Peroxisome Proliferator-Activated Receptors (PPARs) and osteoblasts and adipocytes

Welcome to the wonderful, dynamic and complex world of PPARs

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• History of PPARs
• PPAR-γ
• Bone – fat connection
• Mesenchymal stem cells
  – Adipocyte – osteoblast differentiation
  – Apoptosis
History

• Peroxisomes degrade fatty acids and toxic compounds and catalyze the first two steps in the synthesis of ether phospholipids, which are later used to build membranes.

• Peroxisomes are responsible for oxidation of long-chain fatty acids and thereby generating acetyl groups.

• Peroxisome proliferation in mammalian cells (almost 40 years ago)

• Peroxisome proliferators (10 years later) (hypolipidemic drugs, herbicides, leukotriene antagonists, and plasticizers)

• The first PPAR (PPARα) was discovered during the search of a molecular target for peroxisome (degrade fatty acids and toxic compounds) proliferators (pharmacologically related to fibrates), as they increased peroxisomal numbers in rodent liver tissue (absent in humans) (Isseman and Green, Nature 347:645; 1990).

• Three PPARs were originally identified in Xenopus frogs as receptors that induce the proliferation of peroxisomes in cells. (Dreyer et al., Cell 68:879; 1992)

  • PPARα, PPARβ/δ, and PPARγ

• PPARδ was identified in humans and appeared to be closely related to the PPARβ identified in Xenopus (Schmidt et al. Mol Endo 6:1634; 1992)
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It turned out that PPARs played a much more versatile role in biology than only peroxisome proliferation have emerged as key players in the regulation of mammalian metabolism.

**Functions of PPARs**

**PPARα**
(liver, heart, small intestine)
- Nutrient metabolism (lipid, glucose, amino acids)
- Peroxisome and hepatocyte proliferation (rodents only)
- Inflammation

**PPARγ**
(adipose tissue, lung)
- Lipid and glucose metabolism
- Cell cycle control
- Inflammation

**PPARβ/δ**
(ubiquitous)
- Fatty acid oxidation
- VLDL production
- Wound healing
Clinical features exhibited by adult subjects harboring loss-of-function mutations in PPARγ

(numerator denotes the reported number of affected individuals, denominator denotes the number of subjects for whom relevant information is available)

Gurnell PPAR Res; 2007
PPARs are members of the Nuclear Receptor Family

Functional Domain Structure of Nuclear Receptors

<table>
<thead>
<tr>
<th>A/B</th>
<th>C</th>
<th>D</th>
<th>E/F</th>
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<tbody>
<tr>
<td>N</td>
<td>AF1</td>
<td>DBD</td>
<td>Hinge</td>
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Activation Function 1
- Transactivation
DNA-binding domain
Ligand-binding domain
Activation Function 2
- Transactivation
- Dimerization
- Co-activator recruitment

<table>
<thead>
<tr>
<th>α</th>
<th>β/δ</th>
<th>γ</th>
<th>Subtypes</th>
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<tbody>
<tr>
<td>γ-1</td>
<td>γ-2</td>
<td>Isoforms</td>
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<table>
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<th>Subtypes</th>
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<th>6p21.2-p21.1</th>
<th>3p25</th>
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<td>7/8 exons</td>
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<td>3725 bp</td>
<td>1854/1751 bp</td>
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<tr>
<td>468 aa</td>
<td>441 aa</td>
<td>505/475 aa</td>
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Functional Domain Structure of Nuclear Receptors

Binding to DR1 REs
Heterodimers PPAR:RXR (like VDR:RXR; RAR:RXR)

PPAR:RXR ligand induced modulation
Superfamily: 48 human nuclear receptor genes in 28 classes

**Endocrine Receptors**
High-affinity (K\textsubscript{d} 0.1-1 nM), hormonal lipids

- ER\textalpha, \beta
- PR
- AR
- GR
- MR
- RAR\textalpha, \beta, \gamma
- TR\textalpha, \beta
- VDR

**Adopted Orphan Receptors**
Low-affinity (K\textsubscript{d} 1-1000 µM)
dietary lipids

- RXR\textalpha, \beta, \gamma
- PPAR\textalpha, \beta/\delta/\gamma
- LXR\textalpha, \beta
- FXR
- PXR
- CAR

**Orphan Receptors**
Unknown

- SF-1
- LRH-1
- DAX-1
- SHP
- TLX
- PNR
- NGFI-B\textalpha, \beta, \gamma
- ROR\textalpha, \beta, \gamma
- ERR\textalpha, \beta, \gamma
- RVR\textalpha, \beta, \gamma
- COUP-TF\textalpha, \beta, \gamma
- HNF-4
- TR 2,4
- GCNF

**Ligands:**

- "classical" endocrinology
- "sensors" for lipids and xenobiotics
The ligand-binding pocket (blue) is located in the lower part of the ligand-binding domain and for each member of the nuclear receptor superfamily an individual (and partly dynamic) structure!
Endogenous ligands for PPARγ?

- Fatty acids and eicosanoids
- Oxidized low density lipoproteins
- Oxidized alkyl phospholipids (lysophosphatidic acid, nitrolinoleic acid)
- 15-deoxy-Δ12,14-Prostaglandin J2 (probably too low endogenous conc. to affect PPAR function)

? Whether a highly specific natural ligand exists or acts as a physiological lipid sensor activated by combination of weakly activating fatty acids
Synthetic PPAR agonists have a major clinical significance

**Fibrates (PPARα)**
- Examples: gemfibrozil, fenofibrate, clofibrate, WY14643
- Decrease plasma triglycerides
- Increase plasma HDL
- Decrease free fatty acids

**Thiazolidinediones (PPARγ)**
- Examples: rosiglitazone, troglitazone, pioglitazone
- Decrease plasma triglycerides and (in rodents) free fatty acids
- Improve glycemic control e.g. lower plasma glucose & insulin
• The best-known high affinity PPARγ ligands are the: thiazolidinediones (TZD)

• Rosiglitazone (Avandia®) and Pioglitazone (Actos®)

• Treatment of type 2 diabetic patients

Not all PPARγ ligands exhibit the same effects
  • Receptor binding affinity
  • Impact on receptor conformation
  • Binding of corepressors / coactivators
  • Gene (promoter context)
    In mature adipocytes:
    • in absence of ligand PPARγ together with coactivators bind to fatty acid binding (aP2) promoter
    • In same cells/condition the glycerol kinase (GK) and oxidized low density lipoprotein receptor-1 (OLR-1) require ligand to be induced; in absence of TZD PPARγ recruits corepressors

Selective PPARγ modulation can be achieved by targetting corepressor interaction, separating transactivation and transrepression and favoring specific subsets of coactivators
Activated PPARγ activates pathways leading to net flux of fatty acids from the circulation and other tissues into adipocytes
(a.o. Expression of lipoprotein lipase (LPL), fatty acid transporter protein (FATP))

Increased fat storage would suggest increased adipocyte size

Actually, TZD treatment leads to smaller adipocytes

TZD induces adipocyte differentiation: more smaller adipocytes

Activation of PGC-1a (PPARγ-coactivator 1a) ⇒ mitochondrial biogenesis ⇒ increased fatty acid oxidation ⇒ protects against adipocyte hypertrophy

**TZD induces adipocyte differentiation**

Gurnell et al., Best Practice & Research Clinical Endocrinology & Metabolism; 2005
Okuno et al., JCI; 1998
Wilson-Fritch et al., JCI; 2004
WHY IS THIS IMPORTANT FOR BONE AND BONE DISEASES?

• Effect of PPARγ activation on bone turnover
• Fat – bone connection
• Mesenchymal stem cell lineages
• PPARγ and adipocyte differentiation
• PPARγ expression and effects on osteoblasts
Rosiglitazone decreases markers of bone formation and BMD in normal postmenopausal women.
With aging the composition of the bone marrow changes and results in Osteoporosis: increase osteoclast activity, declined osteoblast activity and more adipocytes

- Osteoporosis is associated with increased marrow fat content
- Adipocyte tissue volume in bone marrow is increased with aging and in patients with osteoporosis

(J. Justesen et al., Biogerontology, 2001)
Osteoblast and adipocyte: mesenchymal origin

Mesenchymal Stem cell

Pre-adipocyte

Pre-chondrocytes

Pre-hypertrophic chondrocytes

Chondrocyte

Pre-Osteoblast

Osteoblast

Osteocyte

Controlled by multiple growth factors, hormones and mechanical loading
PPARγ is an important transcription factor for the adipocytic lineage.
PPARγ alternative splice forms

PPARγ gene

PPARγ transcript variants

PPARγ protein variants

Brudigam et al., FEBS letters; 2008)
PPARγ-1, -3, and -4 are the predominant transcripts in human osteoblasts coding for the PPARγ-1 protein.
Classical PPARγ transcripts follow the expression of PPARγ in human osteoblasts

Bruedigam et al., FEBS letters; 2008)
Male, non-diabetic C57BL/6 mice: from 5 weeks gavaged daily for a period of 90 days with either vehicle (0.25% carboxymethyl cellulose (medium viscosity) aqueous solution (5 ml/kg/day)) alone (CONTROL) or vehicle with 3 mg/kg/day rosiglitazone maleate (TREATMENT)

Soroceanu et al. J Endocrinology 2004
Rosiglitazone treatment leads to increased osteoblast and osteocyte apoptosis
• PPARs are nuclear receptors
• Three isoforms exist: PPARα, PPARβ/δ, and PPARγ
• They act as sensors of diet and xenobiotics
• They play an important role in lipid and glucose homeostasis and metabolic control at whole organism and cellular level
• Involved in: metabolic syndrome, diabetes, obesity, ...
PPARγ Summary

• At least 4 PPARγ splice variants exists

• Leading to the expression of 2 PPARγ proteins: PPARγ-1 and PPARγ-2

• The four PPARγ transcript variants are differentially expressed in human tissues and osteoblast cell lines.

• PPARγ-2 predominantly in adipocytes

• PPARγ-1 is ubiquitously expressed, including in osteoblasts

• Expression of the three transcript variants encoding for PPARγ-1 significantly increases during human osteoblast differentiation.
• PPARγ in unliganded form can regulate gene transcription

• No PPARγ splice variant-specific ligands have not been identified. (they have identical ligand binding domains)

• Activation of PPARγ-2 is considered to be the adipocyte lineage determinant of mesenchymal stem cells

<table>
<thead>
<tr>
<th>Not all PPARγ ligands have similar effects on adipocytes and osteoblasts</th>
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<tbody>
<tr>
<td><strong>Adipocyte</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Netoglitazone</td>
</tr>
<tr>
<td>GW0072</td>
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<td>Troglitazone</td>
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Pei and Tontonoz, JCI; 2004
**PPARγ Summary**

- Activation of PPARγ(-1) in mesenchymal stem does not lead to block of osteoblast differentiation

- Both adipogenic as osteogenic differentiation of human mesenchymal stem cells is accelerated by activation of PPARγ.

- Tempting to speculate:
  - PPARγ acts as an energy sensor during osteoblast differentiation and bone formation
  - There is a switch from glycolysis to respiration and mitochondrial biogenesis (Chen et al. Stem Cells; 2006)

- PPARγ activation induces osteoblast apoptosis while adipocytes are protected against apoptosis
Pei and Tontonoz, JCI; 2004
MSCs with PPARγ2 knockdown or mouse embryonic fibroblasts from PPARγ-2 −/− mice showed decreased **adipogenic** as well as reduced **osteogenic** differentiation and mineralization upon BMP-9 stimulation.

**It is not a single molecule issue**

Systems biological approaches are needed to delineate the regulatory networks of PPARγ adipocyte and osteoblast commitment as well as differentiation.

Kang et al. Stem Cells and Development; 2009
Muruganandan et al. Cell Mol Life Sci; 2009