Thyroid hormone receptors regulate adipogenesis and carcinogenesis via crosstalk signaling with peroxisome proliferator-activated receptors

Changxue Lu and Sheue-Yann Cheng

Laboratory of Molecular Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, 37 Convent Drive, Room 5128, Bethesda, Maryland 20892-4264, USA

(Correspondence should be addressed to S-Y Cheng; Email: chengs@mail.nih.gov)

Abstract

Peroxisome proliferator-activated receptors (PPARs) and thyroid hormone receptors (TRs) are members of the nuclear receptor superfamily. They are ligand-dependent transcription factors that interact with their cognate hormone response elements in the promoters to regulate respective target gene expression to modulate cellular functions. While the transcription activity of each is regulated by their respective ligands, recent studies indicate that via multiple mechanisms PPARs and TRs crosstalk to affect diverse biological functions. Here, we review recent advances in the understanding of the molecular mechanisms and biological impact of crosstalk between these two important nuclear receptors, focusing on their roles in adipogenesis and carcinogenesis.

Journal of Molecular Endocrinology (2010) 44, 143–154

Introduction

Peroxisome proliferator-activated receptors (PPARs) and thyroid hormone receptors (TRs) are ligand-dependent transcription receptors of the subfamily 1 (NR1) in the nuclear receptor superfamily. The NR1 group also includes retinoic acid receptors (RARs), Rev-erb, RAR-related orphan receptors (RORs), oxysterol receptors (LXRs), vitamin D3 receptors (VDRs), and the nuclear xenobiotic receptor (constitutive androstane receptor; CAR). PPARs and TRs share a conserved DNA-binding domain (DBD) and exert their activity partly by heterodimerization with a common partner, the retinoid X receptor (RXR), to regulate the transcription of target genes. PPARs and TRs each have diverse effects on developmental and metabolic processes as well as in diseases such as obesity, diabetes, and cancer. The first part of this review describes current understanding of PPAR and TR biology. The second part highlights recent advances in the understanding of molecular mechanisms underlying the PPAR–TR crosstalks, with particular emphasis on the role of such crosstalk in metabolism and carcinogenesis.

Peroxisome proliferated tracer-activated receptors

PPARs, which consist of PPARα (NR1C1), PPARβ/δ (NR1C2, hereafter referred to as PPARδ), and PPARγ (NR1C3; Fig. 1), are encoded by three different genes (PPARA, PPARD, and PPARG) located at chromosomes 22, 6, and 3 respectively. Upon ligand binding, PPARs are recruited to peroxisome proliferator response elements (PPREs) in the regulatory region of target genes as heterodimers with the auxiliary factor RXR. With the PPAR/RXR heterodimers, either partner can bind cognate ligands and elicit ligand-dependent transactivation (Kliewer et al. 1992). The canonical PPRE contains two direct repeats of the hormone response element (HRE) half site (AGGTCA) with a 1 bp spacer in between (DRI; Kliewer et al. 1992, Tugwood et al. 1992).

Natural and synthetic ligands have been reported for the three PPAR isotypes (Balakumar et al. 2007, Bensinger & Tontonoz 2008; Table 1 and Fig. 2). For PPARα, ligands include natural unsaturated fatty acids (FAs), leukotriene, hydroxyeicosatetraenoic acids (HETEs), and synthetic hypolipemia-inducing drugs such as fibrates. The PPARδ ligands are less well known, but FAs have been suggested to be natural ligands for this subtype of PPAR. The PPARγ ligands are less well known, but FAs have been suggested to be natural ligands for this subtype of PPAR (Fyffe et al. 2006). Recent studies identified a few more PPARγ agonists, namely tetradechyloacetic acid (TTA), L-165041, and GW501516 (Berger et al. 1999, Oliver et al. 2001, Westergaard et al. 2001). For PPARγ, endogenous ligands include polyunsaturated FAs, prostanoids, and oxidized FAs found in low-density lipoproteins (Forman et al. 1995,
glucose metabolism and insulin sensitization. That PPARγ also has a dominant role in adipogenesis is suggested by many loss-of-function studies both in vivo and in vitro (Fajas et al. 2001).

**Thyroid hormone receptors**

In humans, TRs are encoded by two genes, *THRA* and *THRB*, located at chromosomes 17 and 3 respectively. The *THRA* gene encodes three TRα (NR1A1) isoforms, TRα1, TRα2, and TRα3, which differ in their carboxyl terminus as a result of alternative splicing of the primary transcripts (Fig. 1). TRα1 binds triiodothyronine (T3) and activates or represses target genes, whereas TRα2 and TRα3 do not bind T3, and may antagonize T3 action (Izumo & Mahdavi 1988, Mitsushashi et al. 1988, Macchia et al. 2001). The *THRA* gene also yields two truncated proteins known as TRα1 and TRα22, which play a role in intestinal development (Chassande et al. 1997, Plateroti et al. 2001). The *THRB* gene encodes two amino-terminal TRβ (NR1A2) protein variants – TRβ1 and TRβ2. In rodents, the same gene also gives rise to TRβ3 and a truncated protein TR△β3, which lacks the DBD (Williams 2000, Harvey et al. 2007).

TRs bind to thyroid hormone response elements (TREs) in target genes as homodimers as well as heterodimers with RXR. Most naturally occurring positive TREs identified to date include two repeats of the half site 5′-AGGTCA-3′, which is also shared by PPREs arranged as a direct repeat with 4 bps between the two half sites (DR4). Among known TREs, inverted repeats (also called palindromes; e.g. GGTGATGACCT(N6)-AGGTCA, N: any nucleotide) have also been reported (Glass et al. 1988, Brent et al. 1989, Farsetti et al. 1992, Forman et al. 1992). TRs have also been shown to heterodimerize with other nuclear receptors, such as PPARs and VDRs (Bogazzi et al. 1994, Schrader et al. 1994). In the presence of PPARα, TRβ has binding affinity to a DR2 present in the myelin proteolipid protein gene promoter but not to the classical TRE, DR4, located in the malic enzyme gene. Upon thyroid hormone stimulation, the DR2-driven reporter gene is activated by the TRβ–PPARα heterodimer but not by TRβ–PPARγ, TRβ–RXRα, or TRβ–RXRβ. Three amino acids in the D box of the DBD in TRβ are critical for this heterodimerization. The dissimilarity of the D boxes between TRβ and TRα may be responsible for this isoform-dependent protein interaction and the DNA-binding sequence specificity (Bogazzi et al. 1994).

In humans, the DBDs of TRs and PPARs are highly homologous. Importantly, TRs and PPARs share the same proximal box (P-box) sequence in DBD, which is critical in sequence-specific recognition of HRE by NRs, while providing contact surface with the major factors. Therefore, TRs and PPARs are functionally redundant with respect to the metabolism of lipids and carbohydrates.
Table 1 Ligands for peroxisome proliferator-activated receptors (PPARs) and thyroid hormone receptors (TRs)

<table>
<thead>
<tr>
<th>Receptors</th>
<th>Endogenous ligands</th>
<th>Synthetic ligands</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPARβ/δ</td>
<td>Unsaturated or saturated long-chain Fas, prostacyclin, eicosanoids, HODE (oxidized FA; Shureiqi et al. 2003)</td>
<td>Rosiglitazone, pioglitazone, rivotril, etazoline, troglitazone, gigliotazone, farglitazar, GW7845 (Wilson et al. 2000)</td>
</tr>
<tr>
<td>PPARγ</td>
<td>HODE (Schofer et al. 2005), 15d-PGJ2 (Forman et al. 1995, Kliwer et al. 1995), azPC (Davies et al. 2001), LNO2 (unsaturated FA; Schofer et al. 2005)</td>
<td>CO23 (Ocasio &amp; Scanlan 2006) GC-1, KB-141, KB2115, MB07811 (Baxter &amp; Webb 2009)</td>
</tr>
<tr>
<td>TRα1</td>
<td>T4, T3</td>
<td></td>
</tr>
<tr>
<td>TRβ</td>
<td>T4, T3</td>
<td></td>
</tr>
</tbody>
</table>

HETE, hydroxyeicosatetraenoic acid; HODE, hydroxyoctadecadienoic acid; azPC, hexadecyl azelaoyl phosphatidylcholine; LNO2, nitrolinoleic acid.


T₃ is the most active form of thyroid hormone in target tissues, whereas T₄-thyroxine (T₄) is the most abundant thyroid hormone in the blood. Deiodination of T₄ by either type I or type II 5'-deiodinase (DI or DII) in different tissues gives rise to the more active T₃ locally. Several synthetic agonists for TRs have also been developed in the past decade (Table 1). Among them, GC-1 and KB-141 have been extensively studied and show a higher affinity to the TRβ subtype. In animal models, these TRβ-specific agonists decrease plasma cholesterol and triglyceride levels, and induce fat loss without apparent adverse effects on the heart and muscles (Grover et al. 2003, Baxter et al. 2004).

Similar to PPARs, tissue-dependent distribution of TR isoforms regulates thyroid hormone actions in target organs. Both TRα and TRβ are expressed in virtually all tissues but the abundance varies for each isoform (Lazar 1993). TRα1 is abundantly expressed in the skeletal muscle, heart, and BAT, whereas TRα2 mRNA is highly expressed in the brain. TRβ1 is more expressed in the brain, liver, and kidney. TRβ2 has a more restricted expression pattern, with major distribution in the anterior pituitary and hypothalamus (Hodin et al. 1989). The isoform-dependent distribution further adds to the complexity in the regulation of T₃ actions.

Crosstalk of PPARs and TRs in metabolism and adipogenesis

Reciprocal regulation between TRs and PPARs

TRs play important roles, as do PPARs, in lipid mobilization, lipid degradation, FA oxidation, and glucose metabolism. By direct or indirect effect, thyroid
status influences the expression of a number of genes involved in lipid and glucose metabolism. For example, TR isoform-specific regulation of hepatic genes involved in lipogenesis and FA oxidation has been implicated by the cDNA array analysis of TRb knockout mice treated with or without thyroid hormone (Flores-Morales et al. 2002). Among more than 200 hepatic genes responding to T3 treatment, ~60% of them are regulated by TRb and the remaining 40% are regulated through TRs. PPARz is one of the T3-regulated genes (Flores-Morales et al. 2002).

In vivo studies reveal that TR and PPAR signaling can similarly regulate some pathways in the lipid and glucose metabolism. In corticosteroid-induced diabetic mice, a decreased thyroid hormone level is accompanied by increased serum levels of insulin, total cholesterol, triglycerides, and tissue lipid peroxidation. Interestingly, the PPARγ agonist rosiglitazone is able to reverse the effect of dexamethasone and to increase T3 and T4 levels in circulation (Jatwa et al. 2007). Moreover, in rats with a high-fat diet and in a hyperthyroid state, administration of the PPARz agonist Wy14,643 restores glucose tolerance by enhancing glucose-stimulated insulin secretion and relieves the effect of hyperthyroidism. These studies suggest that activation of PPARz activity may restore the islet function affected by abnormal T3–TR signaling (Holness et al. 2008).

Crosstalk between TR- and PPAR-signaling pathways has also been implicated in the gene expression patterns in rats fed a high-fat diet. This diet induces an expression of the PPARz gene with a concomitant decrease in expression in the liver of RARβ, TRz1, and TRβ1. In WAT, this diet increases the expression of PPARγ2 but reduces expression of RARz, TRz1, and TRβ1 (Redonnet et al. 2001). Similar results have been obtained by using a specific PPARz inducer, bezafibrate, in rats. After a 10-day treatment with bezafibrate, PPARz transcription is elevated with an activation of the downstream gene, acyl-CoA oxidase (AOX), and a decrease of maximal ligand-binding capacity of RARβ and TRz1/β1 (Bonilla et al. 2001). However, no RXRz mRNA expression is altered by the treatment (Bonilla et al. 2001, Redonnet et al. 2001).

PPARs have been shown to affect the thyroid hormone functions in thermogenesis in vivo. Administration of the PPARγ agonist rosiglitazone to male rats shifts the energy usage to an anabolic state. Moreover, the activation of PPARγ by rosiglitazone markedly reduces plasma thyroid hormones, and mRNA levels of DI and DII in the liver and BAT respectively. Rosiglitazone also decreases mRNA levels of the TRz1 and TRβ in BAT, and the TRz1 and TRz2 in retroperitoneal WAT. These results explain the functions of PPARγ in up-regulating thermogenesis-related genes in WAT and BAT, while balancing the whole body thermogenesis by down-regulating the transcription activity of TRs in these processes (Festuccia et al. 2008). Regulation of DII by PPARγ activation has also been identified in skeletal muscle in which the expression of DII was found to be induced by PPARγ agonists, e.g., pioglitazone. Thus, via regulation of DII expression, PPARγ provides an additional level of regulation via modulation of thyroid hormone metabolism (Grozovsky et al. 2009).

Studies of energy metabolism in rats show that the PPARγ expression is modulated by T3–TR signaling. By elevating the T3 level in hypothyroid rats, the expression of mitochondrial PPARγ in skeletal muscle is induced along with the increased mRNA level of mitochondria protein uncoupling protein 3 (de Lange et al. 2007). More recently, in a study using transgenic mice overexpressing the putative mitochondrial T3 receptor p43 (an A/B domain truncated form of TRz1 (Casas et al. 1999, Wrutniak-Cabello et al. 2001)) in skeletal muscles, an increased protein level of PPARγ was observed (Casas et al. 2008). Taken together, these findings provide evidence that TR and PPAR can crosstalk by reciprocally affecting each other’s activity.

**Competition for the auxiliary factor RXR by PPAR and TR**

It is reasonable to postulate that TRs and PPARs can regulate some common target genes involved in metabolic functions. As previously mentioned, transcriptional activities of PPARs and TRs are determined by several factors including hormone availability, relative distribution of receptor isoforms, and the abundance of the auxiliary factor, the RXR and other coregulators. On the bases of the sequence homology of HREs and the sharing of the heterodimerization partner RXR, several mechanisms have been proposed to understand the cross-signaling between PPARs and TRs.

An early study examined the effect of PPARα on the binding of TRβ to TREs and transcription activity (Meier-Heusler et al. 1995). Via eletrophoretic mobility shift assay (EMSA), PPARα was shown to reduce the binding of TRβ–RXRz to TRE, but PPARα itself had no affinity to the TRE either as a homodimer or heterodimer with RXRz. In a chloramphenicol acetyltransferase (CAT) activity assay, however, PPARα exerted an inhibitory effect on T3-induced transcription activation by TRβ on the TRE–CAT reporter gene even in the presence of overexpressed RXRz protein in cells. These results suggest that PPARα inhibits the transcriptional activity of TR action by competing for the heterodimerized partner RXR in the nucleus (Meier-Heusler et al. 1995).

In another report, by site mutagenesis, a single leucine replacement by arginine at position 433 (L433R) of PPARα was shown to be critical in the
inhibition of TR action. This L443R alteration abolishes heterodimerization of PPARγ2 with RXR and consequently its selective inhibitory effect on the TR action. A mutational study also supports the idea that PPARγ2 does not compete with TRs for binding of TREs. Mutation in the P-box of hPPARγ2 (C122S), which destroys the DNA-binding ability of PPARγ2, does not affect its interference with TR transactivation. These findings suggest that PPARγ2 inhibits TRz transactivation by competing with TRz for binding to the auxiliary factor RXR (Juge-Aubry et al. 1995).

In a similar manner as PPARs, TRs also inhibit the activity of several peroxisomal FA oxidation enzymes regulated by both of these nuclear receptors. In transgenic mice expressing luciferase under the control of promoter of the rat peroxisomal enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase, T3 inhibits ciprofibrate-induced luciferase activity in a TR-dependent fashion. EMSA analyses reveal that regulation of the PPAR action by TR is through competition for the available RXR. Increasing the amount of RXR in cells partially reverses the TR inhibition on PPAR action, while heterodimerization-defective TR mutants lose the inhibitory activity. These results suggest that the peroxisome proliferators and T3-signaling pathways converge through their common interaction with the heterodimeric partner, RXR (Chu et al. 1995).

Competition for HRE binding by PPARs and TRs

In addition to competition for binding to the common heterodimeric partner (i.e. RXR), TRs compete with PPARs for binding to HREs on certain target genes. EMSA analysis shows that TRz binds to rat AOX PPRE, thus inhibiting the binding of PPAR to PPRE as PPAR/RXRRz heterodimers. However, as shown by transient transfection assays, TRz stimulates transactivation by PPAR/RXRRz heterodimers on the AOX-PPRE luciferase reporter gene in a T3-independent manner with unknown mechanism (Hunter et al. 1996). Moreover, comparison of reporter activity between wild-type and DBD-mutated (C78S) TRz1 shows that TRz1 exerts a DBD-dependent, inhibitory effect on the PPAR-induced AOX gene transcription (Miyamoto et al. 1997).

An in vivo study using mouse models also implicates an interdependent relationship between TRs and PPARs via competition for binding to HREs. Via affecting the mRNA expression levels of many genes including carnitine palmitoyltransferase Iα (CPT-Iα), AOX, acyl-CoA dehydrogenase, male mutant mice harboring a dominant-negative P398H-mutated TRz1 manifest visceral obesity, hyperleptinemia, reduced catecholamine-stimulated lipolysis in WAT, and hepatic steatosis (Liu et al. 2007). In the absence of T3, wild-type TRz1 and P398H mutant significantly reduce PPARγ2-mediated gene transcription in CPT-PPRE luciferase assay. However, thyroid hormone reverses the inhibition of PPARγ2 action by wild-type TRz1 but not by the P398H mutant. In vitro studies suggest that the P398H mutant itself is able to bind PPRE and reduce PPARγ2 binding to PPREs. It is not clear whether P398H mutant competes with PPARγ2 for binding to RXR. The metabolic phenotype exhibited by P398H mutant mice is mediated in part via the unique properties of the P398H mutant receptor in interfering with PPARγ2 signaling (Liu et al. 2007).

TR isoform-specific regulation of PPAR signaling in lipid metabolism

The role of TR isoforms in lipid metabolism has been recently studied in vivo using a loss-of-function approach. Mice expressing an identical mutation (denoted as PV) in the corresponding C-terminal region of TRz1 (TRz1PV mouse; Kaneshige et al. 2001) or TRβ1 were created (TRβ1PV mouse; Kaneshige et al. 2000). The PV mutation was identified in a patient with resistance to thyroid hormone (RTH) who has a frameshift mutation in the C-terminal 14 amino acids of TRβ, resulting in a complete loss of T3-binding and transcriptional capacity. This PV mutation exhibits potent dominant-negative activity. Compared with TRz1PV mice, TRβPV mice exhibit no reduction in white adipose mass but have significant increases in serum-free FAs and total triglycerides (Ying et al. 2007). The impaired adipogenesis in the WAT is mediated by the repression of the expression of PPARγ by TRz1PV, leading to the reduced expression of genes involved in lipid synthesis (Ying et al. 2007). Thus, in the WAT, crosstalk with PPARγ signaling is TR isoform-dependent crosstalk.

The TR isoform-dependent crosstalk with PPARγ is not limited to the WAT. Liver steatosis was observed in TRβPV mice, while a reduced liver mass with scarcity of lipid drops was detected in TRz1PV mice (Araki et al. 2009). In the liver of TRβPV mice, TRβPV activates PPARγ signaling with increased expression of PPARγ and downstream lipogenic genes. With the increased expression of the lipogenesis together with TRβPV-mediated decreased FA β-oxidation activity, excess lipid accumulation is observed in the liver of TRβPV mice. In contrast, TRz1PV functions to decrease the expression of PPARγ and its downstream lipogenic genes, leading to reduced fat mass in the liver of TRz1PV mice (Araki et al. 2009). These results indicate that PPARγ signaling in the liver is distinctively regulated by TR isoforms (Araki et al. 2009). Thus, these in vivo findings highlight the differential regulation by TR isoforms of the PPARγ signaling in lipid metabolism.

At present, the detailed molecular mechanisms by which TR isoforms mediate differential crosstalk with PPARγ signaling in the liver and WAT are not known.
It is possible that similar to the earlier report by Bogazzi et al. (1994), PPARγ could have selectivity in the heterodimerization with TRα or TRβ, resulting in differential functional consequences in lipid metabolism of these two target tissues. Alternatively, the different coregulatory proteins in these two target tissues could play differential modulatory roles, leading to differential regulation in lipid metabolic pathways. In view of the importance of the crosstalk of PPARγ with TRs in the regulation of lipid metabolism, these issues would merit additional studies in the future.

Crosstalk signaling of PPARs and TRs in cancers

PPARs in cancers

PPARs have been implicated in several cancers including colon cancer, thyroid cancer, and pancreatic carcinoma. However, their roles in the tumorigenesis of these cancers are controversial. Recent studies suggest that PPARδ has a role in promoting colon tumorigenesis. Mice with PPARδ gene disruption in colonic epithelial cells have a decreased incidence of chemical carcinogen azoxymethane-induced colon tumors (Zuo et al. 2009). Loss of PPARδ also suppresses the elevated expression of vascular endothelial growth factor in tumor tissue (Zuo et al. 2009). By contrast, in PPARδ−/−ApcMin/+ (adenomatosis polyposis coli gene, APC; multiple intestinal neoplasia, Min) mice, colon polyp formation is significantly greater than in ApcMin/+ mice, suggesting that PPARδ attenuates colon carcinogenesis and therefore can function as a tumor suppressor (Harman et al. 2004).

PPARγ has also been proposed as a tumor suppressor in colon carcinogenesis. In primary colorectal adenocarcinomas, ~8% of patients contain a loss-of-function mutation in one PPARγ allele (Sarraf et al. 1999). PPARγ agonists can decrease premalignant intestinal lesions in rats treated with azoxymethane (Tanaka et al. 2001). When PPARγ is activated in primary colon tumors and colon cancer cell lines by PPARγ agonists, these cells appear to stop proliferation with reduced growth and altered morphology of increased differentiation (Sarraf et al. 1998). Moreover, in Pparγ+/− mice with azoxymethane treatment, a greater incidence of colon cancer with an increase of β-catenin level is observed as compared with wild-type mice (Girnun et al. 2002). However, an oncogenic role of PPARγ in colon cancer has also been suggested in several reports. For example, activating of PPARγ signaling by treating ApcMin mice with the PPARγ ligand troglitazone increases significantly the number of colon polyps as compared with the untreated mice (Saez et al. 1998).

Human pancreatic carcinoma cells express functional PPARγ that upon activation by its ligand, troglitazone, induces growth inhibition associated with G1 cell cycle arrest (Motonura et al. 2000). It has been further shown that p27Kip1 can also play a key role in mediating the inhibitory effect by troglitazone (Motonura et al. 2000). A similar effect is also observed in human pancreatic cancer cell lines by using PPARγ ligands, 15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ2), and ciglitazone. That approach showed 15d-PGJ2 induces apoptosis through activation of caspases and reduces cell invasiveness by decreasing matrix metalloproteinase (MMP)-2 and MMP-9 protein levels and activity (Hashimoto et al. 2002). These findings raise the possibility that PPARγ as a potential therapeutic target for treatment of human pancreatic carcinomas.

The role of PPARγ in thyroid cancer was discovered by the identification of the fusion gene, the paired box box 8 (PAX8)–PPARγ, (PAX8–PPARγ; Kroll et al. 2000). Chromosom al t(2;3)(q13;p25) rearrangement results in the fusion of the thyroid transcription factor PAX8 gene to the PPARγ gene and the expression of a dominant-negative form of PPARγ1 protein (PAX8–PPARγ fusion protein, PPFP). PAX8–PPARγ rearrangement occurs in 25–70% of follicular thyroid cancers (FTC; Kroll et al. 2000). Immortalized human thyrocytes or thyroid cancer cell lines expressing the PPFP have increased cell viability and growth (Gregory Powell et al. 2004, Espadinha et al. 2007). Array analysis shows that the expression of PPFP in cells leads to gene expression profiles with distinct transcriptional signature. PPFP acts to upregulate genes associated with signal transduction, cell growth, and translational control. Concurrently, PPFP represses a large number of ribosomal protein and translational associated genes (Lacroix et al. 2005, Lui et al. 2005, Giordano et al. 2006). However, it is poorly understood how the fusion protein causes FTC.

More recently, a second PPARγ gene rearrangement, CREB3L2–PPARγ, was reported in a patient with FTC. This fusion gene encodes a CREB3L2–PPARγ fusion protein that consists of the transactivation domain of CREB3L2 and functional domains of PPARγ1. CREB3L2–PPARγ occurs infrequently in FTC (<3% of FTC). This fusion protein induces proliferation in primary thyroid cells, but has lost the responsiveness to the PPARγ agonists – troglitazone and rosiglitazone (Lui et al. 2008).

PPARδ has also been implicated in thyroid carcinogenesis. In vivo, a higher expression level of PPARδ is associated with increased proliferation in human thyroid tumors. This increased PPARδ can be induced by thyroid mitogen (e.g. TSH and insulin) in normal primary thyrocytes that are cyclin E1 dependent (Zeng et al. 2008).
TRs in cancers

T₃–TR signaling has also been implicated in the tumorigenesis of the liver, breast, thyroid, and colon. A variety of tumor cells respond to the stimulation of thyroid hormone to proliferate in vitro or in vivo (Short et al. 1980, Zhou-Li et al. 1992, Iishi et al. 1993, Esquenet et al. 1995). For example, thyroid hormone enhances liver cancer cell invasion by inducing the expression of furin protein, a proprotein convertase involved in tumor cell metastasis. The FURIN gene contains putative TREs and Smad-binding sites. Under stimulation by thyroid hormone or the transforming growth factor β (TGFβ), FURIN expression is induced, and the increased protein level subsequently cleaves MMPs such as MMP2 and MMP9 to increase tumor cell mobility (Chen et al. 2008). In xenograft models, the number of metastatic foci in liver and lung is increased with overexpression of furin in HepG2 cells. In addition, an increased T3 level in the presence of K-ras mutations, and more advanced stages of thyroid cancer (see section ‘PPARs in cancers’), a downregulation of the PPARγ gene in thyroid tumors of TRβ PV/PV mice is made evident by decreased mRNA and protein levels. In vitro and in vivo findings indicate that the mutated TRβPV protein acts to inhibit the ligand-dependent transcription of PPARγ by competitive binding to PPREs (Ying et al. 2003, Araki et al. 2005). Cell-based transfection studies using PPRE-containing reporters indicate that TRβ1 acts to enhance the transcription activity of PPARγ in the presence of both T3 and troglitazone. TRβPV also binds to PPRE as TRβ1 does, but due to its lack of T3 binding, TRβPV acts to interfere with the enhancing effect of TRβ1 on the transcription of PPARγ (Ying et al. 2003). Treating TRβ PV/PV mice with the PPARγ agonist rosiglitazone delays tumor progression and blocks metastatic spread (Kato et al. 2006). Moreover, when one allele of the PPARγ gene is disrupted in TRβPV mice, progression of FTC is accelerated (Kato et al. 2006). These data suggest that the mutated TRβPV is a dominant-negative regulator of PPARγ action. Two other PPARγ agonists, ciglitazone and 15d-PGJ2, have also been shown to...
decrease viability of a thyroid cancer cell line, CGRH W-2 (Chen et al. 2006). These two PPARγ agonists induce apoptosis of thyroid cancer cells through the caspase 3 and PTEN/Akt-signaling cascade, and they induce necrosis via the poly (ADP-ribose) polymerase (PARP) pathway (Chen et al. 2006).

Conclusions and future perspectives

Recent studies have indicated that PPARs and TRs can crosstalk to affect diverse cellular functions. Evidence presented in this review reveals three possible molecular mechanisms that may account for the crosstalk at the gene regulation level (Fig. 3). Several lines of evidence support the notion that there is direct competition between PPARs and TRs for HRE binding and/or partnership with RXR. Moreover, the differential binding of PPARs or TRs on DNA may also be determined by the context of the promoter/enhancer of the target gene. The effect of PPARs and TRs may be cooperative or opposing during the crosstalk, but most findings suggest the latter. Crosstalk between PPARs and TRs may also be mediated indirectly, such as a reciprocal regulation of PPAR expression by TRs or vice versa. The TR action may also be regulated indirectly by PPARs, e.g. via its modulating the intermediate enzyme genes, such as DII involved in T₃ metabolism.

Figure 3 Proposed molecular mechanisms in PPAR–TR crosstalk. Three major mechanisms underlying the crosstalk of PPARs and TRs in target gene regulation are illustrated. (A) Reciprocal regulation of the PPAR and TR expression. The liganded PPAR or TR can reciprocally affect the gene expression of the other via direct and/or indirect regulation. (B) Competition for HRE binding. TRs competitively bind to PPRE of PPAR target genes. RA, retinoic acid. (C) Competition for RXR partnership. Either TRs (i) or PPARs (ii) compete with the other receptor for binding to RXR, resulting in decreased availability of RXR to reduce the transcriptional activity of PPAR target genes shown in (i) or the transcriptional activity of PPAR target genes shown in (ii).
Recent exciting developments in the regulation of lipid metabolism and carcinogenesis by PPARs and TRs prompted us to focus in the present review on advances in understanding the crosstalk between PPARs and TRs. However, both PPARs and TRs have other important cellular functions that may also converge as a result of crosstalk between these two receptors. It is of great interest to identify other biological processes and the target genes involved that are modulated by this mode of regulation. Furthermore, while the present review focuses on the crosstalk of these two receptors via nucleus-initiated genomic actions, the crosstalk may also be via nongenomic actions. The nongenomic actions of these two receptors have recently been reported (Bergh et al. 2005, Gardner et al. 2005, Furuya et al. 2007). Thus, the crosstalk of PPARs and TRs could also be mediated via the nongenomic actions. In view of these considerations, a rapid development in the understanding in the biological processes governed by the crosstalks of these two receptors would be forthcoming.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

**Funding**

The present research was supported by the Intramural Research Program of the Center for Cancer Research, National Cancer Institute, National Institutes of Health.

**Acknowledgements**

We wish to thank all colleagues and collaborators who have contributed to the work described in this review.

**References**


Bergh JJ, Lin HY, Lasing L, Mohamed SN, Davis FB, Mousa S & Davis PJ 2005 Integrin alphaVbeta3 contains a cell surface receptor site for thyroid hormone that is linked to activation of mitogen-activated protein kinase and induction of angiogenesis. *Endocrinology* 146 2864–2871.


Chen RN, Huang YH, Lin YC, Yeh CT, Liang Y, Chen SL & Lin KH 2008 Thyroid hormone promotes cell invasion through activation of furin expression in human hepatoma cell lines. *Endocrinology* 149 3817–3821.


Espadinha C, Cavaco BM & Leite V 2007 PAX8PPARγ stimulates cell viability and modulates expression of thyroid-specific genes in a human thyroid cell line. *Thyroid* 17 497–509.


Forman BM, Casanova J, Rauka BM, Glynsaél J & Samuel HS 1992 Half-site spacing and orientation determines whether thyroid hormone and retinoic acid receptors and related factors bind to DNA response elements as monomers, homodimers, or heterodimers. *Molecular Endocrinology* **6** 429–442.


Glass CK, Holloway JM, Devary OV & Rosenfeld MG 1988 The thyroid hormone receptor binds with opposite transcriptional effects to a common sequence motif in thyroid hormone and estrogen response elements. *Cell* **54** 313–323.


Ocasio CA & Scanlan TS 2006 Design and characterization of a thyroid hormone receptor alpha (Tralpha)-specific agonist. *ACS Chemical Biology* **1** 386–395.


Suzuki H, Willingham MC & Cheng SY 2002 Mice with a mutation in the thyroid hormone receptor beta gene spontaneously develop thyroid carcinoma: a mouse model of thyroid carcinogenesis. Thyroid 12 963–969.


Received in final form 28 August 2009
Accepted 6 September 2009
Made available online as an Accepted Preprint 9 September 2009