 (19%)

% Max

VCAM1

- isotype (0%)
- VCAM1 (83%)

% Max

VCAM1

VCAM1

TNNT2
MACS iCM Purification

Matrigel Diff to iCMs → Collagenase V CAM1 MACS → Enriched iCMs → Gelatin/Fibronectin Plating → Assay

isotype (0%) V CAM1 (19%)

% Max

VCAM1

isotype (0%) V CAM1 (83%)

% Max

VCAM1

Actinin DAPI

% Max

VCAM1

TNNT2
CL deficiency in BTHS iCMs
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Seahorses Biosciences
extracellular flux analyzer

Extracellular acidification rate (ECAR): glycolysis

Oxygen consumption rate (OCR): oxidative respiration
Probing mitochondrial function

Graph: OCR vs TIME (Avg)

Diagram: Mitochondrial electron transport chain
- Protons (H+) movement: $\Delta$H+
- Enzymes: NAD+, FAD, Cyt c
- Reactions: NADH + H+ → NAD+ (I), FADH₂ → FAD (II), ATP synthase (IV)
- Products: ATP, ADP + Pi, O₂, H₂O
- Heat dissipation: UCP
Probing mitochondrial function

**OCR vs TIME(Avg)**

- **OCR (pMoles/min)**
  - 1300
  - 1100
  - 900
  - 700
  - 500
  - 300
  - 100
  - 0
  - -100

- **TIME (min)**
  - 0
  - 10
  - 20
  - 30
  - 40
  - 50
  - 60
  - 70
  - 80
  - 90
  - 100
  - 110
  - 120

**Basal Resp**
Probing mitochondrial function

**OCR vs TIME(Avg)**

- OCR (pMoles/min)
- TIME (min)

**Basal Resp**
- ATP Gen
- H^+ Leak

**A. Oligomycin**
Probing mitochondrial function

OCR vs TIME (Avg)

- Basal Resp
- ATP Gen
- H⁺ Leak
- Resp Capacity

A. Oligomycin
B. FCCP
Probing mitochondrial function

OCR vs TIME(Avg)

Basal Resp
ATP Gen
H⁺ Leak
Resp Capacity

Non-mitochondrial

A. Oligomycin
B. FCCP
C. Antimycin
NRVM TAZ Knockdown Model

NRVM → TAZ shRNA adeno → Assay

3 days

Validation of Knockdown

<table>
<thead>
<tr>
<th>No</th>
<th>NT</th>
<th>kd1</th>
<th>kd2</th>
<th>1+2</th>
<th>kd3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>1.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Rel TAZ Expr
NRVM TAZ Knockdown Model

TAZ shRNA adeno
NRVM → 3 days → Assay

Validation of CL Depletion
Mitochondrial morphology in NRVM TAZ Knockdown Model

CTRL

TAZ shRNA

Mito Area/Cell

Area (%) 55

Ctrl TAZkd

Mito Num/CM section

Ctrl TAZkd
NRVM TAZ Knockdown Model

NRVM → TAZ shRNA adeno → 3 days → Assay

Intracellular ATP content

<table>
<thead>
<tr>
<th></th>
<th>pmol/μg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctrl</td>
<td>20</td>
</tr>
<tr>
<td>shTAZ1</td>
<td>10</td>
</tr>
<tr>
<td>shTAZ2</td>
<td>10</td>
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NRVM TAZ Knockdown Model

NRVM → TAZ shRNA adeno → Assay

3 days

Mitochondrial Phenotype

[Graph showing OCR vs TIME (Avg) for BTHH Ctrl]
Mitochondrial function after TAZ depletion in NRVM

- Basal Resp
- Resp Capacity
- ATP Gen
- Resp Reserve

Legend:
- Ctrl shRNA
- TAZ shRNA

Graphs show OCR vs TIME (Avg) with markers indicating Reserve at points A, B, and C.

Bar charts compare Basal Resp, Resp Capacity, ATP Gen, and Resp Reserve between Ctrl shRNA and TAZ shRNA conditions.
**Baseline**

CL deficiency makes F0F1 ATP synthase activity limiting

- Incr transmemb gradient
- Decr ATP

**Stress**

- Reduced ETC activity
- Decr Resp Reserve
Summary: NRVM TAZ kd model of BTHS

1. Rapid TAZ and CL depletion indicate short half-life of both.

2. Mitochondrial functional abnormalities occur prior to detectable morphological abnormalities.

3. Primary effect of CL deficiency at baseline is limitation of F0/F1 ATP synthase activity.
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Analysis of BTHS iCM Mitochondrial Activity

OCR vs TIME (Avg)

Basal Resp
Non-mitochondrial
ATP Gen
H^+ Leak
Resp Capacity

OCR (pMoles/min)

BTHH
Ctrl

TIME (min)
Analysis of BTHS iCM Mitochondrial Activity

Higher basal OCR and decreased ATP content suggest decreased mitochondrial efficiency.
Is the phenotype due to TAZ mutation or to other genetic difference?

1. Analysis of a neonatal rat ventricular CM TAZ knockdown model.

2. TAZ modRNA rescue of the BTHS iPSC phenotype
TAZ modRNA Rescue of BTHS iCM Mitochondrial Phenotype

TAZ Flag

T7

IVT

5me-CTP
Pseudouridine

TAZ modRNA

modRNA daily transfection

iCM diff and purification

Reseed

Assay
Efficient modRNA transfection into NRVMs

NRVM

tx nGFP modRNA

Imaging

DAPI nGFP Actinin
TAZ modRNA Rescue of BTHS iCM Mitochondrial Phenotype

modGFP
TAZ modRNA Rescue of BTHS iCM Mitochondrial Phenotype

modGFP

GFP
Actinin
Merge

modTAZ

Flag-TAZ
mito-RFP
Merge Actinin
TAZ overexpression did not cause substantial mitochondrial phenotype

Control iCMs

[Graph showing OCR over time with 'modGFP' and 'modTAZ' conditions]
TAZ modRNA Rescue of BTHS iCM Mitochondrial Phenotype
TAZ modRNA Rescue of BTHS iCM Mitochondrial Phenotype

OCR vs TIME(Avg)

OCR (pMoles/min)

Basal Resp

Non-mitochondrial

ATP Gen

H^+ Leak

Resp Capacity

OCR (pMol/min/10 µg protein)

Time Point

BTHH iCM + modGFP

Ctrl iCM + modGFP
TAZ modRNA Rescue of BTHS iCM Mitochondrial Phenotype
TAZ modRNA Rescue of BTHS iCM Mitochondrial Phenotype

![Graph showing OCR vs TIME(Avg) with labels for Reserve, Basal Resp, Non-mitochondrial, ATP Gen, H^+ Leak, Resp Capacity.](image)

![Bar graphs showing Basal Resp, Resp Capacity, ATP Gen, Resp Reserve with different conditions.](image)

- **Basal Resp**
- **Resp Capacity**
- **ATP Gen**
- **Resp Reserve**

Legend:
- BTHH iCM + modGFP
- BTHH iCM + modTAZ
- Ctrl iCM + modGFP
Results replicated in BTHC iCMs

- OCR vs TIME (Avg)
- Reserve
- Non-mitochondrial
- Basal Resp
- ATP Gen
- H^+ Leak
- Resp Capacity

Bar graphs showing:

- Basal Resp
- Resp Capacity
- ATP Gen
- Resp Reserve

Comparing:
- BTHC iCM + modGFP
- BTHC iCM + modTAZ
- Ctrl iCM + modGFP
Summary of BTHS iCM mitochondrial abnormalities
Summary of BTHS iCM mitochondrial abnormalities

- Mirror abnormalities seen in NRVM knockdown model.
Summary of BTHS iCM mitochondrial abnormalities

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- modTAZ confirms BTHS iCM metabolic defects are due to TAZ deficiency rather than other genetic differences.
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- Provides positive control for development of assays to screen for therapeutic compounds
Summary of BTHS iCM mitochondrial abnormalities

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- modTAZ confirms BTHS iCM metabolic defects are due to TAZ deficiency rather than other genetic differences.
- Data further demonstrate that the BTHS iCM metabolic defects are rapidly reversible.
- Provides positive control for development of assays to screen for therapeutic compounds
- TAZ modRNA overexpression in control iCMs did not cause measurable abnormalities >>> amenable to gene therapy.
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Targeted Barth Syndrome Treatment

\[ \text{mito PLA}_2 \rightarrow \text{MLCL} \rightarrow \text{CL} \]

TAZ

\[ (18:2)_4 \text{ CL} \]

\[ \text{MLCL} \rightarrow \text{MLCL AT-1} \rightarrow \text{acyl-CoA} \rightarrow \text{CoA} \]
Targeted Barth Syndrome Treatment

Valianpour, J. Lipid Res., 2003: LA increased CL in BTHS fibroblasts
Targeted Barth Syndrome Treatment

\[ \text{mito PLA}_2 \xrightarrow{} \text{MLCL} \xrightarrow{} \text{TAZ} \xrightarrow{(18:2)_4 \text{CL}} \text{MLCL AT-1} \xrightarrow{\text{acyl-CoA}} \text{CoA} \]

- Bromoenol Lactone
- Linoleic Acid

Valianpour, J. Lipid Res., 2003: LA increased CL in BTHS fibroblasts
Malhotra et al., PNAS, 2009: Bromoenol lactone increased CL in BTHS lymphocytes
Targeted Barth Syndrome Treatment

The diagram illustrates the TCA cycle, showing the metabolic pathways involving pyruvate, acetyl CoA, and various intermediates like oxaloacetate, citrate, isocitrate, and α-ketoglutarate. The cycle involves multiple reactions catalyzed by enzymes, resulting in the production of ATP and other metabolic products.
Targeted Barth Syndrome Treatment

R. Kelley: low R and C in some BTHS serum. FS improved with supplementation.
Targeted Barth Syndrome Treatment

ATP Content (arbitrary units)

Rx
- no
- modTAZ
- Linoleic Acid
- PLA2 Inhib
- Arg + Cys

iPSC Ctrl
Targeted Barth Syndrome Treatment

ATP Content (arbitrary units)

Rx
- no
- modTAZ
- Linoleic Acid
- PLA₂ Inhib
- Arg + Cys

iPSC
- Ctrl

ATP Content (arbitrary units)

Rx
- no
- no
- modTAZ
- Linoleic Acid
- PLA₂ Inhib
- Arg + Cys

iPSC
- Ctrl
- BTHH
Rescue of BTHS mitochondrial abnormalities by linoleic acid
Summary: treatments to rescue BTHS iCM mitochondrial abnormalities
Summary: treatments to rescue BTHS iCM mitochondrial abnormalities

- Established a renewable, human cardiomyocyte model for analyzing proposed treatments of BTHS cardiomyopathy.
Summary: treatments to rescue BTHS iCM mitochondrial abnormalities

- Established a renewable, human cardiomyocyte model for analyzing proposed treatments of BTHS cardiomyopathy.
- Showed that linoleic acid and arginine + cysteine supplementation normalize mitochondrial function in BTHS iCMs.
Overall Summary

BTHS skin fibro

↓

OSKM

↓

BTHS iPSC
Overall Summary

BTHS skin fibro

OSKM

CM diff

BTHS iPSC

BTHS iCM
Overall Summary

BTHS skin fibro → OSKM → CM diff → BTHS iCM

inefficient mito fn
  • low ATP, high OCR
  • reduced resp reserve

modTAZ rescue shows rapid reversibility platform for screening of therapeutic strategies
  • linoleic acid, R+C suppl
Recruitment

Seeking additional patients with BTHS for generation of iPS cells.

- informed consent.
- ~2 mm skin punch biopsy, obtained with topical local anesthetic.

Distribution

- Both BTHH and BTHC low passage fibroblasts available from BSF biorepository -- need to address expansion and further distribution.
- Will soon distribute BTHH and BTHC iPSCs lines via a stem cell repository, e.g. Wicell.
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