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Association of COX-2 and PPARs in Carcinogenesis and Chemoprevention

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1. INTRODUCTION

Cyclooxygenase-2 (COX-2) inhibitors have been proven as effective chemopreventive agents for colon cancer in animal models and clinical trials. Although the exact functions of COX-2 products remain to be defined, ample studies have suggested that nuclear receptors such as peroxisome proliferator-activated receptors (PPARs) may function as important downstream mediators of COX-2 activity. This chapter will discuss the general role of several nuclear receptors in human malignancies, involvement of PPARs in tumorigenesis, and use of receptor antagonists or agonists for differentiation therapy and chemoprevention. It is proposed that PPAR γ agonists or PPAR δ antagonists may serve as more specific and less toxic agents for chemoprevention.

Despite significant progress in understanding the molecular genetics of cancer development, cancer-related care and cost remain as one of the biggest burdens imposed on our healthcare system. Effective prevention and early detection of human cancer would significantly ease this burden and improve the long-term survival of cancer patients. For the past decade, several chemopreventive measures have been actively pursued, and more encouraging results are being recorded in both animal studies and clinical trials.

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In the postgenomic era, cancer researchers are gaining information on the molecular mechanisms of tumor development at a rapid pace. The completion of sequencing the human genome will undoubtedly change the practice by which anticancer drugs are screened and developed. More rational approaches based on molecular mechanisms will be utilized to target specific pathways and/or given gene products, which are known to play an important role in tumorigenesis. Thus, mechanism-oriented chemotherapy and chemoprevention will likely take center stage in cancer drug discovery. This is exemplified by the extensive search of COX-2-specific inhibitors (1). Recent studies have suggested that PPARs may function as important downstream mediators of COX functions. It is conceivable that PPARs may serve as more specific targets for cancer chemoprevention. Mechanistically, PPARs are nuclear receptors that were isolated for their ability to modulate lipid metabolism. During the last decade, it has become clear that PPARs also contribute to a variety of different biologic processes, including atherosclerosis, insulin resistance and more recently, cancer. This chapter will discuss the evidence for the different PPARs' role in tumorigenesis, and their potential application for the treatment and prevention of neoplastic diseases.

2. NUCLEAR RECEPTORS AND HUMAN MALIGNANCIES

2.1. Nuclear Receptor Superfamily

Nuclear receptors are ligand-inducible transcription factors present in vertebrates, arthropods, and nematodes (2–4). The nuclear receptor superfamily now includes receptors for steroid hormones, retinoids, thyroid hormones and vitamin D3, and receptors that do not have clearly defined natural ligands, the so-called orphan receptors (Fig. 1). Activation of nuclear receptors requires ligand binding in high-affinity interactions concomitant with the apposition to enhancer elements in the proximity of promoters of their target genes. In general, nuclear receptors regulate key steps of development, lipid homeostasis, proliferation, and differentiation (2–7). The fundamental steps of a nuclear receptor signaling pathway constitute production of the signal molecule (endocrine, paracrine, autocrine, or intracrine), its transport to target organs/tissues, binding of the ligand to the receptor, and transcriptional activation of target genes by the receptor complex.

Recent genetic evidence suggests that nuclear receptors are phylogenetically related proteins whose ligand binding ability may be acquired during nuclear receptor evolution (8–10). The ancestral receptor may be an orphan receptor that may independently give rise to various ligand recognition receptors during evolution (Fig. 1). To date, more than 70 nuclear receptors have been identified. With the exception of a few receptors that appear to contain only regions homologous to the DNA- or ligand-binding domain (DBD and LBD, respectively), all members display an identical structural arrangement with an amino-terminal region A/B, followed by a DBD of two zinc fingers (region C), a linker region D, and a carboxyl-terminal region LBD (11) (Fig. 2A). The transcriptional activity of nuclear receptors originates from two domains, autonomous transactivation (AF-1) in region A/B and ligand-dependent transactivation (AF-2) derived from the LBD (6). Interestingly, the highly conserved features of the receptor structures are in a sharp contrast to the structural and functional diversity of the ligands. At the molecular level, nuclear receptors bind as homodimers (steroid receptors and RXRs), and/or heterodimers (retinoic acid receptors, thyroid hormone receptor, vitamin D receptor, and PPARs) along with the promiscuous heterodimerization partner RXR to cognate response elements of target

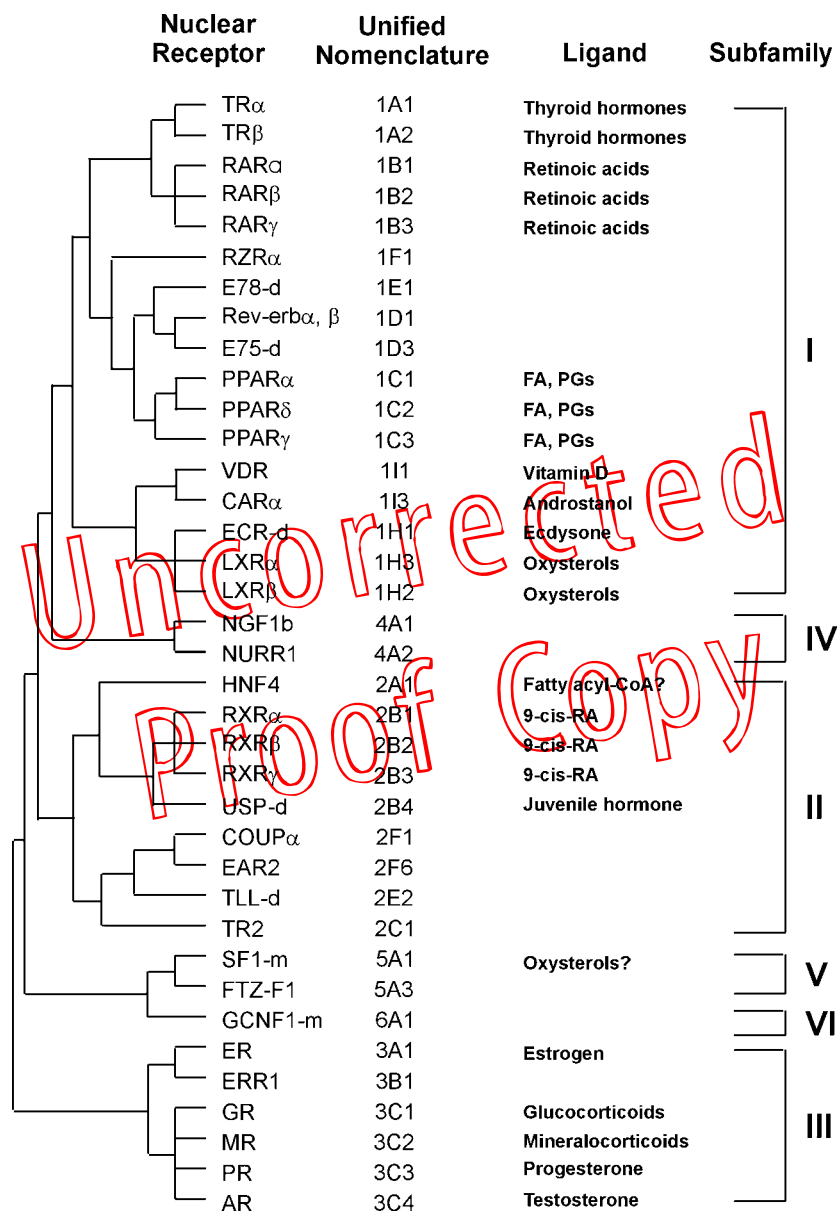


Fig. 1. Phylogenetics of the nuclear receptor superfamily. As indicated, the superfamily is divided into six subfamilies. PPARs belong to Subfamily I. RXRs belong to Subfamily II. Steroid hormone receptors are included in Subfamily III. All listed genes are from human origin, unless stated otherwise (d: *Drosophila*; m: mouse).

genes. A plethora of recent studies on characterization of coactivators and corepressors for the AF-2 domain have further illuminated the regulatory mechanisms of nuclear receptor functions in the context of chromatin structure (12–14). Although these findings have further demonstrated the complexity of nuclear receptor actions, they have also provided a more detailed picture for developing molecules that can modulate the functions of nuclear receptors for therapeutic or preventive purposes.

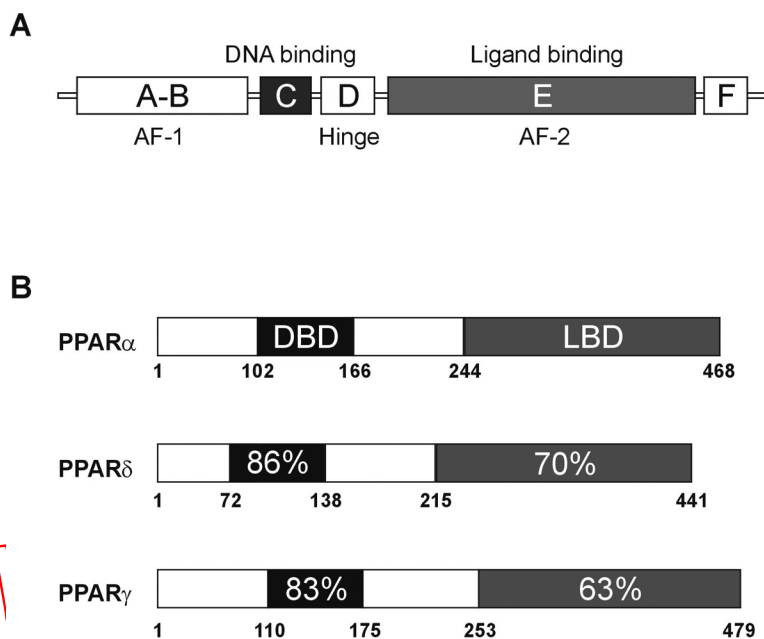


Fig. 2. Structural and functional domains of nuclear receptors and PPARs. **A.** Structural features of a prototypic nuclear receptor. The N-terminal A-B domains confer ligand-independent activation (AF-1). The C-terminal E domain is required for ligand-dependent activation (AF-2) and dimerization. The C domain mediates sequence-specific DNA binding. **B.** Comparison of amino acid identities of human PPAR isotypes. The percentage identity between the DNA-binding and ligand-binding domains are given relative to PPAR α . The numbers of residues from the N-terminus are also given under individual receptors.

2.2. Estrogen Receptors and Breast Cancer

The dysfunction of certain nuclear receptors has long been associated with tumorigenesis in humans and animals (15,16). A wealth of epidemiological data implicates several nuclear receptors and their hormones in the development of so-called hormone-related cancers, such as breast, endometrium, ovary, prostate, testis, and thyroid cancers (17). Currently in the United States, malignancies of hormone-responsive tissues account for >35% of all newly diagnosed cancers in men and >40% of all newly diagnosed cancers in women (18). Essential for development and growth of the mammary gland, estrogen has been associated with promotion and growth of breast cancer (19–21). Animal studies have frequently demonstrated that estrogens can induce and promote breast tumors in rodents, and that the removal of animal ovaries or administration of anti-estrogen drugs can suppress the estrogen-induced carcinogenesis (22–25). Accordingly, some of the most widely accepted risk factors for human breast cancer [e.g., early menarche, late menopause, postmenopausal obesity, and hormone replacement therapy (HRT)] may represent the cumulative effect of estrogen on breast epithelium (17). Most human breast cancers, at least initially, are hormone-dependent and undergo regression when deprived of the supporting hormone (19–21). Because the presence of estrogen receptor α (ER α) is generally considered as an indication of hormone dependence (26), anti-estrogen treatment (e.g., tamoxifen) is the first-line therapy for breast cancers (27,28). Initially demonstrated to induce remission of advanced and metastatic breast cancers, and later used as an adjuvant

for ER-positive primary breast cancers, tamoxifen has improved disease-free status and overall survival of breast cancer patients (27,28). More recently, potential chemopreventive benefit of tamoxifen and raloxifene in women at high risk has been reported (29,30). However, almost without exception, breast cancers that initially respond well to anti-estrogen tamoxifen by tumor regression will eventually resume growth despite continued presence of the antagonist. Although an exact mechanism of the acquired tamoxifen-resistance remains to be defined, it has clearly been demonstrated that estrogen receptors play an important role in the early stage of breast cancer development. The cancer cells that are refractory to anti-estrogen therapy may acquire additional genetic alterations, and the biological outcome of estrogen receptor activation may further be regulated by estrogen receptor coactivators and corepressors (19).

2.3. Androgen Receptor and Prostate Cancer

Prostate cancer is the most common solid tumor among North American men (18). Because the complete molecular mechanism of prostate cancer development remains elusive, an important role of androgen receptor (AR) in prostate tumorigenesis has long been suggested (31,32). Androgens are essential for the development, growth, and maintenance of the prostate. Androgen receptors are expressed in all histological types and stages of prostate cancer. As is the case with normal prostate development, primary prostate cancer cells are largely dependent on androgens for growth and survival (31,32). Most patients respond favorably to androgen ablation and anti-androgen endocrine therapy, which has become a standard treatment of metastatic prostate cancer (34). However, virtually all patients will suffer relapse with clinically defined androgen-independent cancer. The development of hormone ablation resistant prostate cancer is highly reminiscent of tamoxifen treatment of breast cancer. Collective genetic and molecular biological data have suggested that, in order to survive and grow in the low androgen environment, therapy-refractory cancer cells may adopt several mechanisms to bypass or become sensitized to the androgen receptor pathway (31). For instance, the cancer cells can select for mutations in androgen receptor to allow promiscuous activation by different steroids. Alternatively, the androgen receptor may be genetically amplified or activated in a ligand-independent manner by growth factors and cytokines. Moreover, it has been speculated that androgen receptor activity may be further regulated by receptor cofactors (coactivators and corepressors) (33–38). Activity of a steroid receptor in a particular cell depends not only on the expression level of a receptor itself, but also on those of coregulatory proteins. It is conceivable that overexpression of AR coactivators or underexpression of corepressors may lead to hyperstimulation of the androgen signaling cascade and increased expression of AR-regulated genes, most of which are involved in cell cycle regulation and cell proliferation (32). Taken together, the evidence clearly suggests that the androgen receptor plays an important role in prostate cancer development and progression (39).

2.4. Retinoic Acid Receptor α and Acute Promyelocytic Leukemia

Certain leukemias are characterized by chromosomal translocations that result in the generation of chimeric genes leading to nuclear receptor dysfunction. Human acute promyelocytic leukemia (APL) represents about 5% of acute myeloblastic leukemias, and is characterized by an accumulation of hypergranulated and immature promyelocytes (40). In most cases, APL is associated with a chromosomal translocation t(15;17), in which the major part of the RING protein PML fuses with retinoic acid receptor α (RAR α gene,

resulting in the chimeric gene product PML-RAR α (41,42). Because of the balanced translocations, the reciprocal chimera RAR α -PML is also produced, *albeit* at much lower level (43,44), and its role in leukemogenesis seems not to be critical. On the contrary, the causal relationship between fusion protein PML-RAR α and the development of APL has been validated in transgenic animals, in which expression of the fusion protein was under control of myeloid or promyeloid promoters (45–48). Translocations between the RAR α locus and other genes have also been observed in some rare APL syndromes, including PLZF-RAR α t(11;17) (49), NuMA-RAR α t(11;17) (50), and NPM-RAR α t(5;17) (51). PML functions as a tumor suppressor and mediates retinoic acid (RA)-induced differentiation (52). The PML-RAR α fusion protein possesses several unique features including high expression at the G1/S boundary during the cell cycle, preservation of ligand-binding ability by retaining the B-F region of RAR α , maintaining the ability to oligomerize, and significantly impaired ligand responsiveness (40). As a result, the PML-RAR α chimera requires at least a 100-fold higher dose of retinoic acid to achieve full-RA responsiveness in normal cells (40,44). It has become clear that, although PML-RAR α associates normally with the corepressor SMRT, its impaired ligand responsiveness hinders proper dissociation of the corepressor complex, leading to differentiation arrest (40). Consistent with this mechanism, PML-RAR α has been shown to inhibit terminal differentiation of hematopoietic cell lines *in vitro* and to block maturation of myeloid precursors *in vivo* (47,53). As discussed later, the function of RAR α fusion proteins in APL has been exploited for successful differentiation therapy.

3. PPARs AND TUMORIGENESIS

3.1. PPAR Family:

Three Isotypes with Distinct and Overlapping Functions

PPARs were discovered in the early 1990s as a consequence of the speculation that hypolipidemic drugs known as peroxisome proliferators (PP) may act through a nuclear receptor similar to that of the steroid hormones (54). Since the discovery of the first PPAR receptor, now known as PPAR α , two more forms of PPARs have been identified in mammalian cells, termed PPAR γ and PPAR δ (a.k.a., PPAR β , FARR or NUC1) (2,55–58). The human PPAR γ gene gives rise to at least three isoforms, PPAR γ 1, γ 2 and γ 3, by differential RNA splicing and alternative promoter use (59–61). PPAR α is highly expressed in liver and brown fat, and is also found in kidney, heart, and skeletal muscle. PPAR γ is mainly expressed in adipose tissue, and to a lesser extent in colon. PPAR δ is ubiquitously expressed with the highest level found in the gut, kidney, brain, and heart (62).

All three types of PPARs contain four major regions that are highly conserved among the nuclear receptor superfamily: the A/B domain, the C domain, the D domain, and the E/F domain (Fig. 2). The C domain is the DNA binding domain (DBD) and is highly conserved among different receptors. The ligand-binding domain (LBD) comprises the E/F domain (63). Although the structural arrangement and functional domains are very similar among the three PPAR isotypes, they each appear to carry out distinct physiologic functions. For example, PPAR α regulates the transcriptional activity of genes that are involved in lipid metabolism such as fatty acid uptake through plasma membranes, fatty acid binding in the cytoplasm, fatty acid oxidation, and lipoprotein assembly and transport. Animal knockout studies have recently demonstrated that PPAR α is the critical mediator of the peroxisome proliferation phenotype in rodents (64,65). PPAR γ , on the other hand, primarily

regulates the storage of fatty acids in adipose tissue by inducing terminal differentiation of pre-adipocytes (59). Most of the known PPAR γ target genes in fatty tissue are primarily implicated in the lipogenic pathway. In addition, PPAR γ is thought to modulate the body's response to insulin, based on the thiazolidinedione (TZD) class of drugs' ability to lower serum glucose levels. The exact mechanism involved with this remains elusive, as heterozygous PPAR γ knockout mice are not prone to insulin resistance when given a high fat diet, though this phenotype is abrogated when the mice are given a TZD class drug (66–68). Interestingly, PPAR γ homozygous null mice are not viable, though placental rescue experiments convincingly demonstrate PPAR γ 's role in adipogenesis. The physiologic role of PPAR δ had, until recently, not been elucidated. The creation of the PPAR β (PPAR δ) knock-out mouse has demonstrated that this receptor is also involved with lipid metabolism and the inflammatory response (69). Similar to other nuclear receptors, PPARs are ligand-activated transcription factors, in which transcriptional activation of target genes depends on the binding of ligand to the receptor (63). PPARs are fully functional only when they heterodimerize with the 9-*cis* retinoic acid receptor (RXR). The transcriptional activity of the PPAR:RXR complex is further modulated by nuclear receptor coactivators and/or corepressors (70).

3.2. PPAR α and Hepatocarcinogenesis in Rodents

The discovery of PPAR α answered a long-standing question regarding the mechanism of peroxisome proliferation stimulated by hypolipidemic drugs, such as clofibrate, in rodents. Subsequent experiments have confirmed that PPAR α can be activated by a plethora of peroxisome proliferators (64). Downstream target genes regulated by PPAR α have been primarily characterized in hepatocytes. Important target genes are involved in β - and ω -fatty acid oxidation pathways, suggesting an important role for PPAR α in liver lipid homeostasis. Interestingly, rodents chronically fed with peroxisome proliferators, such as Wy-14643, ultimately develop hepatocellular carcinoma (71). Genetic deletion experiments have confirmed that the tumorigenesis is a PPAR α -mediated process, as PPAR α -null animals are refractory to PP-induced hepatocarcinoma (64). Several hypotheses have been suggested for the role of PPAR α in hepatocarcinogenesis. For instance, PPAR α may regulate the expression of genes involved in cell proliferation. It has been reported that CDK-1, CDK-4, and c-Myc are upregulated in wild-type mice fed with Wy-14643, but not in PPAR α null mice (72). Activation of PPAR α by PP may also suppress apoptosis in hepatocytes (73). Irrespective of the molecular mechanism behind the PPAR α -mediated hepatocellular carcinoma in rodents, one of the most intriguing questions is why human and primates are generally refractory to PP-mediated carcinogenesis. The PPAR α ligand clofibrate is used clinically to treat hyperlipidemia. There has been no credible evidence of increased incidence of hepatocellular carcinoma among the subjects who take clofibrates (74). On the contrary, people prescribed these drugs on a long-term basis indeed receive the beneficial lipid-lowering effect, which is mediated by PPAR α activity. Although exact cause of the PPAR α species-specific activity remains undefined, recent studies have demonstrated that expression of PPAR α is significantly lower in human liver than in rodent liver (75,76). Mutant forms or dominant-negative forms of PPAR α are detected in certain human livers (76,77). Thus, the cumulative effect of lower expression, mutated receptors, and inactive peroxisome proliferator response elements (PPREs) in the promoter of the acyl CoA oxidase gene, may contribute to the differential response to PP by human PPAR α (77).

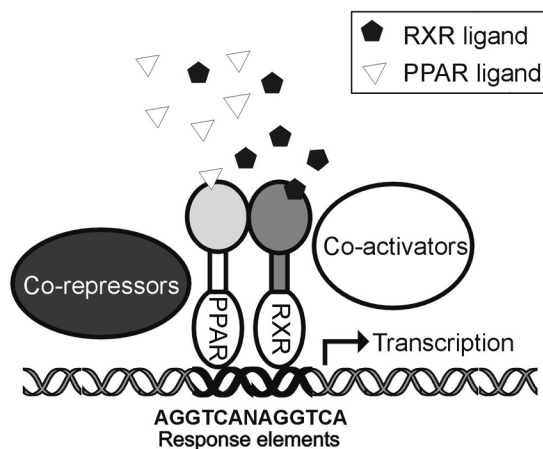
3.3. PPAR γ and Tumorigenesis

PPAR γ is the most widely studied isoform of all three PPARs. The discovery of anti-diabetic TZD drugs as PPAR γ agonists has generated much interest in the role of PPAR γ in diabetes and obesity. More recent studies have suggested that PPAR γ may also play a role in colon cancer development (78,79). In 1998, The research groups of Evans and Auwerx reported that PPAR γ agonists could promote intestinal tumorigenesis in the multiple intestinal neoplasia (Min) mouse model (see later), implicating PPAR γ in the oncogenesis of colorectal tumors (80,81). However, Spiegelman et al. reported that, in the nude mouse model injected with human colorectal tumor cells, the same PPAR γ agonists promoted differentiation and subsequently inhibited the cancer growth (82). Although there is no satisfactory explanation for these contradictory actions of PPAR γ agonists, it is highly reminiscent of the species-specific response to PPAR α ligands (e.g., peroxisome proliferators) in rodents versus humans. A role for PPAR γ in human intestinal tumorigenesis has been further implicated by a recent discover of loss-of-function mutations of this receptor in human colon cancers (83). In this study, one allele of the PPAR γ gene in 4 of 55 sporadic colorectal cancers was mutated, suggesting that PPAR γ may function as a tumor suppressor for colon cancer. However, it remains unclear whether these heterozygous mutations of PPAR γ play any role in colorectal tumorigenesis. Additionally, the role of PPAR γ in colon cancer is also complicated by the findings that some nonsteroidal anti-inflammatory drugs (NSAIDs) can serve as weak ligands for PPAR γ (84). Nevertheless, a growing body of evidence has demonstrated that PPAR γ agonists exhibit anti-tumor and apoptosis-inducing activities in a broad range of human malignancies, including breast cancer, prostate cancer and liposarcomas (85–96).

3.4. PPAR δ and Colorectal Tumorigenesis

The development of colorectal cancer requires the accumulation of multiple genetic alterations (97). The majority of colon cancers are initiated by inactivating mutations in the adenomatous polyposis coli (APC) tumor suppressor gene (97). The role of APC as a gatekeeper for colorectal tumor development is manifested in familial adenomatous polyposis (FAP) patients and the Min mouse model, both of which are caused by germline mutations in the APC gene. One of the most important functions of APC is to target the protein β -catenin for degradation. Originally identified as a cell adhesion molecule, β -catenin has been shown to form a transcription complex with members of the T-cell factor (TcF)/LEF transcription factor family (98,99). More than 90% of human colorectal cancers are initiated by an elevation in β -catenin/TcF activity caused by either inactivating mutations of APC or oncogenic mutations of β -catenin (97). Thus, it is conceivable that identification of the downstream target genes of the β -catenin/TcF pathway may illuminate the mechanisms through which the APC tumor suppressor regulates cell growth. Extensive studies of this pathway have led to identification of several prominent target genes (100–103).

Recently, PPAR δ was identified as a downstream target gene of the APC/ β -catenin/TcF pathway (104). In this study, expression of PPAR δ was repressed by wild-type APC or a dominant-negative mutant form of TcF4. Further analysis revealed that the genomic sequence of the PPAR δ promoter contained two canonical TcF4-responsive elements. More interestingly, a PPAR δ -responsive luciferase reporter was markedly inhibited by NSAIDs, such as sulindac sulfide and indomethacin, both of which have been demonstrated to be effective chemopreventive drugs for colon cancer in humans and animal models. Further-



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Fig. 3. Mechanism of PPAR action. Heterodimerization of PPAR with RXR produces an active transcriptional complex, which binds to a peroxisome proliferator response element (PPRE) and activates the expression of downstream target genes. This process requires ligand binding and is tightly regulated by nuclear receptor coactivators and corepressors.

more, NSAIDs, especially sulindac sulfide, have been shown to directly disrupt the ability of PPAR δ to bind to its responsive elements in *in vitro* DNA-binding assays. These findings suggest a molecular link between the genetic pathways of colorectal tumorigenesis and the mechanism by which NSAIDs can prevent colon cancer. This line of investigation may also provide a molecular basis for the epidemiologic association between dietary fat consumption and the relative risk of colon cancer, because products of fatty acid metabolism may serve as natural ligands for PPAR δ . Thus, it is conceivable that APC or β -catenin mutations may result in increased PPAR δ activity, whereas NSAIDs can potentially compensate for this defect by suppressing PPAR δ activity. Several lines of evidence have supported this notion. Certain forms of prostaglandins (PGs) and other eicosanoids have been identified as PPAR ligands (105, 106). A product of COX-2 metabolism, cPGI, has been shown to bind to PPAR δ . This COX-2 derived ligand can rescue the infertility found in COX-2 null mice, demonstrating that PPAR δ is a downstream effector of COX-2 (107). It has been recently shown that PPAR δ knockout mice are insensitive to the anti-inflammatory effects of sulindac (69). Interestingly, a recent study has shown that homozygous deletion of the PPAR δ gene in a human colorectal cancer cell line exhibited no significant effect on their proliferation rate and, surprisingly, their responsiveness to NSAIDs *in vitro*. However, when xenoplanted in nude mice, the PPAR δ -null cells almost completely lost their tumorigenicity, while the wild-type cells formed full size tumors, confirming that PPAR δ clearly plays an important role in colorectal tumorigenesis (108). These studies have also raised important questions about the validity of *in vitro* studies of NSAID actions, especially the physiological relevance of NSAID-induced apoptosis *in vitro* and their chemopreventive efficacy *in vivo*. One should keep in mind that NSAID-mediated chemoprevention may require a chronic effect on the growth of tumor cells, rather than an acute induction of cell death. Thus, the role of NSAID-mediated chemoprevention is certainly more complex than previously believed, and PPAR δ may serve as a nodal point for multiple pathways affected by NSAIDs.

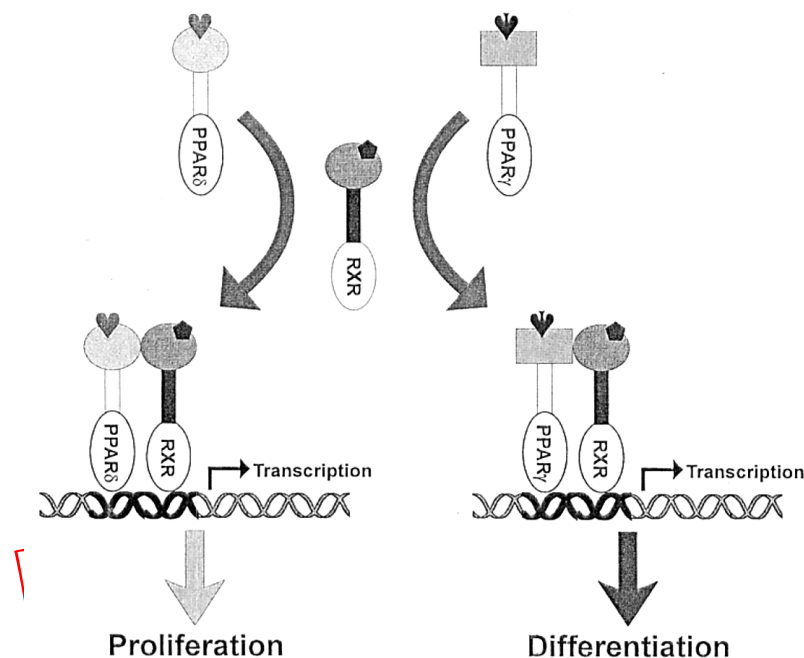


Fig. 4. Balanced regulation between PPAR δ and PPAR γ . Under normal conditions, PPAR δ and PPAR γ compete to form heterodimers with RXR. In cancer cells, this balance can be broken and results in increased activity of PPAR δ . For therapeutic and chemopreventive purposes, either PPAR δ antagonists or PPAR γ agonists can be used to inhibit proliferation of tumor cells.

3.5. PPAR δ vs PPAR γ in Tumorigenesis

Current studies have suggested that in human cancer (at least in colorectal cancer) PPAR δ may facilitate tumor growth and PPAR γ may inhibit tumor growth by promoting terminal differentiation of tumor cells. Because the RXRs are required to form functional heterodimers for both PPAR δ and PPAR γ , it is conceivable that there is normally a balanced regulation between PPAR δ and PPAR γ through their competition for RXRs (Fig. 4). In fact, it has been reported that PPARs and the thyroid hormone receptor can compete with each other for the availability of RXR (57, 109–112). As mentioned above, PPAR δ could promote cellular proliferation, whereas PPAR γ may inhibit cell growth and induce apoptosis. Therefore, colorectal tumorigenesis may be the result of a tipped balance towards PPAR δ ligand binding and activation. If this model proves to be correct, it would further aid efforts to address colorectal cancer chemoprevention, as one could utilize either PPAR δ antagonists or PPAR γ agonists (or both) as chemopreventive agents for colon cancer. Identification of the downstream effectors of these PPARs may even allow for more specific therapies directed toward the prevention and treatment of many different human malignancies.

4. FUNCTIONAL LINKS BETWEEN PPARs AND COX-2

4.1. Fatty Acids and Eicosanoids as Endogenous PPAR Ligands

PPAR ligands can be either endogenous or synthetic (63). Among the synthetic ligands, the fibrates are usually hypolipidemic drugs used to treat hyperlipidemia, most of which

preferentially activate PPAR α . The TZD class of compounds, on the other hand, is more selective for activating PPAR γ , with a high affinity (e.g., 40 nM for rosiglitazone, or several micromoles for troglitazone). Several NSAIDs, such as indomethacin and fenoprofen, have been shown to bind to PPARs with albeit lower affinity and specificity (84). Although a recent study has reported that the compound, L165041, may be a PPAR δ -specific ligand (113), synthetic agonists selectively activating PPAR δ are in general poorly characterized. Extensive molecular investigation of endogenous ligands has led to the identification of fatty acids and derivatives, most notably PGs, as potential natural ligands for PPARs (63,105,106,114–116). Fatty acids have been shown to bind to all three PPAR isotypes. However, among the three types of receptors, PPAR α is the best characterized one with the highest affinity for fatty acids. Particularly, PPAR α has a preference for binding of long-chain unsaturated fatty acids, such as the essential fatty acids linoleic, linolenic and AA, at concentrations that are in agreement with the physiological blood levels of these fatty acids. Similarly, polyunsaturated fatty acids (PUFAs), such as 18:2, 18:3, and 20:4 PUFAs, have been reported as relatively more efficient ligands for PPAR δ . Moreover, AA derivatives generated by the 5-lipoxygenase pathway, such as the inflammatory mediators leukotriene B₄ and 8(S)-hydroxy-eicosatetraenoic acid, have been identified as relatively high-affinity ligands for PPAR α . The most exciting findings in the search for endogenous PPAR ligands has been the identification of COX products, or prostaglandins, as relatively potent and selective agonists for PPARs. Among all the tested prostaglandins, 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂, a metabolite of the eicosanoid prostaglandin J₂, is the most potent natural ligand for PPAR γ so far (116), whereas another product of COX-2 metabolism, cPGI, has been recently shown to be a relatively PPAR δ -selective agonist (105,107).

4.2. Overlapping Phenotypes of COX-2 Knockouts and PPAR Knockouts

The identification and characterization of PPAR ligands have been largely based on *in vitro* transactivation assays. Carefully designed *in vivo* experimentations are warranted because most PPAR ligands are hydrophobic and/or exhibit transient stability in *in vitro* assays. An example of such studies is the gene disruption approach in animal models. For instance, earlier studies of COX-2 knockout animals demonstrated that, in addition to an altered inflammatory response, COX-2 mutants succumbed to severe renal abnormalities and multiple female reproductive failures (117–119). Careful examination of the female sterility has suggested that COX-2 may be important for the early stages of pregnancy, including ovulation, fertilization, implantation and decidualisation. However, it is likely that COX-2 mutants may also exhibit other subtle phenotypes because of the diverse biological functions of COX-2 products.

Recent genetic deletions of PPARs have revealed striking phenotypic similarities with COX-2 mutants. For instance, PPAR γ deficiency causes embryonic lethality, surprisingly owing to the defect in trophoblast terminal differentiation and placental vascularization (66–68). Tetraploid rescue experiments that bypass the placental defect indeed confirm that PPAR γ plays an important role in adipogenesis and glucose homeostasis. In PPAR δ knockout animals, the homozygous mutants were viable, but significantly smaller than their wild type littermates (69). Inflammation induced by O-tetradecanoylphorbol 13-acetate (TPA) in the skin was lower in wild type mice treated with the NSAID sulindac than in similarly treated PPAR δ null animals. Interestingly, breeding experiments of heterozygous progeny with mixed-genetic background yielded significantly fewer null

mice than expected, suggesting possible defects in embryo implantations. Consistent with this possibility, a relatively PPAR δ -specific ligand, prostacyclin (PGI₂) was capable of correcting the embryo implantation defect caused by COX-2 deficiency (107). Thus, these gene knockout experiments provide valuable information on potential functional links between PPARs and COX-2.

4.3. PPARs as Downstream Mediators of COX-2 Function

Collective data from biochemical and genetic studies have strongly suggested that PPARs may serve as one of the most important downstream mediators of COX-2 function, particularly in the context of tumorigenesis and chemoprevention. The identification of PPAR δ as a target for the APC tumor suppressor pathway and potentially NSAID-induced chemoprevention may provide a further mechanistic explanation of their actions in colon cancer. The striking chemopreventive effects of NSAIDs observed in FAP patients and the min mouse model were previously attributed to the inhibition of cyclooxygenases (e.g., COX-2). This inhibition would result in a decreased production of PGs and other eicosanoids, which in turn may decrease cell proliferation normally mediated by PPAR δ . This notion is supported by the fact that expression level of COX-2 and PPAR δ is elevated in some human colorectal tumors (104,120,121). More compelling evidence comes from animal studies in which inactivation of the COX-2 gene in the Min mice is associated with a marked decrease in intestinal polyps (122). However, it is noteworthy that the functional link between COX-2 and PPARs may not be linear, and each may carry out additional biological functions. It is conceivable that further double or triple gene disruption experiments among APC/PPARs/COX-2 may provide valuable evidence to firmly establish their connection in tumorigenesis and chemoprevention.

5. PPARs AS TARGETS OF CANCER CHEMOPREVENTION

5.1. Concept of Differentiation Therapy

The continued increase of cancer incidence and the failure of conventional chemotherapy to reduce mortality indicate that new approaches to cancer control are highly warranted. Given the genotypic and phenotypic heterogeneity of malignant lesions in individual patients, one must wonder just what specific molecular and cellular components, if any, can be targeted for the putative cure of cancer. The misperception of cancer as a disease of excessive cell proliferation has led to an overemphasis on the development of cytotoxic drugs that kill cancer cells. Unfortunately, these chemotherapy drugs are also highly toxic to a wide spectrum of normal cells. Thus, the development of effective treatment for advanced stage cancer remains one of the greatest challenges in modern medicine. Alternatively, we may need to focus more effort on the control of carcinogenesis by chemoprevention, an intervention approach to the arrest or reversal of tumorigenesis. The current paradigm of tumor development considers cancer to be a multiple-staged chronic disease. Chemoprevention may hold a great promise to curtailing cancer mortality by attacking tumor cells at earlier stages and/or preventing further development to next stage. Chemoprevention as a serious and practical approach to the control of cancer has been validated by recent clinical trials with COX-2-specific inhibitors, estrogen receptor antagonists, and retinoids (1).

Collective genetic and molecular biology data have demonstrated that cancer is a genetic disease that requires a series of mutations in genes controlling proliferation, dif-

ferentiation, and apoptosis (97). Many of the deregulated genes are transcriptional factors, whose target genes may play critical roles in tumorigenesis. Historically, cancer was considered as a dedifferentiation disease. From a chemopreventive perspective, the transcriptional factors that are involved in regulating differentiation may be of importance. Activation of these transcriptional factors would promote the differentiation of tumor cells to a histologically lower grade or to a terminally differentiated phenotype, ultimately leading to cell death. Thus, differentiation therapy may represent an alternative regimen to conventional treatment and/or an effective adjuvant therapy for cancer. As discussed below, several commonly used agents for other diseases are now being tested for their chemopreventive potentials.

5.2. Retinoids and Vitamin D as Chemopreventive Agents

Besides COX-2-specific inhibitors, retinoids represent another focus of chemoprevention research (123,124). Retinol (vitamin A) is abundantly presented in the diet as retinyl esters, mostly in animal products (e.g., liver, eggs, milk), or as a carotenoid precursor in green leafy vegetables. Retinoic acid is a natural product derived from retinol, the parent compound for all natural retinoids. Retinoids are essential for the maintenance of epithelial differentiation (124). Retinoids exert their action through binding to their nuclear receptors, the retinoic acid receptors (RARs) with high affinity for all-trans retinoic acids (ATRA) and the RXRs with high affinity for 9-*cis* retinoic acid, respectively. Recently, the retinoids selective for binding to RXRs have been named as “rexinoids.” Retinoic acids as chemotherapeutic agents have been demonstrated by their successful use as a differentiation therapy in acute promyelocytic leukemia (124,125). Almost all newly diagnosed APL cases can be induced into complete remission with ATRA. Randomized clinical trials have shown that the most significant effect of ATRA is an additive or synergistic activity with chemotherapy to reduce the recurrence rate of disease. Further, the relapse rate was significantly lower in cases with concurrent ATRA/chemotherapy than with ATRA therapy followed by chemotherapy. The combination of chemotherapy and RA treatment may be effective in up to 90% of PML-RAR α -caused APL diseases (126). ATRA-induced resistance in APL is highly reminiscent of refractory responses solicited by the antihormone therapy in breast and prostate cancers. In addition to APL, retinoids have been used extensively as chemopreventive agents for skin carcinogenesis (124).

From a chemopreventive point of view, RXRs may represent more important cellular targets because they are more promiscuous and form heterodimers with a broad range of nuclear receptors, including RARs, thyroid hormone receptors, vitamin D receptor (VDR), PPARs, and “orphan” receptors (e.g., LXR, FXR, and PXR). Thus, RXRs play an integrative role in regulating multiple cellular functions mediated by other nuclear receptors. RXRs have been shown to induce apoptosis and, more importantly, RXR-selective ligands (i.e., rexinoids) are less toxic than RAR agonists (124). The first rexinoid used was LGD 1069 (targetin) for prevention of mammary carcinogenesis in rats (127). Interestingly, combination treatment with retinoids and ER antagonists seem to yield a strong synergistic effect in inhibiting the progression of both early and late stages of neoplastic lesions in breast cancer models (127). These findings suggest that the combination of 9-*cis* RA and tamoxifen, either for chemoprevention or for adjuvant therapy, should be considered in clinical trials. As more RXR-specific ligands are being developed, other novel avenues are also being explored by targeting RXR downstream effector genes or by combining with interferon signaling (128,129).

Inspired by encouraging chemopreventive effects of retinoids and rexinoids, many researchers are also focusing on the potential use of vitamin D as a chemopreventive agent (130). The role of 1,25-dihydroxy vitamin D₃ as a regulator of cell growth and differentiation has been well established. However, vitamin D derivatives exhibit weaker differentiation activity and higher cytotoxicity than that of retinoids. Combination use of retinoids and vitamin D analogs seems to exhibit a synergistic inhibitory effect on tumor cell proliferation and angiogenic capability. Thus, vitamin D analogs alone or in combination with other agents are being tested as chemopreventive agents for APL and cutaneous tumors, as well as for prostate, breast, and colon cancer (131–134).

5.3. PPAR γ Agonists and PPAR δ Antagonists as Chemopreventive Agents

Collective data indicate that PPARs, PPAR γ , and PPAR δ in particular, may emerge as new targets for chemoprevention strategies (15, 16, 78, 79). As discussed earlier, although the PPAR γ ligand, troglitazone, was unexpectedly shown to enhance colorectal tumorigenesis in Min mice (80, 81), troglitazone suppressed the growth of human colon cancer cells and promoted expression of differentiation markers in vitro and in xenograft models (82). Numerous studies have demonstrated that PPAR γ is highly expressed in several forms of human tumors (85–96). PPAR γ activation by troglitazone in these cancer cells results in growth arrest, terminal differentiation, and/or apoptosis, suggesting that PPAR γ ligands may be used as therapeutic as well as chemopreventive agents. Further studies demonstrated that inhibition of tumor growth was more pronounced by a combination of troglitazone with retinoids (85). In a pilot study, treatment of liposarcoma patients with troglitazone resulted in antineoplastic prodifferentiation and inhibition of the disease progression (94). It has recently been reported that PPAR γ ligands dramatically inhibited angiogenesis (135), a process necessary for tumor growth and metastasis. Although there is no satisfactory explanation for PPAR γ action in nonadipocyte lineage, it is conceivable that the function of PPAR γ agonists in nonadipocytes, including a variety of human cancer cells, may be to turn on terminal differentiation programs in a nonspecific fashion.

Although the biological function of PPAR δ is least studied among the three PPAR isotypes, current biochemical and genetic data strongly suggest that PPAR δ may play an important role in colorectal tumorigenesis (15, 16, 78, 79). As previously discussed, both PPAR γ and PPAR δ may compete for the availability of RXRs for their activity. Any ligand-stimulated increase of PPAR γ activity would adversely affect PPAR δ 's ability to support cell proliferation, thus enhancing PPAR γ -induced apoptosis. Currently, lack of PPAR δ -specific ligands has significantly hampered the investigations of its biological functions. It is of critical importance to identify PPAR δ -specific agonists as well as antagonists. Given the ubiquitous expression of PPAR δ , it is conceivable that PPAR δ -specific antagonists may serve as more effective and less toxic chemopreventive agents for human cancers. Finally, identification of the important downstream target genes will certainly aid in fully understanding the biologic functions of PPAR δ , which will ultimately lead to the development of better and more efficacious chemopreventive agents.

6. CONCLUDING REMARKS AND PERSPECTIVES

Inhibition of COX-2 activity has been demonstrated as an effective chemopreventive measure for colon cancer (136–138). Collective genetic and molecular biology data have suggested that PPARs may function as major downstream mediators of COX-2 activity.

Currently, it is thought that activation of PPAR γ promotes differentiation and apoptosis, whereas activation of PPAR δ facilitates cell proliferation. Thus, the development of PPAR γ -specific agonists and/or PPAR δ -specific antagonists would provide a new avenue for chemoprevention and/or adjuvant cancer therapy. It should also be emphasized that chemopreventive measures are important for eliminating recurrence or metastasis after the removal of primary tumors.

As widely documented in antihormone therapy of breast and prostate cancers, resistance may also develop when PPAR γ agonists or PPAR δ antagonists are chronically used for therapy or chemoprevention. This seemingly unavoidable phenomenon may reflect the complexity of nuclear receptor regulation. The structures of agonists and antagonists show no clearly predictable patterns. Crystal structures of PPARs' ligand binding domains have suggested that there are ample flexibilities in the ligand-binding pockets (63,139,140). Thus, it is conceivable that very subtle conformational changes acquired through somatic mutations during tumor progression may convert an agonist into an antagonist, or vice versa. Combination use of different agents that target multiple cellular pathways may overcome this ligand-induced refractory effect and provide the most efficacious chemoprevention.

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