Functional roles of chaperone molecule (HSP70) for muscle atrophy inhibition: future implication

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Muscle atrophy

1. A significant decrease in mass or volume of skeletal muscle, with loss of myofibrillar proteins and tone.
2. Caused by various factors including physical unloading and diseases.
3. Molecular mechanisms underlying the atrophic process are much shared in the causal states.

Space microgravity
Bed rest
Spinal injury – denervation
Muscle injury
Inactivity by cast
Cancer
AIDS
Sepsis

Space physiology → good solutions → the quality of life
Muscle atrophy occurs steadily despite elaborate exercise program in space.

- Muscle force production ↓
- Performance and work ↓
- Mission capability ↓
- Responsiveness to emergency ↓
- Muscle atrophy during prolonged spaceflight
- Severe muscle damage by reloading (landing)

We may need another means of countermeasure to effectively prevent muscle atrophy!
Muscle mass = balance between protein synthesis rate and degradation rate

**Figure 1** The proteins Akt1 and Foxo at the decision point of atrophy versus hypertrophy.

Inhibition of C2C12 myotube atrophy by a novel HSP70 inducer, celastrol, via activation of Akt1 and ERK1/2 pathways

**Previous reports: muscle atrophy prevented by HSP70 overexpression**


**Celastrol (CEL): general features**

- A quinine methide triterpene
- Heat Shock Protein 72 inducer
- Diverse functions: antioxidant activity, neuroprotection, and inhibition of proteasome activity
Cell line: **mouse C2C12 myotube**

Concentration of CEL used for this study: **1.5 μM for 6 h treatment**

The cells exposed to four doses of CEL for 6 h

FACS analysis

Data were obtained from three independent experiments (each experiment performed in triplicate) and are presented as mean ± SEM.
Experimental design

Myotube diameter affected by CEL and DEX.

- **DEX (150µM): time-dependent test**

  The cell diameter was significantly decreased in myotubes exposed to 150 µM DEX for ≥12 h.

- **CEL abolished DEX-induced myotube atrophy**

  Comparison of the cell diameters among the four groups

  Data are presented as the mean ± SEM. Cell counts: 220 - 250 cells per group. The symbols a, b, c and d indicate statistical significance among the denoted groups at $P < 0.05$ (one-way ANOVA and SNK post hoc multiple comparisons test).

White bar = 100 μm
Inhibition of C2C12 myotube atrophy by a novel HSP70 inducer, celastrol, via activation of Akt1 and ERK1/2 pathways

Expression of HSP72 upregulated by CEL.

Elevation of HSP72 protein expression by CEL

Upregulation of HSP72 via heat shock factor 1 (HSF1) transcriptional activity

(A and B) Whole cell lysates subjected to immunoblot analyses with antibodies reacting with total or Ser230-phosphorylated HSF1. Mean ± SEM (n = 6), a versus b, P < 0.05. (C) Confocal immunofluorescent analysis to visualize the expression and subcellular localization of p-HSF1 in C2C12 cells. The cells were cultured on chamber slides and treated as indicated in the Methods, and stained with p-HSF1 antibody followed by a secondary antibody conjugated to Rhodamine. The nucleus was stained with DAPI (blue).

Scale bars = 10 μm.

Data: mean ± SEM (n = 6). a versus b, P < 0.05.
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Anabolic signalling activities upregulated; catabolic signaling activities abolished by celastrol

Data: Mean ± SEM (n = 6). a versus b, P < 0.05.

Data: Mean ± SEM (n = 6). *P < 0.05.
HSP70 overexpression induced by celastrol abolished the atrophic effect of HSP siRNA in L6 myotubes

HSP72 expression was affected by HSP72 siRNA and CEL in the L6 myotubes

- HSP72 mRNA and protein expression was upregulated by CEL treatment despite Hsp72 gene knock down.
- HSF1 was transactivated and accumulated in the nucleus by CEL treatment.

Elevation of HSP72 expression by CEL

![Graph showing elevation of HSP72 expression by CEL](image)

Data: mean ± SEM (immunoblot analysis; n = 6, real-time PCR analysis; n=4) The symbols a, b, c and d; significant differences among the designated groups at P < 0.05.

White bars = 10 μm.
Myotube diameters compared among the four groups.

The cell diameter was significantly decreased by HSP72 siRNA

CEL abolished the atrophic effect of HSP72 siRNA on muscle cell

(A) NC siRNA, (B) HSP72 siRNA, (C) NC siRNA+CEL and (D) HSP72 siRNA+CEL

White bars = 100 µm

Data: mean ± SEM (n = 178 -183 cells per group with 3 independent experiments).
The symbols a, b and c indicate significant differences among the denoted groups at $P < 0.05$. 

HSP70 overexpression induced by celastrol abolished the atrophic effect of HSP siRNA in L6 myotubes
Conclusions

1. The anti-atrophic effect of CEL is mediated by activation of HSF1 and consequent overexpression of HSP72.

2. HSP overexpression by CEL treatment not only activated the Akt1, S6K and ERK1/2 signaling pathways but also suppressed FoxO activation, E3 ligase expression and proteasome activity by DEX in C2C12 myotube.

3. The lack of HSP70 was directly responsible for the L6 myotube atrophy, and CEL abolished such atrophy of the myotube.

4. An *in vivo* study on the anti-atrophic potency of celastrol is currently under way with a direct application of CEL in the presence or absence of siRNA in the skeletal muscle of a mouse model.
Thank you for your attention!

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