The Role of Post-Translational Modifications in SERCA2a-Related Cardiac Dysfunction

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Cardiac SR Ca\textsuperscript{2+}-ATPase (SERCA2a) is a Promising Therapeutic Target in Heart Failure

- Impaired Ca\textsuperscript{2+} re-uptake resulting from (i) decreased expression and (ii) reduced SERCA2a activity is a hallmark of heart failure

- Gene therapy: Phase 2b/3 human clinical trials of SERCA2a replacement therapy (CUPID)
Isoelectric Point (pI) Distribution of SERCA2a is Altered in Porcine Failing Hearts

HF, Heart Failure
HFS, Failing heart with SERCA2a gene transfer
Diverse Lysine Modifications: 
Key Role of Lysine Residues

- Ubiquitination
- Acetylation
- Methylation
- SUMOylation
- Glycation
- Phosphoglycerylation

“Lysine”

Competition
Cooperation
\[ \Delta \text{ Activity} \]
Key Findings from **Small Ubiquitin-like Modifier 1 (SUMO-1)** Gene Transfer Experiments in Animal Models of Heart Failure

Levels of SUMO-1 protein and SUMOylation of SERCA2a are reduced leading to an enzymatic dysfunction and de-stabilization of SERCA2a during HF.

Increased SERCA2a SUMOylation via SUMO-1 overexpression rescues HF phenotype in murine and porcine models of HF. This beneficial effect is SERCA2a dependent.

Overexpression of SUMO-1 by gene transfer protects SERCA2a from oxidative stresses.

Combined delivery of SUMO-1 and SERCA2a suggests additional beneficial effects on the molecular level in a porcine model of HF.

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Lee et al (2014) Antioxidant Redox & Signaling, in-press
Hypothesis

Post-translational mechanisms causing impairment of SERCA2a function may play an important role in the setting of heart failure.

Aims

To understand the biological consequences of acetylation of SERCA2a on cardiac function and the possible relationships of acetylation to SUMOylation.
SERCA2a Acetylation is Significantly Increased in Failing Hearts

A. Mouse

B. Human

Sham        2M TAC

Acetyl SERCA2a

IP: SERCA2a
IB: Acetyl-Lys

SERCA2a

IP: SERCA2a
IB: Acetyl-Lys

Sham       2M TAC

Ac-SERCA2a/SERCA2a Band intensity (AU)

kDa
100

p < 0.05

Normal             HF

Acetyl SERCA2a

IP: SERCA2a
IB: Acetyl-Lys

SERCA2a

kDa
100

p < 0.05

Normal             HF

Ac-SERCA2a/SERCA2a Band intensity (AU)
SUMOylation of SERCA2a Antagonizes its Acetylation

**A**

AAV9. Scramble  
AAV9. shSUMO1

- **Acetyl SERCA2a**
  - kDa: 100
  - IP: SERCA2a, IB: Acetyl-Lys

- **SUMOylated SERCA2a**
  - kDa: 250, 150
  - IP: SERCA2a, IB: SUMO-1

- **SUMO-1**
  - kDa: 15, 37

- **GAPDH**
  - kDa: 15, 37

**B**

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>TAC</th>
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<tr>
<td>AAV9.GFP</td>
<td>AAV9.GFP</td>
<td>AAV9.GFP</td>
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</table>

- **Acetyl SERCA2a**
  - kDa: 100
  - IP: SERCA2a, IB: Acetyl-Lys

- **SUMOylated SERCA2a**
  - kDa: 250, 150
  - IP: SERCA2a, IB: SUMO-1

- **SUMO-1**
  - kDa: 15, 37

- **GAPDH**
  - kDa: 15, 37
Sirt1 (Sirtuin 1, NAD+-dependent deacetylase) is identified as a SERCA2a Deacetylase

**A**

IP : IgG  
SERCA2a  
2-DE gel

**B**

IP : IgG  
SERCA2a  
Sirt1  
Adult myocyte lysates

**C**

NAD+  
Sirt1  
Acetyl

Substrate  
Acetyl-Lys  
NAM + O-AADPR  
in vitro assay  
SERCA2a

**Identified as a SERCA2a Deacetylase**
Sirt1 Expression and its Cofactor, NAD⁺, levels are Decreased in Failing Hearts

A

Sham

2M TAC

Sirt1

GAPDH

kDa

100

37

B

p < 0.05

Band intensity (AU)

p < 0.02

Sham

TAC

NAD⁺ (nmol/g tissue)

Sham

TAC
Sirt1 Knockdown Increases SERCA2a Acetylation and Reduces its Function

A

Scramble | si-Sirt1
---|---
100 kDa Acetyl SERCA2a
IP: SERCA2a
IB: Acetyl-Lys

100 kDa SERCA2a

100 kDa Sirt1

37 kDa GAPDH

25 kDa P-PLN (Ser16)

25 kDa Total PLN

B

SR Ca^{2+} uptake (% V_{max})

\[ \text{[Ca}^{2+}] \] (μM)

Scramble | si-Sirt1
---|---

ATPase activity (nmol/min/mg)

Scramble | si-Sirt1
---|---

*
Sirt1 Silencing Increases SERCA2a Acetylation and Induces Cardiac Dysfunction in Mice

A

IP: SERCA2a
IB: Acetyl-Lys

Acetyl SERCA2a
SERCA2a
Sirt1
GAPDH

B

SR Ca^{2+} uptake (%V_{max})

pCa

AAV9.Scr
AAV9.shSirt1

C

FS (%)

AAV9.Scr
AAV9.shSirt1

p < 0.001

LVIDs (cm)

AAV9.Scr
AAV9.shSirt1

p < 0.001
Sirt1-Targeted Acetylated Residues in SERCA2a may be Important for ATP Binding and SUMOylation

TAC mouse heart treated with Sirt1 activator
Mutants Mimicking Acetylation in Sirt1-Targeted Lysines Decrease SERCA2a’s Function

K-Q mutant: mimicking constitutive acetylation
Summary & Conclusion

- Our data suggests a pathological role of acetylated SERCA2a in the setting of heart failure.
- SUMOylation of SERCA2a may prevent its ability to be acetylated.
- We provide evidence that SERCA2a is a direct substrate of Sirt1 deacetylase.
- Our findings highlight the pathological importance of post-translational modification and suggest that SERCA2a activity might be manipulated by post-translational modification.
Proposed Model

NORMAL

SERCA2a

S

S

HEART FAILURE

SERCA2a

Ac Ac

Therapeutic Approaches

• Small Molecule Activation of SERCA2a SUMOylation

• Gene therapy (SUMO-1 or SUMO-SERCA2a)

• Blocking SERCA2a Acetylation
Acknowledgements

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Thank you for your attention!
Sirt1-mediated Serca2a regulation could be a target for Pharmacologic Invention: *Beta-Lapachone, a Modulator of NAD Metabolism*

Natural quinone compound from the bark of the lapacho tree.

![Diagram](image-url)
NAD⁺-Dependent Activation of Sirt1 Provides Cardioprotection in Mice

**Graphs and Data**

- **Acetyl SERCA2a**
  - IP: SERCA2a
  - IB: Acetyl-Lys

- **FS (%)**
  - Veh bL Veh bL
  - Sham TAC
  - p < 0.05
  - n = 4
  - n = 3
  - n = 3
  - n = 4

- **SR Ca²⁺ uptake (% Vmax)**
  - pCa
  - Sham Veh
  - Sham bL
  - TAC Veh
  - TAC bL
  - p < 0.05

- **LVIDs (cm)**
  - Veh bL Veh bL
  - Sham TAC
  - p < 0.05
  - n = 4
  - n = 3
  - n = 3
  - n = 4
SERCA2a Acetylation is Regulated by Sirt1 Activity

Ex527, small molecule Sirt1 inhibitor
REV (Resveratrol), Sirt1 activator
Antibody Test: SERCAK492Ac

+Ex527

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<th>WT</th>
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<th>K451A</th>
<th>K492A</th>
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<td>IP: Flag (SERCA2a)</td>
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<tr>
<td>IB: SERCAK492Ac</td>
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<tr>
<td>Acetyl SERA2a at K492</td>
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IP: Flag (SERCA2a)  IB: Acetyl-lysine

Acetyl SERA2a

IB: Flag (SERCA2a)  

SERA2a

Ex527, small molecule Sirt1 inhibitor