Novel Dual-function Small-molecule AMPK Activator Ameliorates Metabolic Syndrome

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AMPK: an Energy Sensor, with multifunctions in metabolism

- $\alpha_1$ and $\alpha_2$
- $\beta_1$ and $\beta_2$
- $\gamma_1$, $\gamma_2$ and $\gamma_3$

- 75% homology between $\alpha_1$ and $\alpha_2$, mainly in kinase domain
- $\alpha_1$ is universally expressed
- $\alpha_2$ is expressed mainly in heart, skeleton muscle and liver

Annu. Rev. Pharmocol. Toxicol. 2006, 47, 1
Glucose, Insulin and AICAR Tolerance Test in AMPKa2 KO Mice

α2 KO mice are glucose intolerant, insulin resistant and AICAR resistant

**Glucose, Insulin and AICAR Tolerance Test in AMPKa1 KO Mice**

**GTT**

![GTT Graph]

- **α2-WT**: Blood glucose [mM]
- **α2-KO**: Blood glucose [mM]
- **WT\_AUC**: 505 +/- 58
- **KO\_AUC**: 710 +/- 82 *

**ITT**

![ITT Graph]

- **α2-WT**: Blood glucose [mM]
- **α2-KO**: Blood glucose [mM]
- **WT\_AUC**: -308 +/- 44
- **KO\_AUC**: -111 +/- 33 *

**ATT**

![ATT Graph]

- **α2-WT**: Blood glucose [mM]
- **α2-KO**: Blood glucose [mM]
- **WT\_AUC**: -268 +/- 33
- **KO\_AUC**: -131 +/- 30 *

**α1 KO mice have normal glucose tolerance and insulin sensitivity and are not AICAR resistant**

*J.Biol.Chem. 2004, 279, 1070*
AMPK Pathway was Damaged by Insulin Resistance in Skeletal Muscle

BBRC 2006, 339, 701
Am J Physiol Endocrinol Metab 2006, 290, 251
Impact on onset Type 2 Diabetes

Indicators in onset type 2 diabetes:

- High circulating lipid, high ectopic fat deposition, hyperglycemia, hyperinsulinia, and insulin resistance

AMPK activation will lead to the improvement of onset type 2 diabetes through:

- Decreasing circulating lipid; decreasing ectopic fat deposition, decreasing insulin secretion, decreasing glucose level and increasing insulin sensitivity
Proof of concept through Pharmacological Activators of AMPK

- Indirectly activate AMPK
  - AICAR (ZMP)

- Antidiabetic drugs
  - Metformin
  - TZDs: rosiglitazone, troglitazone, pioglitazone

- Directly activate AMPK
  - Small-molecule activator: A-769662 (Abbott)
  - Identified through HTS on $\alpha_2\beta_1\gamma_1$ enzyme

Autoinhibition hypothesis

Inactive
Unphosphorylated
Unmasked Destruction Domain
Rapid Turnover

Active
Phosphorylated
Masked Destruction Domain
Slow Turnover

AMP + AMPKK

+PP2C or PP2A
+Beta and Gamma

N

312

392

312

β

γ

C

N

392
Autoinhibition mechanism study

Kinase domain

Graphs showing time vs. activity and incorporation of CPM.
The effect of deletions on the activity of human AMPK α subunits
Structural model of human AMPK α1 subunit including the kinase domain and autoinhibitory domain.
Effects of mutations of predicted interacted residues on $\alpha1(1-394)$ activity
Overexpression of the deleted AMPK α mutant (Δα394), V298G and L328Q stimulate ACC phosphorylation in COS7 cells, respectively.
Overexpression of deleted AMPK α mutant (Δα394), V298G and L328 Q enhances glucose uptake in HepG2 cells following glucose deprivation.
Hypothesis Based on Knowledge

• Knowledge
  – Autoinhibition by autoinhibitory domain, which impacts on the conformation of kinase domain
  – Activation by interaction with β and γ subunits, which change the conformation of kinase domain
  – Allosteric activation by AMP

• Hypothesis
  – Will conformational change remove autoinhibition?
  – Is conformational change a key and prerequisite step?
  – Can conformational change be achieved by small-molecule activators?
Novel Strategy for AMPK small-molecule activator screening

• Screening AMPK small-molecule activators with inactive AMPK truncations
# Hits Finding

Compounds screened: 3600 diverse small-molecule pure chemicals  
Concentration: 40 μg/mL

<table>
<thead>
<tr>
<th>compound</th>
<th>EC50 (mM)</th>
</tr>
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<tbody>
<tr>
<td>PT1</td>
<td>8.7</td>
</tr>
<tr>
<td>PT2</td>
<td>21.6</td>
</tr>
<tr>
<td>PT3</td>
<td>15.6</td>
</tr>
<tr>
<td>PT4</td>
<td>17.8</td>
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<tr>
<td>PT5</td>
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<tr>
<td>PT7</td>
<td>43.4</td>
</tr>
<tr>
<td>PT8</td>
<td>11.8</td>
</tr>
</tbody>
</table>

Activation curve (EC50) for hits of AMPK
The effects of PT1 on $\alpha_1\beta_1$ dimer, $\alpha_2(1-398)$, truncated $\alpha_1$ proteins
PT1 lowers lipid contents in HepG2 cells

A

PT1 (μM) - 5 10 20 40 80 -
2 mM Metformin - - - - - +
pAMPK
AMPK
pACC
ACC
β-actin

40 μM Compound C
40 μM PT1 - - - - + -
2 mM Metformin - - - - + -
pACC
ACC
β-actin

B

Lipid Content (μg/mg protein)

DMSO Metformin 5 10 20 40 80

P<0.01

P<0.05

TG Content (μg/mg protein)

DMSO 40 μM PT1 2 mM Metformin
PT1-docking model with human AMPK α1 subunit

Modification and discovery of YLF466

**A**

![Chemical structure of YLF466D](image)

**B**

![Graph showing concentration vs. AMPK activity for different treatments](image)

**C**

![Graph showing concentration vs. AMPK activity for different treatments](image)

**D**

![Graph showing concentration vs. AMPK activity for different treatments](image)

**E**

![Graph showing concentration vs. AMPK activity for different treatments](image)

**F**

![Graph showing concentration vs. AMPK activity for different treatments](image)
Predicted interaction mode between AMPK α subunit and YLF466D
Addictive effects with AMP or A-769662

Graphs showing the AMPK activity (fold of basal) with varying concentrations of AMP, A-769662, and YLF466D.
YLF466D activate AMPK pathway in L6 Myotubes
Compound C blocked glucose uptake by YLF466D
YLF466D activated AMPK and lowered lipid contents in HepG2 cells.
Primary PK data

![Graph showing concentration over time for i.v. and p.o. administrations]
Oral treatment with YLF466D on db/db for 4 weeks has no effects on food consumption and body weight.
Oral treatment with YLF466D on db/db for 4 weeks improved metabolic syndrome
Oral treatment with YLF466D on db/db for 4 weeks improved glucose tolerance
Oral treatment with YLF466D on db/db for 4 weeks enhanced AMPK pathway in liver and muscle
Oral treatment with YLF466D on db/db for 4 weeks inhibited gene expression in liver and triglyceride in muscle
Oral treatment with YLF466D on DIO mice for 4 weeks improved metabolic syndrome
Oral treatment with YLF466D on DIO mice for 4 weeks improved glucose tolerance and insulin sensitivity.
Thank You!