The Role of Autophagy in Ischemic Heart Disease

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DISCLOSURE INFORMATION
No relevant financial relationship exists
Autophagy
(Greek for “self-eating”)

- A major mechanism of degradation for cytoplasmic materials (proteins and organelles) through lysosome
- Sequestration by double membrane vesicles termed autophagosomes (macroautophagy)
- Relatively non-specific and large capacity but could be targeted, such as mitophagy
Autophagy is activated during both ischemia and reperfusion with distinct signaling mechanisms

(Matsui et al Circ Res, 2007)
Distinct roles of autophagy in the heart during ischemia and reperfusion

Myocardial ischemia

I/R injury

ATP↓

AMPK

eEF-2K mTOR p27 Autophagy genes

Autophagy

Apoptotic cell death

Autophagic cell death

(Matsui et al Circ Res 2007)
Contents

• Dichotomous function of autophagy in the post MI heart
• Signaling mechanisms mediating adaptive autophagy: The role of FoxO1-Sirt1 pathway
• Regulation of autophagy by Mst1: An endogenous regulator of Beclin1-Bcl-2 interaction
Ablation of ULK1 attenuates ischemia/reperfusion injury

(Collaboration with Dr. Kundu)
Excessive autophagy may facilitate death of myocytes during acute MI

WT Day 4  Beclin 1 +/- Day 4

WT  beclin 1 +/-

2 days after MI

Beclin 1

GAPDH

Control  MI

WT  beclin1+/-

perfused

infarced

survived

without autophagy
Oxidative Stress Mediates Upregulation of Beclin1 and Autophagy during I/R

MPG = 2-mercaptopropionil glycine (ant-oxidant)

Tg-αMHC-mRFP-GFP-LC3

Oxidative stress

Beclin1

Autophagy

(ARS in press Hariharan et al, 2010)
The survival rate was significantly higher in the early phase but not different in the chronic phase in \textit{beclin 1 +/-} mice compared to WT mice.

No longer significant at 28 days.

\*p<0.05 vs WT
Cardiac-specific knockout of Atg7 exacerbates LV function of chronic myocardial infarction (2 weeks)

Chronic Myocardial infarction → LV remodeling → Autophagy → LV dysfunction

(Collaboration with Dr. M Komatsu)
Autophagy has *phase-dependent* functions after MI:

: *mediated by distinct signaling mechanisms?*

**Dramatic activation of autophagy at **acute** phase:**
- Strong upregulation of Beclin 1 and Lysosomal enzymes
- Detrimental
  - Greater MI size
  - Higher mortality
  - Reduced LV function

**Modest activation of autophagy at **chronic** phase:**
- ?
- Protective
  - Reduced MI expansion?
  - Wound Contraction?
  - Inhibition of hypertrophy
  - Suppression of apoptosis
  - Suppression of aggresome (Protein QC)
Chronic MI upregulates FoxO1, an inducer of autophagy, in the border zone.
FoxO1 Overexpression Increases Autophagic Flux In Vitro

(Courtesy Dr. T Yoshimori)
FoxO1 is Required for Glucose Deprivation-Induced Autophagy

**p<0.05, **p<0.01

*Autophagosomes (Yellow dots)/cell
Autolysosomes (Free red dots)/cell

<table>
<thead>
<tr>
<th>Condition</th>
<th>p62</th>
<th>FoxO1</th>
<th>Tubulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad-LacZ + GD</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ad-sh-FoxO1 + GD</td>
<td>-</td>
<td>+</td>
<td>-</td>
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</table>

**p<0.05, **p<0.01

**p<0.05, **p<0.01

**p<0.05, **p<0.01

FoxO1

sh-FoxO1

Autophagy flux
FoxO Is Deacetylated in Response to Glucose Deprivation through a Sirt1-Dependent Mechanism

**Diagram Description:**
- **Ad-LacZ** and **Ad-LacZ + GD (2h)**
  - Ac-FKHR
  - Sirt1
  - Tubulin

- **Relative NAD⁺ content**
  - Ad-LacZ
  - Ad-LacZ + GD

- **GD**
- **Sirt1**
- **sh-Sirt1, DN-Sirt1**
- **Deacetylation of FoxO1**
Acetylation of FoxO1 Inhibits GD-Induced Autophagy

Nakae J, JCI (2006)

Murine/Human FoxO1: PGLLETLLTSDS
Murine/Human FoxO3: NQLQDLNASDS
Murine/Human FoxO4: SQALESLLTSDT

3A/LXXAA → p300
FoxO1 Deacetylation

Autophagy

Collaboration with Dr. J Nakae
**Functional FoxO1 Is Required for Starvation Induced Autophagy In Vivo**

<table>
<thead>
<tr>
<th>NTg</th>
<th>DN-FoxO1</th>
<th>FoxO1-CKO</th>
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<tbody>
<tr>
<td>B</td>
<td>S</td>
<td>B</td>
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</tbody>
</table>

- **p62**
- **LC3**
- **Tubulin**

**NTg**: Non-Transgenic mice.

**DN-FoxO1-Tg**: Transgenic mice with cardiac specific over-expression of FoxO1 3A/LXXAA mutant.

**FoxO1-CKO**: Mice with cardiac specific knockout of FoxO1.

**B**: Baseline, **S**: 48 hours starvation

(Collaboration with Dr. RA DePinho)
Functional FoxO1 and Autophagy Are Required for Maintenance of LV Function during Starvation

Starvation → Sirt1-mediated Deacetylation of FoxO → Autophagy → LV dysfunction

- FoxO1 (LXXAA) or FoxO1 KO

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>48 hour starvation</th>
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<td>NTg</td>
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<tr>
<td>Control</td>
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<tr>
<td>beclin1 +/-</td>
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Fractional Shortening (%)
Sirt1-dependent transcriptional activation of FoxO plays an essential role in mediating autophagy in the heart

Glucose Starvation

Sirt1

Deacetylation FoxO

Rab7

other mechanisms

Lysosomal biogenesis

Fusion

Phagophore → Autophagosome → Autolysosome → Lysosomal degradation
Ubiquitinated Proteins And p62/SQSTM1 Are Accumulated in Tg-Mst1 Mice, a Mouse Model of DCM

Mst1 = Mammalian sterile 20 like kinase 1

(Yamamoto et al JCI 2003)

Anti-ubiquitin antibody staining

p62/SQSTM1: LC3 binding protein known to be degraded by autophagy

NTg Tg-Mst1

NTg Tg-Mst1

NTg Tg-Mst1

NTg Tg-Mst1

NTG TG-Mst1

P<0.01
Autophagosome formation is **decreased** in Tg-Mst1 mice.

**Genetic cross between Tg-Mst1 and Tg-GFP-LC3**

- Mst1 (-); Tg-GFP-LC3
- Mst1 (+); Tg-Mst1 x Tg-GFP-LC3

**Starvation**

- NTG
- TG-Mst1

Increased autophagosomes

# p<0.01 vs NTG
Hypothesis: Basal autophagy is required for protein QC during heart failure

Pathological stress
Chronic ischemia
Increased wall stress

Mst1

Phosphorylation of Beclin1

Suppression of Autophagy

Accumulation of p62
Aggresome formation

Progression of Dilated Cardiomyopathy

(Levine 2007)

Increased susceptibility to death in response to stress
Cellular homeostasis
Death through excessive self-digestion
Mst1 activity is decreased during starvation

< Cultured cardiomyocytes >

AA(-): Amino Acid deprivation, Glu(-): Glucose deprivation

< Mouse >

AA deprivation

Ctrl. 1hr 2hr 4hr

Mst1 Kinase activity (Fold vs. Ctrl.)

p < 0.05
Conclusions

• Oxidative stress induces strong upregulation of Beclin1/autophagy in response to I/R, which is detrimental.
• Deacetylation of FoxO stimulates autophagy, through upregulation of stimulators of autophagy, such as Rab-7, which is protective.
• Mst1 is an endogenous regulator of Beclin1-Bcl-2 interaction. Stress-induced activation of Mst1 inhibits autophagy through phosphorylation of Beclin1, which is detrimental for the heart.
Current Lab members

March 2010

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