Role of PPARα and Relevance of Human Tumors

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1. Is there any new evidence since the publication by Klaunig et al. (2003) to suggest that PPARα agonists or peroxisome proliferation may lead to carcinogenesis in humans?

Nothing compelling

2. Is there any new evidence since the publication by Klaunig et al. (2003) to support the conclusion that PPARα agonists are not likely to pose a cancer risk to humans?

The recent findings from the humanized PPARα mouse lines and the proposed mechanism elucidated. I will elaborate more on this later.

3a. Ito et al. (2007) reported that DEHP caused liver tumors in PPARα-null mice. The authors have suggested a PPARα-independent mode of action. How do these findings affect the conclusion of Klaunig et al. that PPARα-agonists are not likely to pose a cancer risk to humans? Is it plausible that some PPARα agonists may induce tumorigenesis by a PPARα-independent mode of action (Ren et al. 2010)?

Raises questions. Impossible to determine whether these findings affect the conclusion that PPARα-agonists are not likely to pose a cancer risk to humans due to many limitations of the Ito study:

Limited to 2 doses; no consistent dose-dependent changes in liver tumors found in either genotype. Statistical analysis is limited to trends.

The phenotype in the *Ppara*-null mice may not be relevant to humans; polymorphisms to date only show a receptor with enhanced activity (L162V).

Ppara-null mice develop liver tumors with age; most likely due in part to lipid accumulation and dysregulated inflammation (enhanced inflammation). The trend of liver tumors in null mice could reflect an entirely different MOA, not found when PPARα is present.

Does PPARα protect against liver cancer? Remains possible of more than one MOA for DEHP including the PPARα MOA.

3b. Ito et al. (2007) reported that DEHP caused liver tumors in PPARα-null mice. The authors have suggested a PPARα-independent mode of action. How do these findings affect the conclusion of Klaunig et al. that PPARα-agonists are not likely to pose a cancer risk to humans? Is it plausible that some PPARα agonists may induce tumorigenesis by a PPARα-independent mode of action (Ren et al. 2010)?

Yes, it is plausible.

But this does not rule out the possibility that the established PPARα MOA can also be a central MOA for any given chemical.

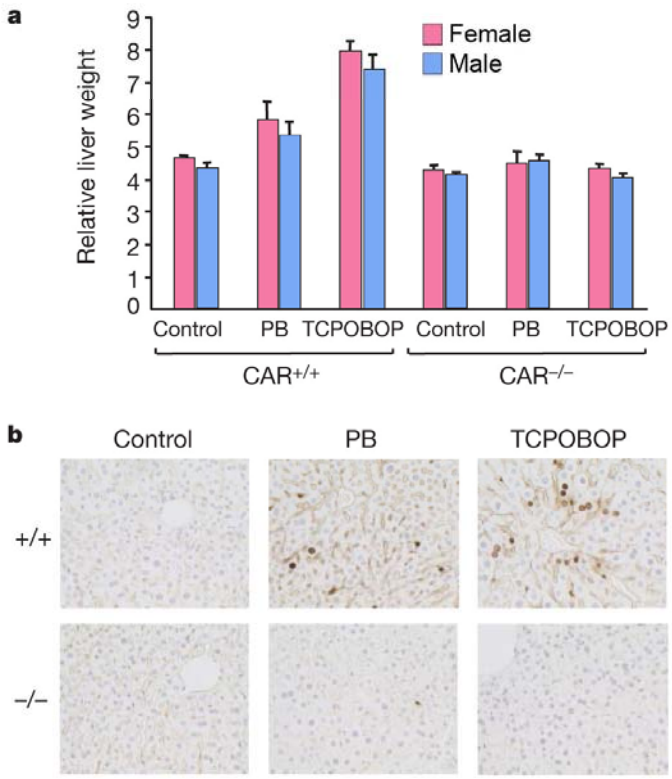
Difficult to reconcile how *Ppara*-null mice do not develop liver tumors in response to long term exposure to PPARα agonists, without accepting the fact that PPARα is required to mediate the hepatocarcinogenic of PPARα agonists.

Peters, JM et al Carcinogenesis (1997) 18: 2029-2033 [Wy-14,643]

Hays, T et al Carcinogenesis (2005) 26: 219-227 [Bezafibrate]

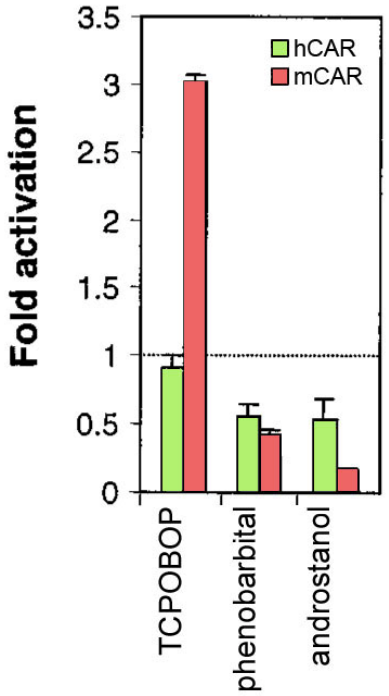
Is CAR involved?

CAR-mediates PB and TCPOBOP-inducd liver hyperplasia in mice.



Wei, P., et al. *Nature*, 407: 920-923, 2000

Species differences in ability of different CAR agonists to activate rodent versus human CAR



Moore, L.B., et al. *J. Biol.Chem.*, 275: 15122-15127, 2000

4a. Recently, a strain of “humanized” mice have been described that express human PPARα (Yang et al. 2008). PPARα agonists induce peroxisome proliferation and reduce lipid levels in humanized mice, essentially as they do in the wild-type mice. However, cell proliferation and tumorigenesis are not induced in the humanized mice. How do these studies support the conclusion of Klaunig et al. that PPARα-agonists are not likely to pose a cancer risk to humans? Can these studies be interpreted as evidence of PPARα-independent cancer modes of action in humans (Guyton et al. 2009)?

Clarification: There are 2 unique lines of humanized mice that have been developed, one liver-specific and one where PPARα is expressed globally.

Cheung, C et al Cancer Research (2004) 64: 3849-54 [liver-specific]

Morimura, K et ak Carcinogenesis (2006) 27: 1074-1080 [liver-specific]

Yang, Q et al Tox Sci (2008) 101-132-139 [global expression]

The regulation of lipid catabolism is observed in humanized mice (both models) but limited changes in cell proliferation (both models) or tumorigenesis (liver-specific) are observed.

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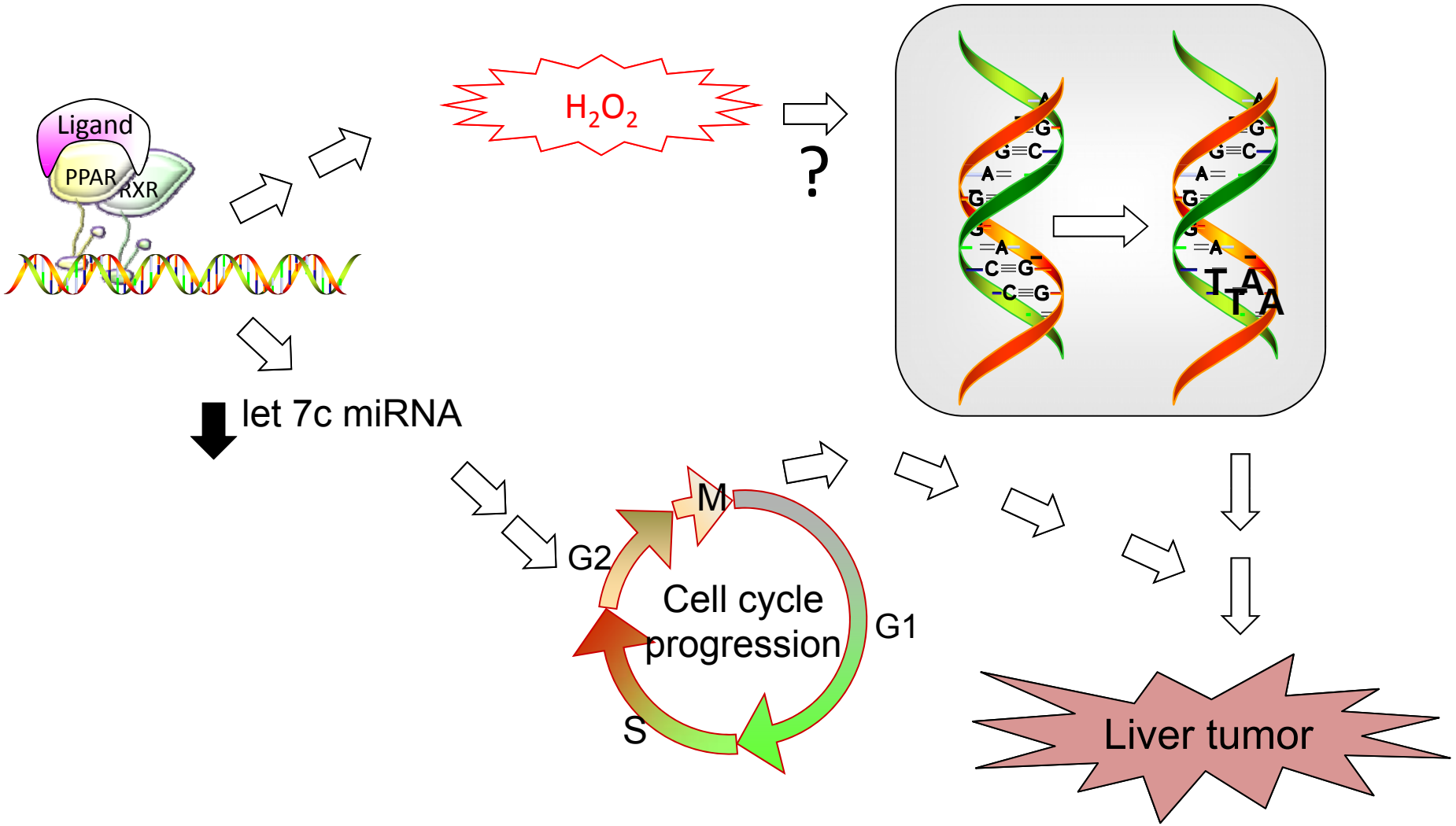
Demonstrate that the human PPARα modulates lipid catabolism in vivo; basis for the ongoing therapeutic use of fibrates for treating dyslipidemias in humans.

Demonstrate that activating the human PPARα in a mouse does not cause liver cancer after chronic treatment with Wy-14,643 which is known to cause liver cancer through a PPARα-dependent mechanism (Peters, 1997).

Most likely explanation for this difference in activity is differences in molecular targets due to differences in transcriptional co-factor recruitment…….

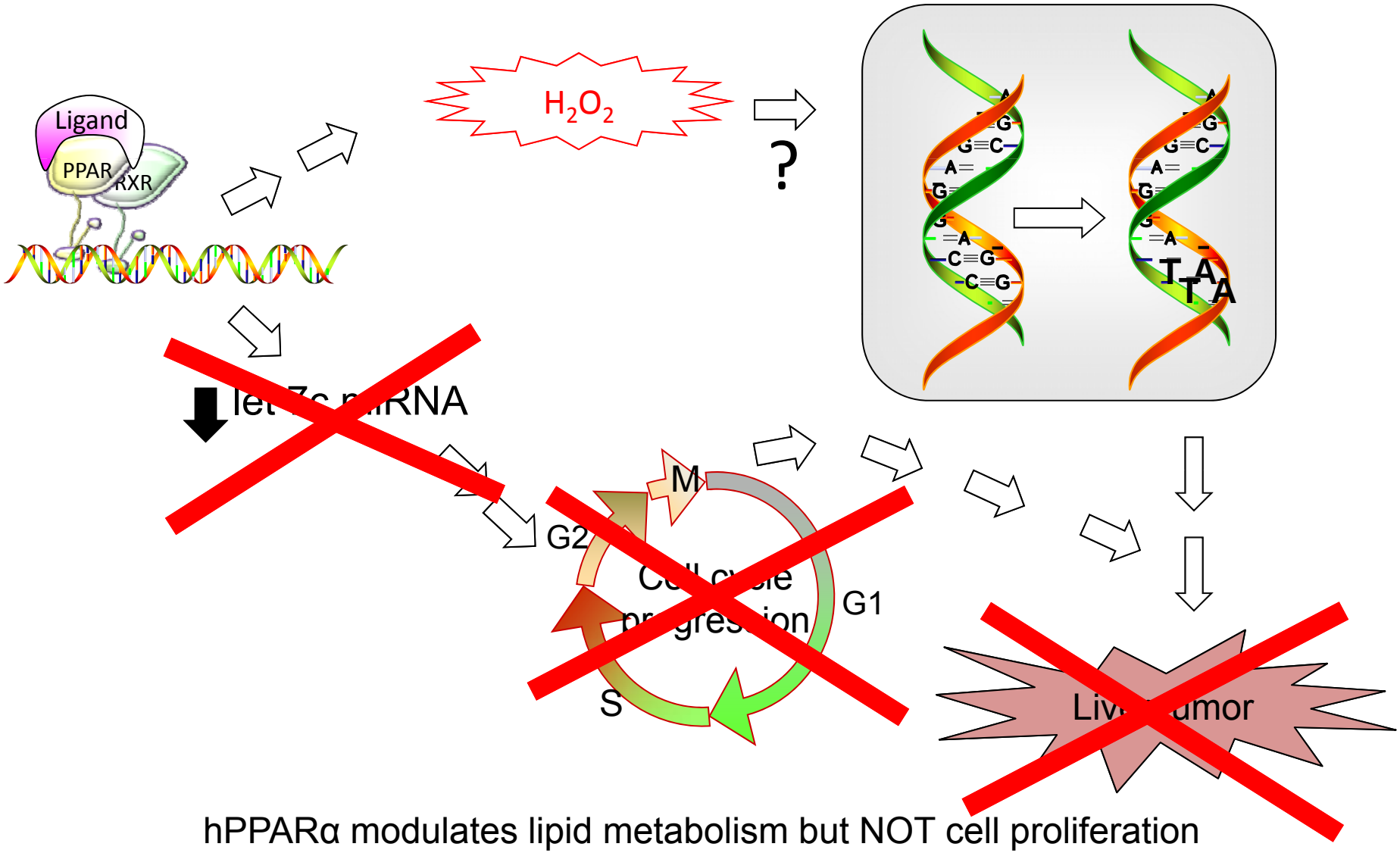
Mechanism For Species Difference



mPPARα modulates lipid metabolism AND cell proliferation

Shah, YM et al, Mol. Cell. Biol. (2007) 27: 4238-4247

Mechanism For Species Difference



hPPARα modulates lipid metabolism but NOT cell proliferation

Shah, YM et al, Mol. Cell. Biol. (2007) 27: 4238-4247

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The arguments presented by Guyton et al suggested the Morimura study was limited because mice were only exposed for 38 weeks, there was mortality in the wild-type mice, small number of animals studied, and that the human PPARα may not function the same in the mouse due to differences in transcriptional co-factor recruitment.

How does one explain the lack of liver tumors in mice when the human PPARα is expressed and does respond to ligand activation by modulation of lipid catabolism?

The humanized mice studies do not provide compelling evidence of PPARα-independent cancer modes of action in humans.

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Guyton's argument of a PPARα-independent MOA is based primarily on 2 papers:

1) Ito (limitations discussed previously)

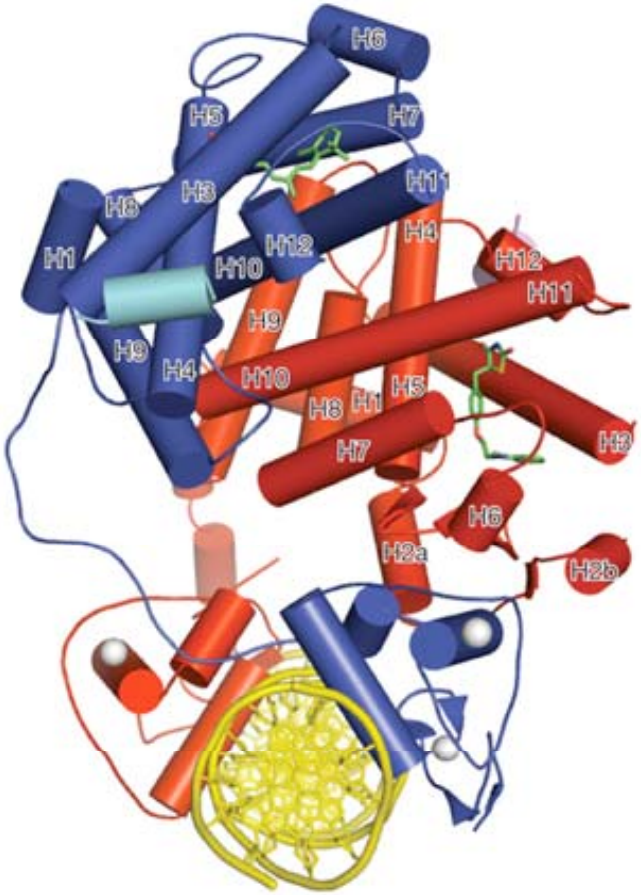
2) Yang, Q et al (2007) Carcinogenesis 28: 1171-1177

Lack of change in hepatic cell proliferation despite increased expression of lipid catabolizing enzymes in a transgenic mouse expressing a VP16-PPARα fusion protein.

Guyton: "…. PPARα activation (by the VP16 fusion PPARα) is not sufficient to induce hepatocarcinogenesis.", "These data are therefore inconsistent with the hypothesis that effects mediated through PPARα activation constitute a complete MOA for carcinogenesis."

There is a major problem with this interpretation.

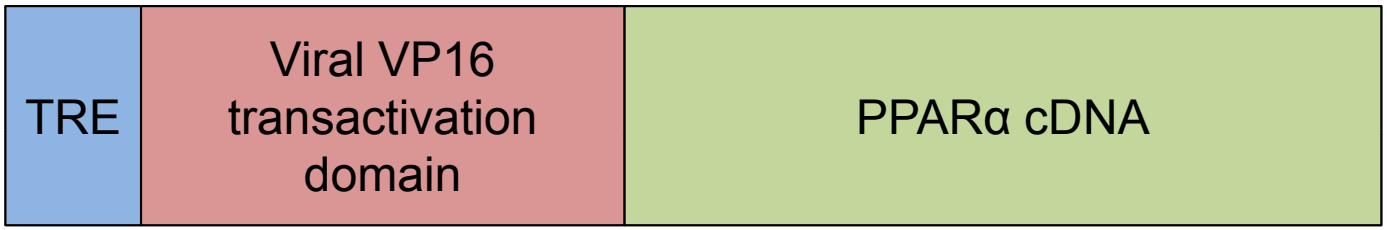
Structure of PPAR/RXR Complex on DNA



Ligand activation causes conformational changes in protein structure that allows dissociation of co-repressors and recruitment of specific co-activators, scaffolding proteins, RNA polymerase etc.

The ligand has a major role in modulation of receptor function (troglitazone vs. pioglitazone)

Chandra, V. et.al. Nature 456: 350-356, 2008



VP16 fusion PPARα

Fusion PPARα ≠ Endogenous PPARα

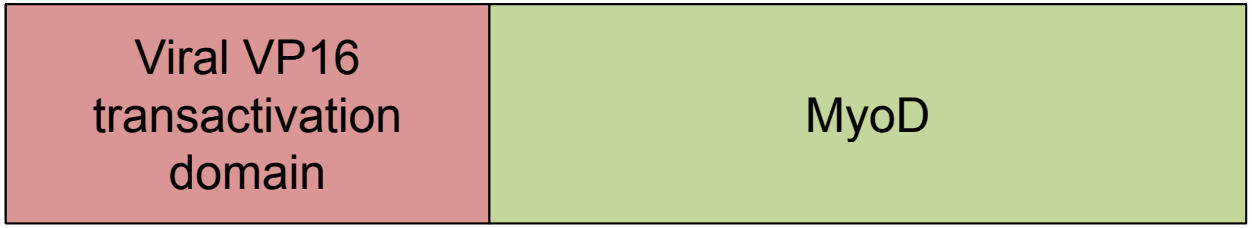
VP16 fusion protein relies on a viral transactivation domain for activity.

VP16 transactivation domain increases transcription of fusion proteins by a number of mechanisms:

protein-protein interactions with general transcription factors TFIIA, TFIIB, the TATA-binding protein and TAFII40 components of the multisubunit TFIID, and direct recruitment of RNA polymerase.

Contrast with transcription mediated by ligand-receptor mechanisms.

Lack of change in cell proliferation in the VP16 fusion PPARα transgenic mouse may simply reflect differences in the ability of this protein to modulate targets that can regulate cell proliferation (e.g. let 7c miRNA, etc)



MyoD transcription factor (muscle TF that modulates differentiation) has a transactivation domain (TAD), similar to PPAR

Replacement of MyoD TAD with VP16 TAD results in fusion protein

Fusion protein transactivates MyoD reporters, but does not induce myogenesis

Fusion protein transcription factor ≠ Endogenous transcription factor

Schwarz, J. et.al. M.C.B. 12: 266-275, 1992

5. Many phthalates are capable of activating both murine and human PPARα and PPARγ (Bility et al. 2004; Peraza et al. 2006). Are PPARα and PPARγ required for, or contribute to, the toxic effects of phthalates in rodents including:

PPARγ

A. Cancer? Highly unlikely, ongoing chemoprevention/chemotherapeutic studies in humans.

B. Liver toxicity? Uncertain. Not been examined with null mice. Increased activity of PPARγ associated with lipid accumulation in liver, but typically very low expression. Some evidence of hepatoprotective effects due to anti-inflammatory activities.

C. Kidney toxicity? Uncertain. Not been examined with null mice.

D. Reproductive and developmental effects? MEHP, PPARγ agonists inhibit aromatase mRNA in rat granulosa cells, antagonist mitigates. No evidence of developmental toxicity due to PPARγ agonists.

Other health endpoints? Addressed later.

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PPARα

A. Cancer?

Liver: clear role, but other mechanisms can also not be clearly ruled out.

PACT, LCT: unclear role.

Other tumors: Not been examined in detail.

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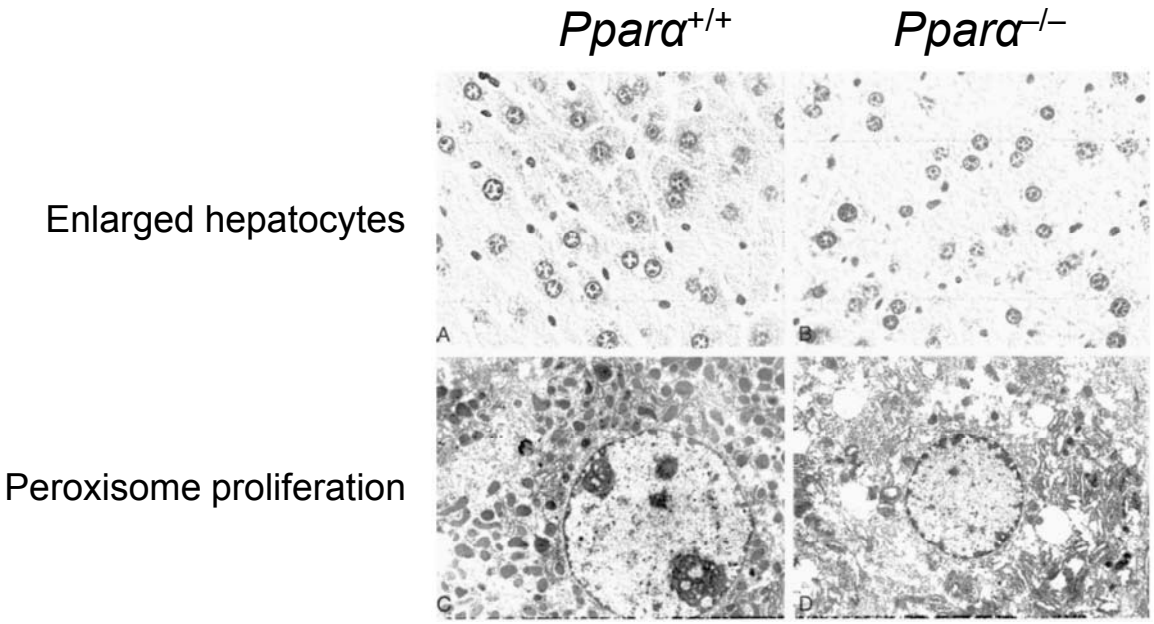
B. Liver toxicity? Yes. DEHP causes marked diffuse hepatocytomegaly and cytoplasmic granular hepatocyte eosinophilia (peroxisome proliferation) only found in wild-type, not *Pparα*-null mice.



(Ward, JM, et al (1998) Toxicol Pathol 26: 240-246)

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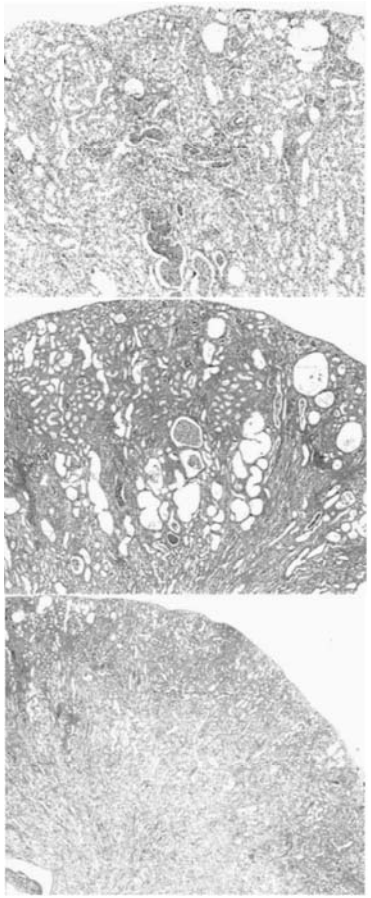
PPARα

C. Kidney toxicity? Yes and no.

After 4 weeks of DEHP, nephropathy, focal tubular degeneration, atrophy and regenerative tubular hyperplasia in wild-type mice but not in *Pparα*-null mice.

After 8-16 weeks of DEHP, severe cystic renal tubules, this effect was diminished in *Pparα*-null mice.

After 24 weeks of DEHP, severe nephropathy in *Pparα*-null mice. No comparison with wild-type because they had all died.



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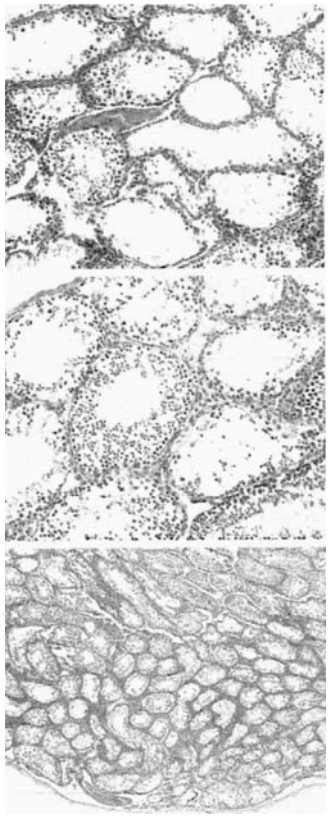
PPARα

D. Reproductive and developmental effects? Yes and no.

After 8 weeks of DEHP, no spermatogenesis in most tubules in wild-type mice.

After 8 weeks of DEHP, only a few tubules where normal spermatogenesis is not found in *Pparα*-null mice.

After 24 weeks of DEHP, diffuse tubular aspermatogenesis in *Pparα*-null mice. No comparison with wild-type because they had all died.



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D. Reproductive and developmental effects? Yes and no.

DEHP-induced developmental toxicity is similar in both wild-type mice and *Pparα*-null mice.

TABLE 1. Influence of DEHP treatment on GD10 pregnancy outcome in wild-type and PPARα (−/−) mice*

Geno.[1]	Treatment group	N[2]	Liver/BW (g/kg BW)[3]	Sites[4]	% Live[5]	% Resorption[6]	CR (mm)[7]	% NTD[8]
+/+	Control	10	45.1 ± 2.8[a]	7 ± 1[a]	88 ± 4[a]	17 ± 4[a]	5.0 ± 0.2[a]	8 ± 4[a]
+/+	DEHP	10	66.3 ± 2.1[b]	8 ± 1[a]	34 ± 9[b]	18 ± 8[a]	3.7 ± 0.4[b]	78 ± 7[b]
−/−	Control	10	49.3 ± 2.5[a]	8 ± 1[a]	80 ± 5[a]	10 ± 6[a]	4.7 ± 0.1[a]	12 ± 6[a]
−/−	DEHP	10	61.9 ± 2.6[b]	8 ± 1[a]	28 ± 9[b]	17 ± 7[a]	3.6 ± 0.4[b]	90 ± 3[b]

*Values represent the mean ± S.E.M. Values within a column with different superscripts are significantly different at $P \leq 0.05$.
[1]PPARα genotype.
[2]N, number of litters examined.
[3]Relative maternal liver weight.
[4]Implantation sites per litter examined.
[5]Percentage of live embryos (those with a visible heartbeat) per total number of fetuses examined.
[6]Resorptions per total number of implantation sites.
[7]Crown-rump length.
[8]NTD, percentage of embryos with neural tube defects, primarily open hind- and mid-brain.

TABLE 2. Influence of DEHP treatment on GD18 pregnancy outcome in wild-type and PPARα (−/−) mice*

Geno.[1]	Treatment group	N[2]	Sites[3]	% Live[4]	% Resorption[5]	Fetus wt. (g)	CR (mm)[6]	% Abnormal[7]
+/+	Control	12	8 ± 1[a]	84.3 ± 4.3[a]	14.7 ± 4.3[a]	1.17 ± 0.03[a]	22.5 ± 0.3[a]	3.1 ± 1.6[a]
+/+	DEHP	10	8 ± 1[a]	28.2 ± 10.2[b]	71.8 ± 10.2[b]	1.07 ± 0.05[b]	22.6 ± 0.5[a]	40.0 ± 14.9[b]
−/−	Control	14	8 ± 1[a]	85.3 ± 4.2[a]	12.3 ± 3.5[a]	1.23 ± 0.03[a]	22.8 ± 0.2[a]	3.4 ± 1.6[a]
−/−	DEHP	13	7 ± 1[a]	37.1 ± 9.3[b]	62.9 ± 9.3[b]	1.07 ± 0.06[b]	22.2 ± 0.3[a]	49.6 ± 11.7[b]

*Values represent the mean ± S.E.M. Values within a column with different superscripts are significantly different at $P \leq 0.05$.
[1]PPARα genotype.
[2]N, number of litters examined.
[3]Number of implantation sites per litter examined.
[4]Percentage of live fetuses per total number of fetuses examined.
[5]Percentage of resorptions per total number of implantation sites.
[6]CR, crown-rump length.
[7]Percentage of fetuses with external malformations, primarily exencephaly.

(Peters, JM, et al (1997) Teratology 56: 311-316)

6. PPARα and possibly PPARγ induce a different suite of genes in humans, as compared to rodents. PPARα and PPARγ may contribute to health effects in rodents. What are the implications of interspecies differences in PPAR function regarding human health risk?

Whether PPARα and/or PPARγ induce a different suite of genes in humans as compared to rodents may be overstated. Differences likely exist but also many similarities (e.g. basis for modulation of lipid metabolism by fibrates, modulation of glucose by TZDs)

Interspecies differences are likely important for all xenobiotic receptors, but probably exist on many levels:

- Receptor itself
- Distribution of receptor, co-factors in different tissues
- Response elements
- Epigenetic differences

6. PPARα and possibly PPARγ induce a different suite of genes in humans, as compared to rodents. PPARα and PPARγ may contribute to health effects in rodents. Are adverse effects in rodents mediated by PPARα and PPARγ relevant to humans?

Context dependent, minimally requires data from knockout/knockdown experiments to demonstrate requirement of PPAR and strong dataset from humans for comparison.

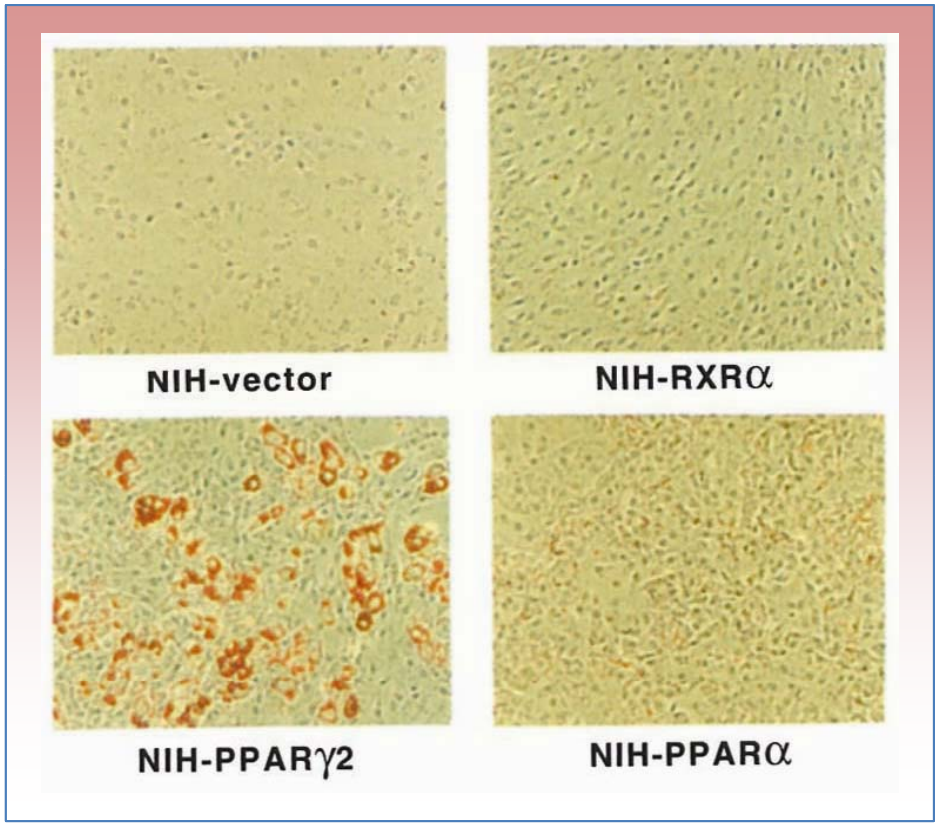
Fairly strong weight of evidence that at least for some PPARα agonists (fibrates), liver cancer observed in rodents is likely not relevant.

Other examples less clear, PACT, LCTs.

PPARγ-dependent increase in osteoclast activity observed in mice correlates well with decreased bone mass observed in some humans treated with PPARγ agonists.

Some toxicities observed in humans following exposure to PPAR agonists not always seen in rodent models (e.g. CHD)

6. PPARα and possibly PPARγ induce a different suite of genes in humans, as compared to rodents. PPARα and PPARγ may contribute to health effects in rodents. What is known about the function of PPARγ in humans?



Activation of PPARγ causes fibroblasts to differentiate into adipocytes

First evidence to demonstrate that PPARγ promotes differentiation of adipocytes

Tontonoz, P. et al Cell 79: 1147-1156, 1994

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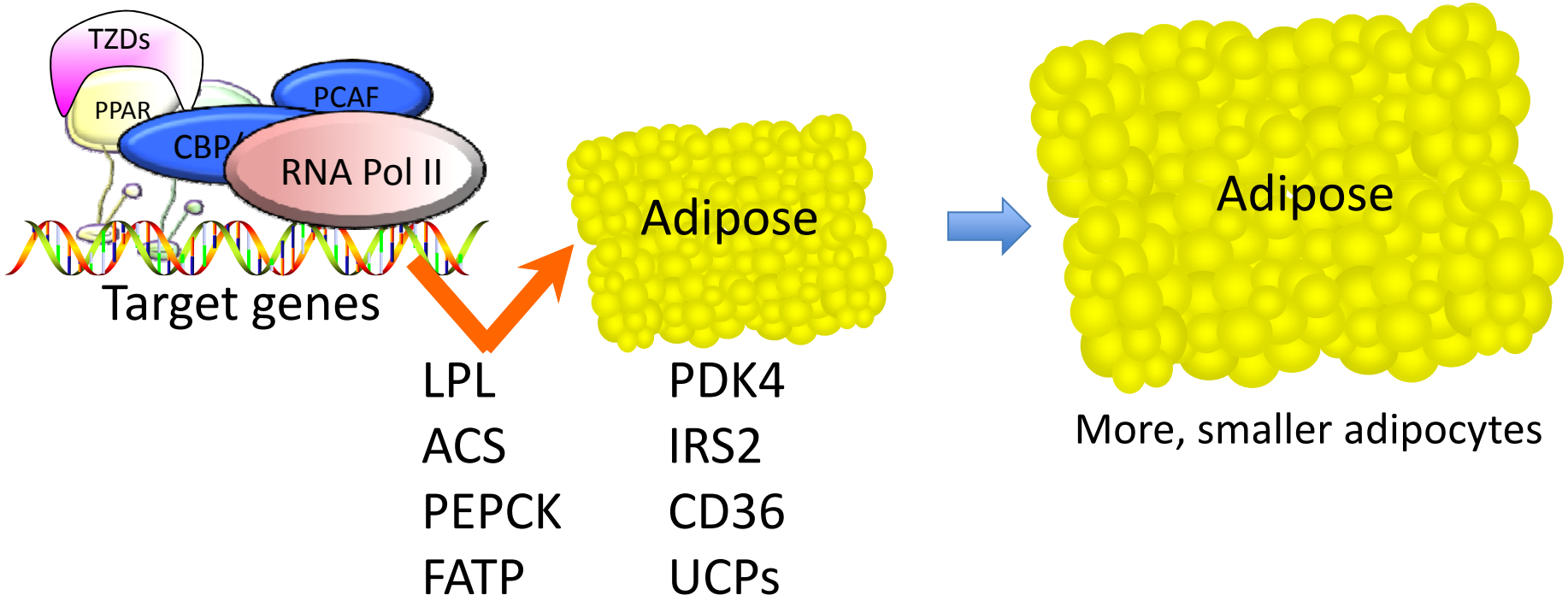

TZDs do not exert hypoglycemic effects in the absence of PPARγ expression in adipose: Adipose is a primary target tissue of PPARγ

6. PPARα and possibly PPARγ induce a different suite of genes in humans, as compared to rodents. PPARα and PPARγ may contribute to health effects in rodents. What is known about the function of PPARγ in humans?

PPARγ agonists; ongoing clinical trials to determine the efficacy of chemoprevention/chemotherapy-modulates terminal differentiation, inhibits cell proliferation, increases apoptotic signaling

PPARγ has anti-inflammatory activity in immune cells: transrepression

PPARγ inhibits differentiation of Th17, but not Th1, Th2 or regulatory T cells; immunointervention in Th17-mediated autoimmune diseases such as MS?

Klots, L. et al J. Exp. Med 206: 2079-2089, 2009

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Adverse Side Effects Associated With TZDs

- Hepatotoxicity (Rezulin)
- Fluid retention (Epithelial Na+ Channel)
- Edema (fluid retention, endothelial permeability?)
- Weight gain (adipogenesis, fluid retention?)
- Congestive heart failure
- Bone fractures (osteoclast activity)

7. PPARα-agonists are most commonly associated with liver cancer in rodents. Are there any other cancer sites that the CHAP should consider in its risk assessment of phthalates such as pancreas or testes?

Previous CHAP used mononuclear cell leukemia for DINP

Uncertain whether PACT or LCT are PPARα-dependent, there is some controversy whether these tumors are relevant due to species differences.

8. Are you aware of any ongoing studies that may be helpful to the CHAP during the next year or so?

Ongoing bioassay of wild-type, *Pparα*-null and humanized PPARα mice with GW7647 (highly potent PPARα agonist [nM], greater affinity for human versus mouse PPARα).

Have heard there may be other long term bioassays with DEHP, but uncertain of specifics.