Identification of a Mitochondrial Target of Thiazolidinediones (mTOT)


Metabolic Solutions Development Company, Kalamazoo, MI, USA.

Southwest Michigan Innovation Core Life Science Lab, Kalamazoo, MI, USA.

Thiazolidinediones (TZDs) are insulin sensitizing compounds with proven clinical efficacy but with no completely understood antidiabetic pharmacology and significant side effects that have limited their clinical utility. Here, we describe a selective photoconvertable affinity probe that identifies a mitochondrial target of TZDs. This probe is composed of a thiazolidinedione core chemically covalently linked to a tetramethylrhodamine dye, which is convertible to rhodamine red via a visible light-driven photoisomerization reaction. To determine the degree to which the complex is involved in the actions of many mitochondrial targets, we have identified the ability of various analogs, including PPARδ-independent T2D016, T2D017, and MSDC-0160, to enhance differentiation of brown adipose tissue (BAT) progenitors with human tissue equivalents. Addition of these compounds enabled the identification of several key components (C163, C160, and C169) that are not present in the active compounds. Consistently, a targeted mutation that deletes the first 16 amino acids from the amino terminal sequence results in a marked enhancement of differentiation response to the active compounds. In addition, a series of other chemical classes, including MRL-24, also exhibit a similar trend. The probe enables quick screening for potential targets in a range of different biological samples, including cells, tissues, and cells with human tissue equivalents. The probe is not sensitive to compounds that block the action of the active compounds, such as LiCl or specific inhibitors of GSK-3β. This new target is part of a phylogenetically conserved and highly selective complex in the mitochondrial membrane, which is responsible for binding of these agents to mitochondrial membranes. To determine the degree to which the complex is involved in the actions of many mitochondrial targets, we have identified the ability of various analogs, including PPARδ-independent T2D016, T2D017, and MSDC-0160, to enhance differentiation of brown adipose tissue (BAT) progenitors with human tissue equivalents. Addition of these compounds enabled the identification of several key components (C163, C160, and C169) that are not present in the active compounds. Consistently, a targeted mutation that deletes the first 16 amino acids from the amino terminal sequence results in a marked enhancement of differentiation response to the active compounds. In addition, a series of other chemical classes, including MRL-24, also exhibit a similar trend. The probe enables quick screening for potential targets in a range of different biological samples, including cells, tissues, and cells with human tissue equivalents. The probe is not sensitive to compounds that block the action of the active compounds, such as LiCl or specific inhibitors of GSK-3β. This new target is part of a phylogenetically conserved and highly selective complex in the mitochondrial membrane, which is responsible for binding of these agents to mitochondrial membranes.
Identification of a Mitochondrial Target of Thiazolidinediones (mTOT)

WILLIAM G. MCDONALD¹, GREGORY S. CAVEY², SERENA L. COLE¹, DANIELLE D. HOLEWA¹, ANGELA S. BRIGHTWELL-CONRAD¹, CINDY WOLFE¹, JEAN WHEELER¹, KRISTIN COULTER¹, ROLF F. KLETZIEN¹, JERRY R. COLCA¹

¹METABOLIC SOLUTIONS DEVELOPMENT COMPANY, LLC, KALAMAZOO, MI, USA
²SOUTHWEST MICHIGAN INNOVATION CENTER, KALAMAZOO, MI, USA
Background

- Insulin sensitizing thiazolidinediones were discovered empirically without regard to mechanism.
- It is generally thought that both the dose limiting side effects and the positive pleiotropic pharmacology occur through the adipocyte master regulator PPARγ.
- TZDs have PPARγ-independent effects (Chen et al, J. Biol. Chem. published 23 May 2012, 10.1074/jbc.M112.363960 http://www.jbc.org/cgi/content/abstract/M112.363960v1).
- We propose that significant insulin sensitizing pharmacology occurs through modulation of a mitochondrial target (mTOT) that has remained to be identified.

Overview

- We used drug analog photoaffinity crosslinking and MS proteomics to identify a phylogenetically conserved protein in the inner mitochondrial membrane as part of the TZD recognition complex.
- Proof of identity has been accomplished by expression and knockdown.
- Two previously uncharacterized protein family members play a role.
- Two papers published online by two separate groups in the May 24, 2012 Science Express have coincidentally found that these two proteins, which they renamed Mpc1 and Mpc2, are part of a pyruvate carrier mechanism.
- Together these findings place the TZD recognition complex (mTOT) at the “crossroads of metabolism” and provide a new way to look at insulin sensitizers.
The TZD photoaffinity probe crosslinks a specific protein at about 14kDa.

Crosslinking is specific as it is reduced by competition with biologically active TZDs (including pioglitazone) but not the inactive (e.g., 1473) analogs.

This protein is not mitoNEET and remains to be identified.
Purification and Identification

- The labeled complex ran at about 150 kDa on Blue Native Gels (A).
- Separation of this complex on a second dimension SDS gel focused 3 spots (B).
- MS/MS identified the 4 peptides shown in red suggesting the crosslinked protein is BRP44.
Expressed BRP44 localizes to mitochondria and is crosslinked by the TZD probe

A. GFP fusion locates to mitochondria

B. Hexhis fusion is crosslinked by the probe

C. BRP-44 localizes to the inner membrane

A and C, courtesy of Sandy Wiley and Anne Murphy, UCSD.
The photoprobe also specifically crosslinks a fly protein of larger size

- BRP44 is phylogenetically conserved but the *Drosophila* ortholog CG9399 has an N-terminal extension that runs at about 19kDa on SDS-PAGE and is crosslinked by the TZD probe.
- Knockdown of *either* the BRP44 ortholog (CG9399) or CG14290, ortholog of related family member BRP44L, prevents crosslinking (genes 2, 3, 5 are unrelated).

**A. Crosslinking in Drosophila**

**B. Crosslinking in knockdown flies - both family members required**

**C. Western blots for fly knockdowns**

Both family members are required for crosslinking in *Drosophila*.

Collaboration with Medros, St. Louis (See also PO-2363).
Two recently published reports have identified BRP44 and BRP44L as components of a pyruvate carrier system that are phylogenetically conserved in yeast, drosophila, and mammals.

<table>
<thead>
<tr>
<th>Mammals</th>
<th>Fly</th>
<th>Yeast</th>
<th>New Term</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRP44</td>
<td>CG9399</td>
<td>YHR162W</td>
<td>MPC2</td>
</tr>
<tr>
<td>BRP44L</td>
<td>CG14290</td>
<td>YGL080W</td>
<td>MPC1</td>
</tr>
</tbody>
</table>

These proteins are now referred to as MPC1 and MPC2 for Mitochondrial Pyruvate Carrier protein family 1 and 2, in the order found by Bricker et al., 2012.

We will refer to the TZD recognition complex as mTOT (mitochondrial target of thiazolidinediones). This complex contains MPC2 and MPC1, as well as other proteins that appear to be tissue specific.
Common findings with emerging data for pyruvate carrier family

- The proteins migrate in a complex that runs at about 150 kDa on Blue Native gels.
- The complex contains both BRP44 (Mpc2) and BRP44L (Mpc1) and these proteins can be co-immunoprecipitated.
- These proteins are localized to the inner mitochondrial membrane.
- UK-5099, an inhibitor of the pyruvate carrier activity also blocks the crosslinking of the TZD probe.

MSDC-0160 directly inhibits pyruvate oxidation in permeabilized cells. See PO-2484.

The mTOT complex is involved in the regulation of pyruvate utilization.
Conclusions

- We have utilized drug analog photoaffinity crosslinking to identify a previously uncharacterized complex of proteins in the inner mitochondrial membrane.

- The protein crosslinked by this process is BRP44. This protein has been renamed Mpc2 by recent independent publications that show BRP44 is a key member in a protein family that constitutes the mitochondrial pyruvate carrier.

- The identity of the protein was confirmed by knockdown of expression as well as the expression of C-terminally tagged proteins.

- Modulation of the complex that regulates pyruvate entry into mitochondria places drug action at the crossroads of metabolism. These findings provide new ground toward understanding the pleiotropic effects of insulin sensitizers and how changes in metabolism can predict onset and corrections of diabetes.

See also Phase 2B data with prototype mTOT Modulator™- P-966; and 2363-PO and 2484-PO.