PPAR\(\gamma\), phosphorylation and the anti-diabetic PPAR\(\gamma\) ligands

a new look at an old friend

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UNIST
Metabolic Syndrome

- Hypertension
- Obesity
- Insulin resistance
- Atherogenic dyslipidemia

Type 2 diabetes
Cardiovascular disease
Certain cancers
Obesity trends among U.S. adults

(*BMI $\geq 30$, or about 30 lbs. overweight for 5’4” person)
David, after a short stay in America
What should we do for managing metabolic syndrome?
The major strategies for treating diabetes are:

A. Diet and Exercise
B. Oral hypoglycaemic therapy
C. Insulin Therapy
Exercise and diet

“It’s not a rash, it’s moss. You need to start being more active than a tree.”

Cumulative Incidence of Diabetes (%)

- Placebo
- Metformin
- Lifestyle

Year

n>1000

# Oral hypoglycemic medications

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Drug Name</th>
<th>Brand Name</th>
<th>Mechanism of Action</th>
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<tbody>
<tr>
<td>Biguanides</td>
<td>Metformin</td>
<td>Glucophage</td>
<td>Inhibit glucose production by the liver</td>
</tr>
<tr>
<td>Sulfonylureas (second-generation)</td>
<td>Glimepiride</td>
<td>Amaryl</td>
<td>Increase insulin secretion by pancreatic beta cells</td>
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<tr>
<td></td>
<td>Glipizide</td>
<td>Glucotol</td>
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<td></td>
<td>Glyburide</td>
<td>Diabeta Glynase</td>
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<td></td>
<td></td>
<td>PresTab Micronase</td>
<td></td>
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<tr>
<td>Meglitinides</td>
<td>Repaglinide</td>
<td>Prandin</td>
<td>Increase insulin secretion by pancreatic beta cells</td>
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<tr>
<td></td>
<td>Nateglinide</td>
<td>Starlix</td>
<td></td>
</tr>
<tr>
<td>Thiazolidinediones (PPAR_γ specific full agonist ligands)</td>
<td>Pioglitazone</td>
<td>Actos</td>
<td>Increase glucose uptake by skeletal muscle</td>
</tr>
<tr>
<td></td>
<td>Rosiglitazone</td>
<td>Avandia</td>
<td></td>
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<tr>
<td>Alpha-glucosidase inhibitors</td>
<td>Acarbose</td>
<td>Precose</td>
<td>Inhibit carbohydrate absorption in the small intestine</td>
</tr>
<tr>
<td></td>
<td>Miglitol</td>
<td>Glyset</td>
<td></td>
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What is PPARγ?

C/EBPα

C/EBPβ

C/EBPδ

RXR

PPARγ

Insulin

ADD1/SREBP1C

Fat Cell-Specific Gene Expression
Fat Differentiation
Insulin Sensitivity

Natural Ligands (?)

Synthetic TZDs

Fat (Cell Specific (Gene Expression)

Lipogenesis

Fat (Differentiation

Synthetic TZDs

Natural Ligands (?)

Fat (Cell Specific (Gene Expression

Lipogenesis

Fat (Differentiation

Role of TZDs in PPARγ-mediated glucose metabolism

Dominant-negative PPARγ mutation in human

Severe Insulin Resistance
Limb and buttock lipodystrophy
Severe dyslipidaemia
Early Onset Hypertension

Nature (1999)
Diabetes (2003)
1. Partial loss of function mutations in PPARγ in humans unambiguously cause severe insulin resistance.
2. PPARγ agonists improve insulin-resistance and diabetes.
3. Most PPARγ target genes are already fully “ON” in obesity.
4. Severe side effects of PPARγ full agonists (TZDs) such as heart failure, weight gain, fluid retention.
5. Some PPARγ ligands with poor agonist activity (partial agonists) still have marked anti-diabetic actions.

PPARγ full agonist ligands: TZDs (rosiglitazone etc.)
PPARγ partial agonist ligands: MRL24, nTZDpa etc.
1. Partial loss of function mutations in PPARγ in humans unambiguously cause severe insulin resistance.
2. PPARγ agonists improve insulin-resistance and diabetes.
3. Most PPARγ target genes are already fully “ON” in obesity.
4. Severe side effects of PPARγ full agonists (TZDs) such as heart failure, weight gain, fluid retention.
5. Some PPARγ ligands with poor agonist activity (partial agonists) still have marked anti-diabetic actions.

Can we separate PPARγ agonism from anti-diabetic actions? Are there novel mechanisms linking PPARγ to insulin-resistance?
Schematic model of PPARγ and anti-diabetic PPARγ ligands

Obesity, high fat diets

Inflammatory cytokines (TNF-α, IL-6 etc.)

Lipids, FFAs

CDK5

p25

Specific target genes (adiponectin, adipisin etc.)

Insulin Sensitivity, browning (?)

Anti-diabetic PPARγ ligands (TZDs, MRL24)

Choi et al., Nature (2010)

New PPARγ ligands with no agonism (SR1664, 1824)

Choi et al., Nature (2011)
Cdk5 phosphorylates PPARγ

A

<table>
<thead>
<tr>
<th>Protein</th>
<th>Ser273</th>
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<tbody>
<tr>
<td>PPARγ2</td>
<td>TTDKSPFVIY</td>
</tr>
<tr>
<td>PPARγ1</td>
<td>TTDKSPFVIY</td>
</tr>
<tr>
<td>PPARα</td>
<td>TSNNPFPVIH</td>
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<tr>
<td>PPARδ</td>
<td>SSHNAPFVIH</td>
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<td>Bovine</td>
<td>TTDKSPFVIY</td>
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<tr>
<td>Mouse</td>
<td>TTDKSPFVIY</td>
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<td>Rabbit</td>
<td>TTDKSPFVIY</td>
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<tr>
<td>Pig</td>
<td>TTDKSPFVIY</td>
</tr>
<tr>
<td>Chicken</td>
<td>TTDKSPFVIY</td>
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B

Proteins: BSA, Histone or Rb, PPARγWT, PPARγS273A
CDKs: +, +, -, -

CDK5/p35
- pPPARγ
- pHistone
- PPARγ

CDK1/cdc2
- pRb

CDK2/Cyclin A
- pRb

CDK2/Cyclin E
- pRb

CDK4/Cyclin D1
- pRb

C

<table>
<thead>
<tr>
<th>Protein</th>
<th>PPARγWT</th>
<th>PPARγS273A</th>
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<tr>
<td>Vector</td>
<td>CDK5WT</td>
<td>CDK5KO</td>
</tr>
<tr>
<td>Vector</td>
<td>CDK5WT</td>
<td>CDK5KO</td>
</tr>
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</table>

I.P.: α-FLAG Ab.
- pPPARγ
- PPARγ

Total cell lysates
- CDK5

Choi et al., Nature (2010)
What is Cdk5?

CDK5 KO: perinatal death
  Defect on corticogenesis
  Cerebellar defolidation

Bioassay (2004)
Nat. Med. (2005)
Nat. Rev. MCB (2001)
Obesity-induced phosphorylation of PPARγ in fat tissues

A

<table>
<thead>
<tr>
<th></th>
<th>3 weeks</th>
<th>7 weeks</th>
<th>13 weeks</th>
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<tr>
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<td>SD</td>
<td>HF</td>
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<td>pPPARγ</td>
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<td>pCDK5</td>
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<td>CDK5</td>
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<tr>
<td>p35</td>
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<td>p25</td>
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<th>13 weeks Epi.</th>
<th>13 weeks Ing.</th>
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<tr>
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<td>SD</td>
<td>HF</td>
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<tr>
<td>pPPARγ</td>
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<tr>
<td>PPARγ</td>
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C

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<tr>
<th></th>
<th>7 weeks Chow</th>
<th>7 weeks HFD</th>
<th>13 weeks Chow</th>
<th>13 weeks HFD</th>
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<tbody>
<tr>
<td>Weight (grams)</td>
<td>26.2 ± 0.3</td>
<td>33.8 ± 1***</td>
<td>29.4 ± 0.6</td>
<td>44.5 ± 1.1***</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>88.2 ± 6.4</td>
<td>92.6 ± 5.4</td>
<td>115.4 ± 8.9</td>
<td>124.4 ± 6.5</td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>0.1 ± 0.03</td>
<td>0.18 ± 0.06</td>
<td>0.24 ± 0.03</td>
<td>1.07 ± 0.13**</td>
</tr>
</tbody>
</table>

Choi et al., Nature (2010)
What is the biological role of phosphorylation?
Specific fat cell genes regulated by phosphorylation

Choi et al., Nature (2010)
Specific fat cell genes regulated by phosphorylation

Specific gene sets regulated by phosphorylation

Phosphorylation of PPARγ might play an important role in the context of obesity and diabetes.

PPARγ ligands block the phosphorylation of PPARγ

Anti-diabetic PPARγ ligands directly block the phosphorylation independent of PPARγ agonism.

What is the physiological role of phosphorylation *in vivo*?
Anti-diabetic effects of PPARγ ligands in HFD mice

Choi et al., Nature (2010)
Modulation of PPARγ phosphorylation by rosiglitazone during the therapy of human type 2 diabetes

Newly diagnosed T2D, male, 6 months treatment, 4mg/day

Phosphorylation of PPARγ tightly correlates with insulin sensitivity.

Choi et al., Nature (2010)
MRL-24 increases metabolic rate and thermogenic gene expression *in vivo*

**Ucp1**

**Pgc1α**

**Whole-body oxygen consumption**
Proposed model of PPARγ phosphorylation and PPARγ ligands

- **Obesity, high fat diets**
  - Inflammatory cytokines (TNF-α, IL-6 etc.)
  - Lipids, FFAs

- **Anti-diabetic PPARγ ligands** (TZDs, MRL24)

- **CDK5**
  - p25

- **PPARγ**
  - S273 Phosphorylation
  - Specific target genes (adiponectin, adipsin etc.)

- **Insulin Sensitivity, browning (?)**

Many unanswered questions?

- The TZD drugs are PPAR$_\gamma$ agonists and block Cdk5-mediated phosphorylation. Can PPAR$_\gamma$ ligands be developed with no classical agonism and still block Cdk5-mediated phosphorylation of PPAR$_\gamma$?
- Would such compounds have anti-diabetic activity?
- Would they have a better therapeutic window than TZDs?
Many unanswered questions?

- The TZD drugs are PPAR$_\gamma$ agonists and block Cdk5-mediated phosphorylation. Can PPAR$_\gamma$ ligands be developed with no classical agonism and still block Cdk5-mediated phosphorylation of PPAR$_\gamma$?
- Would such compounds have anti-diabetic activity?
- Would they have a **better therapeutic window** than TZDs?

Withdrawal of Avandia because of its side effects
Development of chemical derivatives of Compound 7b

Choi et al., Nature (2011)
SR1664 has no effect on adipogenesis

Choi et al., Nature (2011)
SR1664 & SR1824 block PPARγ phosphorylation

A

<table>
<thead>
<tr>
<th>Rosiglitazone</th>
<th>SR1664</th>
<th>SR1824</th>
</tr>
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<tbody>
<tr>
<td>NT</td>
<td>2 μM</td>
<td>0.2 μM</td>
</tr>
<tr>
<td>IB: α-CDK sub. Ab.</td>
<td>pPPARγ</td>
<td>PPARγ</td>
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<tr>
<td>IB: α-PPARγ Ab.</td>
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<tr>
<td>IB: α-CDK5 Ab.</td>
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B

<table>
<thead>
<tr>
<th>Rosiglitazone</th>
<th>SR1664</th>
<th>SR1824</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT</td>
<td>2 μM</td>
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<tr>
<td>IB: α-CDK sub. Ab.</td>
<td>pRb</td>
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C

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<tr>
<th>TNF-α</th>
<th>SR1664</th>
<th>SR1824</th>
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<tbody>
<tr>
<td>NT</td>
<td>Rosi (10 μM)</td>
<td>Rosi (0.1 μM)</td>
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<td>pPPARγ</td>
<td>PPARγ</td>
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<td>IB: α-PPARγ Ab.</td>
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</table>

Choi et al., Nature (2011)
What is the physiological role of SR1664 in whole body metabolism?

1. DIO mouse model
2. Ob/ob mouse model
SR1664 improves glucose tolerance in DIO mice

SR1664 improves glucose tolerance in DIO mice (CLAMP)

5 days injection in DIO mice (twice/day)
Dr. Gerald Shulman, Yale Univ.

Choi et al., Nature (2011)
SR1664 improves glucose tolerance in ob/ob mice

Would SR1664 have a better therapeutic window than TZDs (less side effects)?

1. Bone mineralization
2. Weight gain, Fat % change
3. Hemodilution
No changes in bone mineralization by SR1664

A

B

No changes in weight, fat % and hemodilution by SR1664

**Proposed model of full- or non-agonist PPARγ ligands**

- **Full-agonist PPARγ ligands** (TZDs)
  - Improve insulin sensitivity
- **Non-agonist PPARγ ligands** (SR1664, 1824)
  - Side effects (fluid retention, weight gain, bone fracture etc.)

**Choi et al., Nature (2011)**
Burning key questions

• What is the molecular mechanism regulating the specific gene expression program controlled by phosphorylation?
  1. Different DNA occupancy by phosphorylation?
  2. Specific modulators binding to PPARγ?

![Graph showing relative gene expression for various genes under different conditions]
No difference of DNA occupancy by phosphorylation

Choi et al., Nature (2010)
Identifying PPARγ-binding proteins in phosphorylation-dependent manner

Vector  PPARγ\textsuperscript{WT}  PPARγ\textsuperscript{S273A}

PPARγ KO MEFs →

treat cells with TNFα →

isolate PPARγ-binding proteins

<table>
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<tr>
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<th>NT</th>
<th>TNFα</th>
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<tr>
<td>PPARγ\textsuperscript{S273A}</td>
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M.W. (kDa)

191 → 97 → 64 → 51 → 39 → 28 → 14

PPARγ
Thyroid hormone receptor-associated proteins (TRAP/MED)

Thrap3 (thyroid hormone receptor-associated protein 3) TRAP150

- A subunit of the transcription regulatory complex TRAP/Mediator (no LXXLL motif)

- A component of the spliceosome

- It activates pre-mRNA splicing and promotes nuclear mRNA degradation (R/S-rich domain)

- Thrap3 is a real subunit of TRAP/Mediator????

- The exact function of Thrap3 remains unclear.
Tissue distribution of Thrap3

![Bar chart showing relative gene expression of Thrap3 in different tissues]
Thrap3 interacts with phosphorylated PPARγ
Direct interaction between phosphorylated PPARγ and Thrap3

Thrap3 binds to phosphorylated PPARγ at Ser273.
Domain mapping of Thrap3

A

1 164 597 955

Thrap3WT

Thrap3AN

Thrap3AC

Thrap3ANC

R/S rich domain

Homologous Bclaf1

B

Vector Thrap3WT Thrap3AN Thrap3AC Thrap3ANC

Total cell lysates

IP : PPARγ

Thrap3

PPARγ
Specific gene regulation by Thrap3

- Scr shRNA
- Thrap3 shRNA

![Bar graph showing relative gene expression for various genes under Scr and Thrap3 shRNA conditions.]

- Significant differences indicated by stars above the bars.

![Western blot images of Thrap3 and Tubulin expression under Scr and Thrap3 shRNA conditions.]

![Legend for gene expression levels: unaltered, increased by 1.5, increased by 2, increased by 2.5.]

- Gene names: aP2, Nr3c1, Agrp2, Apn2, Rarres2, cyp24a, Ddx17, Rybp, Peg10, Car3, cldn24a, Txnip, Nr1d1, Slendp1, Adiponectin, Adipsin, Thrap3.
Conclusion

- Cdk5 is activated in obesity and modifies PPARγ at serine 273
- Cdk5 phosphorylation of PPARγ is sufficient to cause dysregulation of several fat cell genes, including some known to be aberrantly regulated in obesity, like adiponectin.
- Anti-diabetic PPARγ ligands block this Cdk5-mediated phosphorylation directly, an activity completely separate from classical agonism.
- These data now indicate that anti-diabetic PPARγ ligands work therapeutically, at least in part, by inhibition of the Cdk5-mediated phosphorylation of PPARγ.
- The development of entirely new classes of PPARγ-targeted drugs with no classical agonism is feasible
- Thrap3 may regulate Cdk5/PPARγ-specific gene reprogramming
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- Dr. Scott Busby

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- Dr. Mark Jedrychowski

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