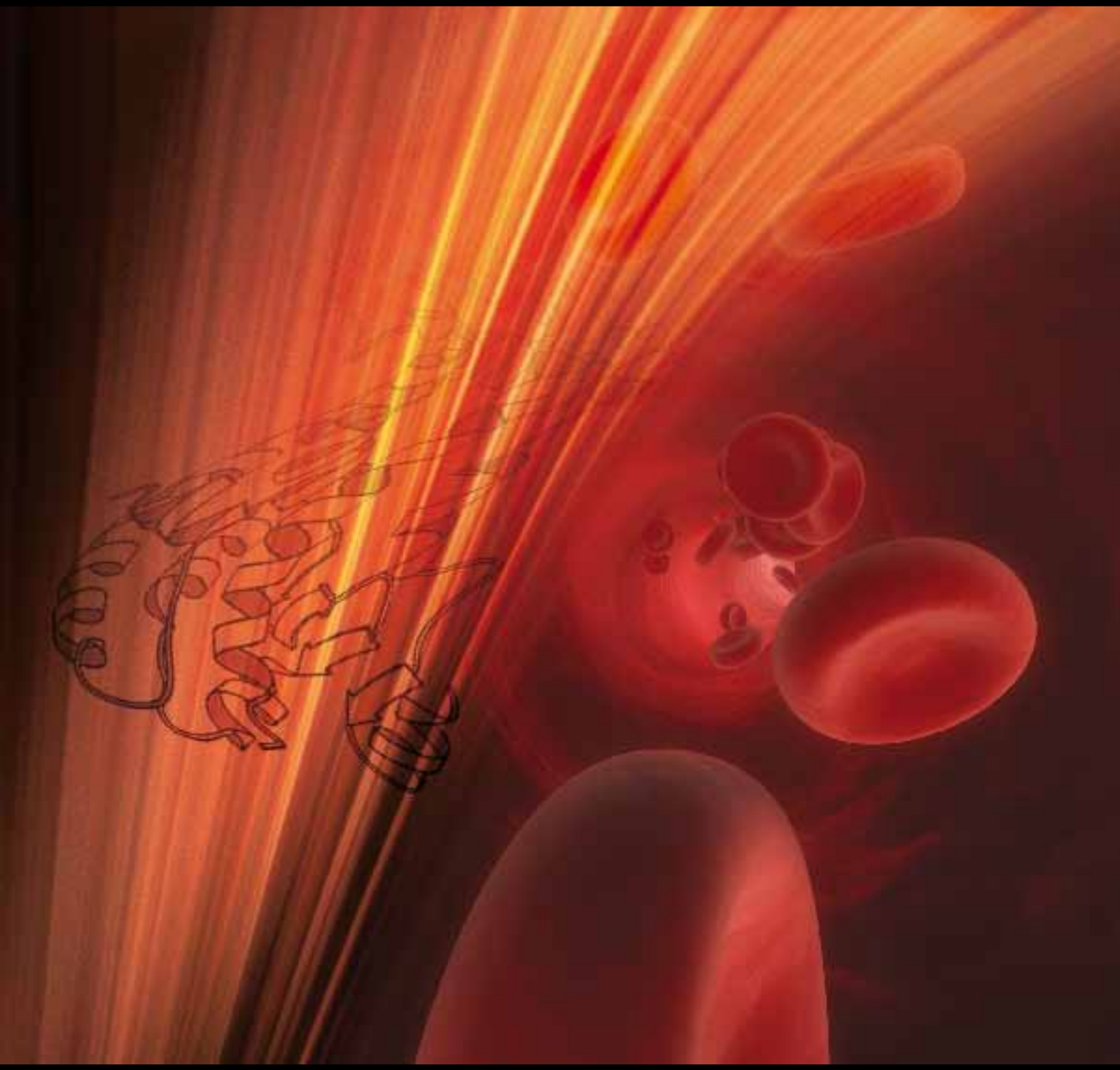
PPAR Research

PPARs and Gastrointestinal Cancer

Guest Editors: Valerio Pazienza, Manlio Vinciguerra,
and Gianluigi Mazzoccoli



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Editorial Board

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Editorial

PPARs and Gastrointestinal Cancer

Valerio Pazienza

Research Laboratory of Gastroenterology Unit, IRCCS "Casa Sollievo della Sofferenza" Hospital, 71013 San Giovanni Rotondo, Italy

Correspondence should be addressed to Valerio Pazienza, v.pazienza@operapadrepio.it

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Since the discovery of the peroxisome proliferator-activated receptors (PPARs) in *Xenopus* frogs as receptors that induce the proliferation of peroxisomes in cells, the study of these factors has received widespread interest. After the characterization of the different isoforms (PPARα, PPARβ/δ, and PPARγ) of these nuclear receptors in the 90s, numerous ligands have been described. Although the responsibility of PPARs in cancer development and progression is still controversial, many scientists consider PPARs as potential biomarkers/targets for cancer prevention and therapy.

Numerous mechanisms of PPARs action have been well reported; however the behaviour and function of PPARs differs within the context of different types of tissues and also depends on etiologic agents which could be one of the reasons to explain the conflicting literature.

The aim of this SI was to assemble different studies in order to better establish the functions of PPARs in the context of different types of gastrointestinal cancer (GI).

There are two research papers published by our group and nine reviews covering the main aspects of all types of GI cancer with the aim of elucidating the role of PPARs by confining their function to the different organs taken in consideration by the different authors.

In our research article entitled "*Time-qualified patterns of variation of PPARγ, DNMT1, and DNMT3B expression in pancreatic cancer cell lines,*" we assessed the time-related patterns of variation of PPARγ and DNMTs in pancreatic cancer (PC) cell lines after synchronization in order to understand the circadian behaviour of these factors. Our data demonstrated the temporal influence (over 24 h) on PPARγ expression in PC cells. The circadian fluctuation of PPARγ expression is an important finding that could help to understand the many disagreeing studies. A temporal variation of PPARγ mRNA expression over the day has to be taken into account when performing an "*in vitro*" or "*in vivo*" study.

In our study "*Correlations among PPARγ, DNMT1, and DNMT3B expression levels and pancreatic cancer*," we sought to investigate the relationship among PPARγ and the DNA-methyltransferases in PC patients and in "*in vitro*" models of PC cell lines to better understand the role of PPAR in epigenetic modification. We demonstrated that PPARγ expression is positively associated with DNMT1 but not with DNMT3B whose higher expression, however, was significantly associated to a lower mortality in a cohort of PC patients.

A. Stravodimou et al. described the regulation of these nuclear receptors by the ubiquitin-proteasome system in pancreatic cancer suggesting that inhibition of specific ubiquitination enzymes (instead of the proteasome) could also be a solution for a more specific pharmacologic interventions.

In the review article "*The role of peroxisome proliferator-activated receptors in the esophageal, gastric, and colorectal cancer*" by A. Fucci et al., the authors stressed out the promising translational outcome of the reported studies on nuclear receptors, raising the possibility of identifying PPAR alterations in premalignant lesions so that they can be used as prognostic biomarkers, whilst J.-I. Park and J.-Y. Kwak, in the review "*The role of peroxisome proliferator-activated receptors in colorectal cancer*," highlight the role of PPARs as target for cancer therapy. An interesting message appears in "PPARγ in inflammatory bowel disease" by V. Annese et al. After describing the potential of PPARγ in inflammatory-induced mechanisms, the authors reflected on the link between microbiota and PPARγ receptor, considering it worth further studies, since some commensal bacterial or natural ligands of foods may directly activate and increase the expression of PPARγ, thus determining a "biologic" anti-inflammatory action.

A paper by J.-M. Lee et al. about "*The role of PPARγ in Helicobacter pylori infection and gastric carcinogenesis*" reported that in gastric epithelial cells of *H. pylori*-infected

patients a strong nuclear staining was present as compared to *H. pylori*-negative subjects.

As for the liver, a reconciling model based on mitochondria-related features to resolve the conflicting results on PPARs-Liver-HCV is offered by F. Agriesti et al. in "*PPARs and HCV-related hepatocarcinoma: a mitochondrial point of view*."

Conversely the following two reviews indicate possible future research directions. M. Peyrou et al., in "*PPARs in liver diseases and cancer: epigenetic regulation by microRNAs*" dealt with the epigenetic regulation of PPARs expression and activity by miRNAs considering this new field of research to still be in its infancy and suggesting that alterations of the expression/activity of PPARs isoforms by distinct miRNAs could represent critical molecular mechanisms involved in the physiopathology of each organ undergoing a PPAR-dependent control; alternatively G. P. Ables, in "*Update on Pparγ and nonalcoholic fatty liver disease*," invites the investigators to elucidate the effect of specific conformational and structural differences between the nuclear receptor and its ligands concluding that the advent of the development of SPPARMS moves in the direction of specifically eliciting the desirable effects of PPARγ activation.

In conclusion, even though PPARs could have prognostic and/or therapeutic roles, there is an urgent need to shed light on the favorable potential or harmful risk of their modulator. Taking into account all the contributions made by the authors to this SI, our hope is that a step forward is made to resolve, at least partially, the conflicting data existing in the literature and to give interesting future research directions for a wide range of researchers.

Finally, I wish to thank the two guest coeditors, Dr. Manlio Vinciguerra and Dr. Gianluigi Mazzoccoli, for their work on this SI, and a warm thank you to the authors who have contributed to this special issue dedicating our effort to all those individuals who lead a brave daily battle against cancer.

Valerio Pazienza

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Review Article

Peroxisome Proliferator-Activated Receptor Gamma and Regulations by the Ubiquitin-Proteasome System in Pancreatic Cancer

Athina Stravodimou,[1] Gianluigi Mazzoccoli,[2] and Ioannis A. Voutsadakis[1]

[1] *Centre Pluridisciplinaire d'Oncologie, Centre Hospitalier Universitaire Vaudois, BH06, Bugnon 46, 1011 Lausanne, Switzerland*
[2] *Division of Internal Medicine and Chronobiology Unit, Department of Medical Sciences, IRCCS Scientific Institute and Regional General Hospital "Casa Sollievo della Sofferenza", San Giovanni Rotondo, Italy*

Correspondence should be addressed to Ioannis A. Voutsadakis, ivoutsadakis@yahoo.com

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Pancreatic cancer is one of the most lethal forms of human cancer. Although progress in oncology has improved outcomes in many forms of cancer, little progress has been made in pancreatic carcinoma and the prognosis of this malignancy remains grim. Several molecular abnormalities often present in pancreatic cancer have been defined and include mutations in K-ras, p53, p16, and DPC4 genes. Nuclear receptor Peroxisome Proliferator-Activated Receptor gamma (PPARγ) has a role in many carcinomas and has been found to be overexpressed in pancreatic cancer. It plays generally a tumor suppressor role antagonizing proteins promoting carcinogenesis such as NF-κB and TGFβ. Regulation of pathways involved in pancreatic carcinogenesis is effectuated by the Ubiquitin Proteasome System (UPS). This paper will examine PPARγ in pancreatic cancer, the regulation of this nuclear receptor by the UPS, and their relationship to other pathways important in pancreatic carcinogenesis.

1. Introduction

Pancreatic cancer is one of the most common and most deadly cancers with the incidence approaching mortality [1]. Reasons contributing to this lethality are the delayed diagnosis and the anatomic position and close relationships of the organ that precludes complete resection in many instances even in localized cases. Nevertheless, the majority of patients that have been completely rejected recur. This fact attests for the presence of occult micrometastases in early stages and an intrinsic aggressiveness of pancreatic cancer. Despite advancements in the molecular biology of pancreatic cancer and discovery of key molecular lesions playing a part in the pathogenesis such as K-ras, p53, p16, and DPC4 (Deleted in Pancreatic Cancer 4 or Smad4), this progress has not been translated in therapeutic results. In clinical practice, drugs used in pancreatic cancer such as gemcitabine, the basic backbone of therapy for many years [2] and the more recently introduced combination regimen of 5-FU, Folinic acid, Irinotecan, and Oxaliplatin [3] are given in a non-discriminatory way to all metastatic patients that can tolerate them. Currently there are no clinically applicable predictive markers of response despite a wealth of preclinical data that pinpoint to subsets of tumors which would potentially respond better than others [4]. Thus there is a need to further delineate clinically such subsets.

Peroxisome Proliferator-Activated Receptor gamma (PPARγ) is a nuclear receptor family transcription factor that is expressed in several types of cancers among which gastrointestinal and pancreatic cancers. It appears that the subset of pancreatic cancers with the higher expression of PPARγ constitutes a more aggressive group [5] and thus research on the regulation of this transcription factor in pancreatic cancer may present an opportunity for defining targets and eventually better treatments. The Ubiquitin Proteasome System (UPS) is a multi-protein molecular machinery that has a well-established role in most carcinogenesis processes and regulates PPARγ in multiple

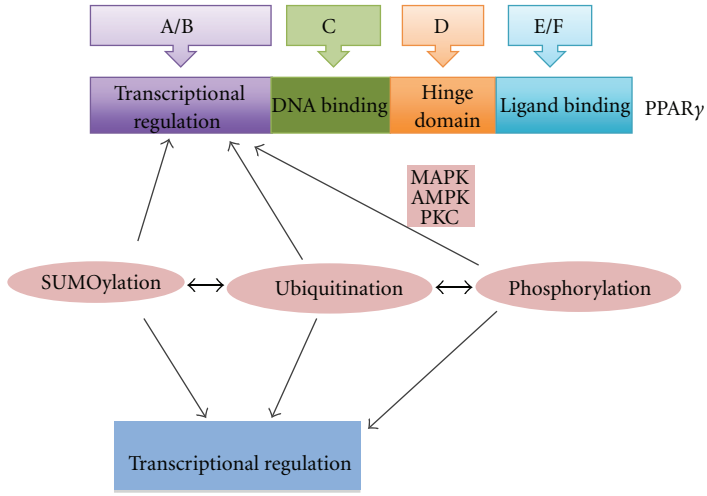

Figure 1: A schematic representation of the PPARγ molecule and its domains with their function. The molecule of PPARγ consists of an aminoterminal domain (also called A/B domain), which is responsible for ligand-independent transcriptional regulation. The following domain (also called domain C) contains two zinc finger-like and α-helical DNA-binding motifs typical of transcription factors. The C domain interacts with DNA through a PPRE (Peroxisome Proliferator Response Element) sequence. More carboxy terminal is the hinge domain (or D domain) which allows independent movement of the next and last domain of PPARγ molecule, domain E/F. This is the ligand-binding domain and potentiates the ability of PPARγ to dimerize with RXRα and recruit coactivators for transcription. Several post-translational modifications such as phosphorylation, ubiquitination, and SUMOylation modulate PPARγ activity.

ways. This regulation as it pertains to pancreatic cancer will be discussed in this paper.

2. PPARγ Structure and Function

PPARγ is transcribed from a gene in the short arm of human chromosome 3 (3p25) [6]. Alternative splicing of PPARγ gene results in two isoforms. PPARγ1 isoform has a wide tissue distribution and PPARγ2 has an expression restricted to adipose tissue [7]. PPARγ is already expressed in the mesodermal and endodermal layers of human embryos in the seventh week of gestation [8] and displays comparable to adult levels of expression in several organs during midgestation [9]. Pancreatic beta cells are among the tissues that physiologically express PPARγ.

The structure of PPARγ is similar to other nuclear receptor transcription factors (Figure 1). It includes an aminoterminal AF1 (Activation Function 1) domain that mediates recruitment of transcription cofactors, the DNA-binding domain (DBD) followed by a hinge region centrally, and the ligand-binding domain (LBD) together with a second AF2 domain in the carboxy-terminal part of the molecule [10]. Following ligand binding, PPARγ associates with another nuclear receptor, RXRα (Retinoid × Receptor α), and binds to specific DNA elements called PPREs (PPAR Response Elements), recruiting cofactors such as PGC-1 (PPARγ Coactivator-1) and the basal transcription machinery for transcription initiation. PPREs consist of a direct repeat sequence of six nucleotides divided by a single spacer nucleotide. The 5′-part of the repeat is bound by PPARγ and the 3′-part by RXRα. The two other members of the PPAR family, PPARα and PPARβ/δ, use similar DNA-binding sequences as expected by the high conservation of their DBD [11]. The specificity of the transcription program between the three PPAR nuclear receptors is provided by the cellular context, the chromatin landscape and ligands and cofactors availability [11]. In tissues where it has its highest expression, PPARγ physiologically contributes to the regulation of differentiation, metabolic control, and inflammation suppression [10]. These effects are mediated by transcription of targets genes such as lipid metabolism regulators (e.g., adipophilin and liver fatty acid binding protein) and differentiation-related genes (e.g., cytokeratins 18, 19 and 20 and members of the Carcinoembryonic Antigen family) as well as suppression of immune mediators (e.g., interferon γ and interleukin 2).

Both natural and synthetic ligands of PPARγ exist and may mediate PPARγ activation. Natural PPARγ ligands include prostaglandin D_2 (PGD_2) metabolite 15-deoxy-$\Delta^{12,14}$-PGJ_2 (15d-PGJ_2), linoleic acid derivative nitrolinoleic acid, other conjugated linoleic acid derivatives, eicosapentaenoic and arachidonic acids, 9-hydroxyoctadecadienoic acid (9-HODE), 13-HODE, 15-hydroxyeicosatetraenoic acid (15-HETE), and 13-oxooctadecadienoic acid. The anti-diabetic class of drugs thiazolidinediones such as pioglitazone, troglitazone, and rosiglitazone are PPARγ agonists. The realization that PPARγ is, at least in part, the mediator of their effect has contributed in bringing the receptor to the spotlight as a potential pharmacologic target in diseases beyond diabetes such as cancer [12].

Several transduction cascades can affect nuclear receptors function in parallel with their ligands and PPARγ is no exception. These cascades act through posttranslation modifications of the receptor [13]. Phosphorylation of PPARγ in both AF1 and AF2 domains is carried out by MAPK kinases downstream of growth factors, AMP-activated protein kinase and PKC (Protein Kinase C) and results in transcription

repression and in some cases subsequent ubiquitination and proteasome degradation [14, 15]. Regulation of PPARγ by ubiquitination will be discussed in the next section after a brief discussion of ubiquitination machinery.

3. Ubiquitination, the Ubiquitin Proteasome System and Regulation of PPARγ

Ubiquitination is a post-translational modification that consists of attachment of the 76 aminoacids protein ubiquitin to target proteins. This attachment is taking place through a cascade of enzymatic reactions mediated by three types of enzymes. The first step involves E1 (or ubiquitin-activating enzyme) which loads an ubiquitin molecule in an ATP-dependent manner onto a second type of enzyme, E2 (or ubiquitin conjugating enzyme). Ubiquitin is linked to E2 through a thioester bond and is subsequently transferred to a target protein by a third type of enzymes called ubiquitin ligases or E3 [16]. Human genome encodes for two E1 enzymes (UBA1 and UBA6), about 30 to 40 E2 enzymes and several hundred E3 ligases [17, 18].

E3 ligases belong to two families characterized by specific domains, RING (Really Interesting New Gene) and HECT (Homologous to Human Papillomavirus E6 Carboxy-terminal domain) family. Despite differing in their catalytic mode of action, both types of E3s execute ubiquitin ligation to the target protein [19]. There exists a third type of E3s, U-box ligases that can be considered either a separate family or a subfamily of RING E3 ligases due to the similarity of U-box domain to the RING domain. RING domains of E3 ligases constitute the interactive surface with the ubiquitin-conjugating enzyme E2 bound to ubiquitin. Some E3s are single polypeptides that possess both the RING E2-binding domain and the substrate-binding domain. Other E3s represent complexes of several distinct proteins. One of them is the RING domain E2-binding protein. Another protein of the complex binds the target (substrate) protein to be ubiquitinated while often a third peptide serves as a linker between them [19]. HECT ligases are constituted by various aminoterminal domains while their carboxy terminus is occupied by an HECT domain first identified and named after E3 ligase E6-AP (Human Papillomavirus E6-Associated Protein). HECT domain has two subdomains, one of which binds the E2 ubiquitin-conjugating enzyme and the other binds the substrate protein.

RING-type E3s are the most common ubiquitin ligases and represent about 95% of human E3s, while there are less than 30-HECT type E3s in human genome [20]. Like phosphorylation, ubiquitination is a reversible modification. De-ubiquitination is carried out by deubiquitinizing enzymes belonging to five families. The process preserves cellular ubiquitin stocks and amends inappropriate ubiquitination [21]. Deubiquitinases attack the isopeptide bond between the carboxy-terminal glycine of ubiquitin and the ε-amino-group of a lysine of another ubiquitin molecule or of a target protein.

Ubiquitin molecule has seven lysine residues at positions 6, 11, 27, 29, 33, 48, and 63. Attachment through each of these lysine residues as well as through the aminoterminal ubiquitin methionine residue has been confirmed to possess signaling potential [22, 23]. The number of ubiquitin molecules attached encodes also for different outcomes [24]. A target protein may become mono-ubiquitinated (a single ubiquitin molecule attached), multi-ubiquitinated (one ubiquitin molecule attached in several different lysine residues), or polyubiquitinated (a chain of ubiquitins attached in the same lysine residue). Lysine 48 ubiquitin chains of at least four molecules are the trigger for recognition of the target protein by the proteasome and subsequent degradation [24]. Occasionally, lysine 6, 11, and other lysines-mediated ubiquitin chains have been observed to signal for target protein proteasome degradation [25]. Lysine 63-mediated ubiquitin attachment leads less often to proteasome degradation but serves mostly as signal for lysosome-mediated proteolysis [26]. Moreover, it serves non-proteolytic functions including DNA repair and receptor kinases endocytosis [26, 27]. Other processes requiring ubiquitination include cell cycle progression, DNA transcription, and DNA damage tolerance [28, 29]. The general mode of regulation by ubiquitination is based on the recognition of an ubiquitin molecule or chain or more complex module on the decorated protein by another protein that bears an ubiquitin-recognizing domain in order for the two proteins to interact [30]. Recognition by a subunit of the proteasome is a specific scenario that leads to subsequent degradation.

The proteasome (also called 26S proteasome) is a cylindrical multiprotein structure made of two substructures, a core particle (CP or 20S proteasome) covered in one or both sides by a regulatory particle (RP or 19S proteasome) [31]. RP is built by a lid and a base subcomplex and its role includes the recognition of ubiquitinated proteins, unfolding them, deubiquitination which allows ubiquitin molecules to be recycled and delivery of the target proteins to the CP [16]. The different subunits of RP possess specific activities to accomplish these functions. Three subunits of the base subcomplex possess ubiquitin recognition domains that allow them to recognize polyubiquitin chains. Mammalian subunit S13 of the lid subcomplex is a de-ubiquitinase and recycles ubiquitin from proteins that had been recognized. The 19S base subcomplex includes six ATPases that belong to the AAA (ATPases associated with various cellular activities) family and are able to hydrolyze all four nucleotide triphosphates and to alter the conformation of substrate proteins, preventing their aggregation before they enter the CP to be degraded [16].

CP is made of four rings of seven member proteins each that are stacked one on the other. The two identical peripheral rings are named α rings (with subunits α1 to 7) and the two similarly identical central rings are called β rings (with subunits β1 to 7) [32]. The proteasome cleaves target proteins through three enzymatic activities, a trypsin-like (postbasic residues cleavage) activity, a chymotrypsin-like (posthydrophobic residues cleavage) activity and a post-glutamyl (caspase-like or postacidic residues cleavage) activity, that reside in subunits β2, β5, and β1, respectively [31]. With these activities the proteasome has the ability to cleave almost any peptide bond-producing fragments of 4 to 14 aminoacids in length [33].

A general role of UPS in transcription function of nuclear receptors has emerged [34] and has been discussed for Androgen Receptor [35]. Transcription activity of nuclear receptors and possibly of other transcription factors is coupled with their proteasome degradation. This degradation participates in the replacement of repression complexes by transcription activation complexes during transcription initiation [36]. Components of the UPS are recruited in transcribed gene promoters and eventually lead to degradation of the nuclear receptor shutting off transcription and favoring loading of new molecules onto the promoter only if the ligand signal persists. This permits the tight control of hormonal signalling. As mentioned, PPARγ is a proteasome degradation target and this degradation is coupled with activation consistent with the above model [37]. Other proteins of the PPARγ transcription machinery such as its partner RXRα [38] and coactivators PGC-1α [39], SRC-1 [40], and SRC-3 [41, 42] are also proteasome substrates.

SUMOylation is a post-translational modification similar to ubiquitination that refers to the attachment of protein SUMO (Small Ubiquitin-like Modifier) to target proteins using also a cascade of enzymes similar to ubiquitin. A major mode of action of SUMOylation involves modulation of ubiquitination, most often preventing it but occasionally facilitating subsequent ubiquitination of target proteins [43]. SUMOylation plays a role in PPARγ activity regulation. The nuclear receptor is a substrate for this modification which results in transcriptional repression of target genes [44]. The transcription coactivator C/EBPβ which is a positive regulator of expression of PPARγ is regulated by SUMOylation, in this instance leading to subsequent ubiquitination and proteasome degradation [45]. Another example of SUMO-modified PPARγ cooperating proteins is coactivator PGC-1α. SUMOylation on a specific lysine residue of PGC-1α represses transcriptional activity by facilitating the interaction with corepressors [46].

Ubiquitination and SUMOylation may simultaneously or consecutively affect the same proteins or different interacting proteins and constitute a post-translation modification code that integrates multiple input signals to produce a final PPARγ activity output [47]. In some instances, modifications involve the proteasome and lead to degradation while in others lead to nondegradative outcomes. It is also evident from the above discussion that the UPS may indirectly regulate PPARγ by affecting the transcription machinery that serves, besides itself, other transcription factors that interact with it. Other modifications such as phosphorylation and nitration are also participating in PPARγ regulation [48].

4. PPARγ in Pancreatic Cancer

PPARγ has been investigated in multiple preclinical studies in pancreatic cancer. PPARγ activation by troglitazone reduced the proliferation of pancreatic cancer cell lines in vitro and had an additive effect with 9-cis-retinoic acid, a ligand for RXRα [49]. Cyclin D1 mRNA and protein expression was decreased after troglitazone treatment. Another in vitro study of several pancreatic cell lines showed variable proliferation inhibition and cell cycle arrest in G1 phase after troglitazone treatment [50]. Despite PPARγ expression, some pancreatic cell lines were troglitazone resistant. CDK inhibitor p21 was upregulated possibly due to mRNA stabilization. Troglitazone treatment also promoted differentiation of pancreatic cancer cells with duct structure and tight junctions formation [50]. The natural PPARγ ligand 15d-PGJ_2-induced apoptosis in a pancreatic cancer cell line with concomitant activation of MAPKs JNK, p38, and ERK [51]. Apoptosis was dependent on MAPK p38, as the pharmacologic inhibition of this kinase before 15d-PGJ_2 treatment prevented apoptosis induction. In contrast pharmacologic inhibition of the ERK branch of MAPKs had apparently no role in PPARγ-induced apoptosis after 15d-PGJ_2 treatment in this cell line [51]. Troglitazone treatment of pancreatic cancer cell lines inhibited their invasiveness in vitro and induced a rounding of cells that was reversible upon removal of the drug from the culture [52]. In another study a different thiazolidinedione, ciglitazone, and 15d-PGJ_2 inhibited pancreatic cancer cell invasion [53]. This effect was PPARγ dependent as it was negated by a PPARγ antagonist or adenoviral transfection of cells with a dominant-negative PPARγ and appeared to be at least partially mediated by components of the uPA (urokinase-type Plasminogen Activator) system. Other investigators reported an increase of PAI-1 (Plasminogen Activator Inhibitor 1) and a decrease in cell invasion in pancreatic cancer cell lines treated with rosiglitazone or pioglitazone but these effects seemed to be independent of PPARγ activation because they were observed even in cell lines that did not express the nuclear receptor [54]. The same team of investigators showed that rosiglitazone- or pioglitazone-induced inhibition of anchorage-independent growth of pancreatic carcinoma cells was PPARγ dependent [55]. PPARγ ligands also induced a more differentiated morphology and differentiation markers Carbonic Anhydrase II (CA II) and cytokeratin 7, as well as CDK inhibitors p21 and p27 in these cells, but they had no apoptosis induction effect [55]. Expression of PPARγ in pancreatic cell lines needs to be accompanied by transcriptional functionality in order to be able to mediate inhibition effect; In a study of several cancer cell lines among which were pancreatic cell lines KMP-2 and BxPC3, only KMP-2 could be inhibited by various thiazolidinediones [56]. This cell line was expressing a functional PPARγ while in BxPC3 cells, PPARγ, although robust expression was not functional in a transactivation assay [56].

In an in vivo study in Syrian golden hamsters, pioglitazone feeding reduced the incidence of N-nitrosobis(2-oxopropyl)amine (BOP-)induced pancreatic cancer [57]. These hamsters, in contrast to other rodents, have low lipoprotein lipase (LPL) activity, develop hypertriglyceridemia, and hypercholesterolemia and are particularly sensitive to BOP carcinogenesis. Pioglitazone, in parallel with decrease of pancreatic cancer development in these animals, reduced the incidence of cholangiocarcinoma and induced LPL expression [57]. In another in vivo study, rosiglitazone treatment reduced human pancreatic xenograft tumor size in nude mice and decreased microvessel density evaluated by endothelial cell staining for collagen IV [58]. Pharmacologic inhibition of PPARγ by specific inhibitor

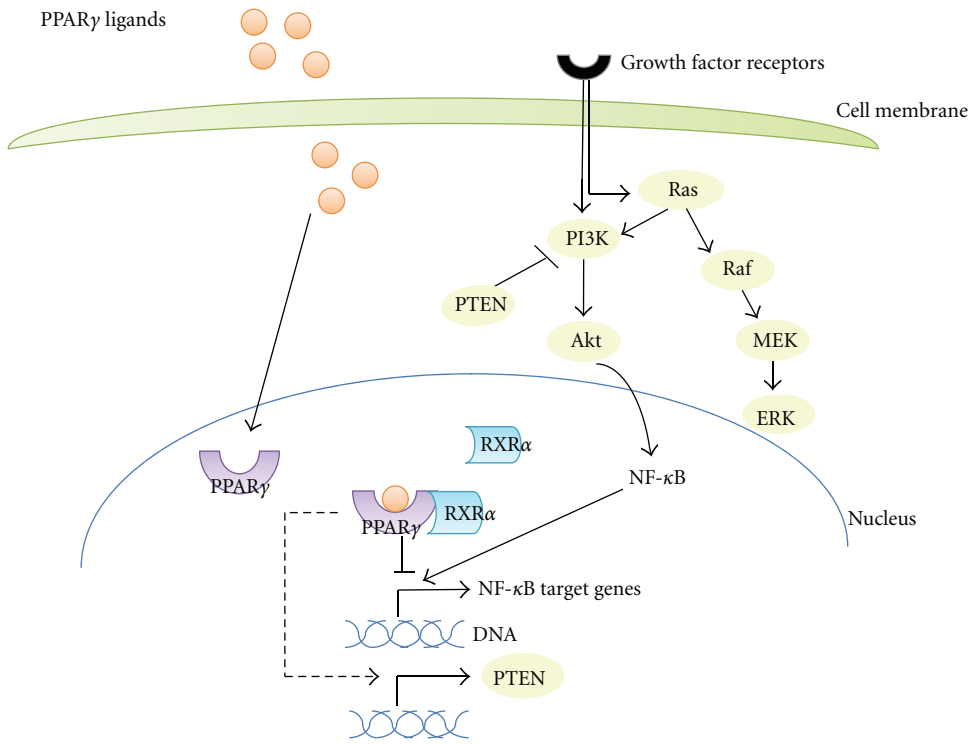

Figure 2: The role of ubiquitination and PPARγ in the PI3K-AKT and MAPKs signaling pathways. Proteins that are regulated by ubiquitination are depicted as yellow cycles. Examples of regulation of these pathways by PPARγ include transcriptional activation of phosphatase PTEN and antagonism of the transcriptional activity of NF-κB.

T0070907 unexpectedly also reduced pancreatic cancer cells migration in vitro and metastasis formation in an SCID mouse xenograft model in vivo [59]. T0070907 treatment induced membranous p120 catenin accumulation and GTPases Cdc42 and Rac-1 inhibition, events that would be expected to contribute to cell adhesion stabilization and motility reduction.

Overall, these data argue for a role of PPARγ in pancreatic cancer cell proliferation, differentiation and invasiveness. The picture painted from available experimental evidence speaks for a role of PPARγ activation in inducing cell cycle arrest and a more differentiated phenotype and in reducing cell invasiveness. Nevertheless, most data come from in vitro studies and have limitations. One of these limitations relates to the use of pharmacologic activators to infer effects of PPARγ activation on cellular properties. Thiazolidinediones for example, have effects that are PPARγ independent making the evaluation of PPARγ contribution particularly difficult. Use of pancreas targeted PPARγ knockout models in vivo or PPARγ RNA interference in vitro instead or in addition to pharmacologic activators would help resolving these problems. Other discrepancies may relate to technical issues, antibodies used, and cell lines identification. For example, a cell line used in one of the above discussed studies [54] and reported not to express PPARγ and thus contributing to the argument that effects seen were PPARγ independent was found to robustly express the nuclear receptor in another study [59]. In addition, other contradictory effects may stem from differences in the cellular environment that could alter the effects of PPARγ directly or indirectly, for example, through phosphorylation of the receptor or availability of cofactors.

5. Molecular Lesions in Pancreatic Cancer and Relationship with PPARγ and the UPS

Common molecular lesions in pancreatic cancer include K-ras-activating mutations and Cyclin Dependent Kinases (CDK) Inhibitor $p16^{INK4A}$ loss of function which are present in the great majority of cases, p53-inactivating mutations that are present in half to three-fourths of patients, and Smad4-(also called DPC4, Deleted in Pancreatic Cancer 4) inactivating mutations that are present in about half of pancreatic cancers [60]. Proteins and pathways involved in these lesions are regulated by the UPS and are interconnected with PPARγ.

K-ras-activating mutations are an early event in pancreatic cancer and result in the activation of several downstream pathways among which are the Raf-MAPKs and the PI3K-Akt both having important cancer-promoting properties mediated by activation of procarcinogenic effectors or inhibition of tumor suppressors [61]. Activation of PPARγ plays an antagonistic role towards K-ras-initiated cascades. PPARγ induces phosphatase PTEN which is an inhibitor of PI3K pathway [62, 63]. NF-κB is an example of proteins activated by Akt kinase (Figure 2). The NF-κB family of transcription factors is comprised of five proteins that form homo- or heterodimers in order to perform

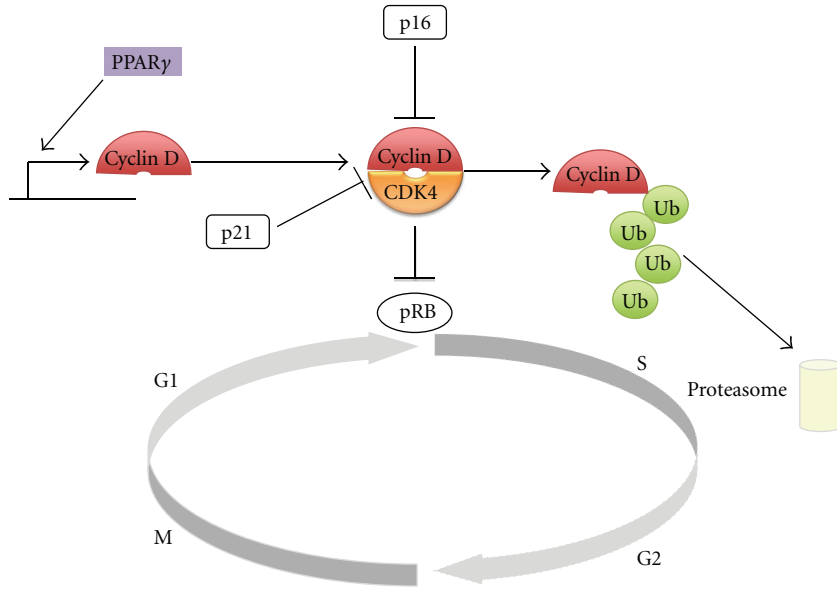

Figure 3: The role of PPARγ and the UPS in the regulation of p16/CDK4/Cyclin D/Rb axis. Dysfunction of this axis promotes cell proliferation and carcinogenesis. Cyclin D is a transcriptional repression target of PPARγ and a substrate for ubiquitination leading to degradation by the proteasome.

their transcription function resulting in inhibition of apoptosis and modulation of the inflammatory response. PPARγ antagonizes the activity of NF-κB and reciprocally NF-κB inhibits PPARγ transcription activity. Both PTEN and NF-κB cascade are regulated by the UPS. PTEN is a direct target of ubiquitination for proteasomal degradation [64]. The NF-κB cascade is regulated in multiple levels by ubiquitination that leads to proteolytic or non-proteolytic outcomes [65]. Other components of signalling downstream of activated K-ras such as kinases Raf [66], ERK1 and 2 [67], and ERK3 [68], the regulatory subunit p85 of PI3K [69], and kinase Akt [70] are subjects of regulation by ubiquitination.

CDK Inhibitor $p16^{INK4A}$ is a regulator of cell cycle and functions by inhibiting the CDK4/Cyclin D complex leading to the release of Rb from the negative regulation by the complex and cell cycle arrest at the G1/S transition [71]. Its inactivation in the great majority of pancreatic cancers promotes cell proliferation and synergizes with K-ras mutations to promote pancreatic carcinogenesis [72]. Dysfunctioning $p16^{INK4A}$/CDK4/Cyclin D/Rb axis may still be regulated by PPARγ which is a transcriptional repressor of Cyclin D. Furthermore, this Cyclin is regulated by the UPS by being a target protein for ubiquitination and degradation [73] (Figure 3). In addition, PPARγ interacts with Rb protein and the PPARγ/Rb complex recruits histone deacetylase 3 (HDAC3) and causes cell cycle arrest at the G1 phase of the cell cycle in mouse embryo fibroblasts [74].

Tumor suppressor p53 mediates PPARγ induction of apoptosis in various cell types and as a result its inactivation in pancreatic cancer may interfere with the ability of PPARγ to induce apoptosis [75, 76]. However, in other cell types PPARγ-induced apoptosis may be p53 independent [77]. The effect of p53 inactivation on the ability of PPARγ to mediate apoptosis in pancreatic cancer has not been specifically studied. Nevertheless, the fact that the nuclear receptor retains the ability to promote apoptosis in pancreatic cells, which are often p53 mutant, argues for at least a partially p53-independent ability of PPARγ to induce apoptosis. p53 is a short-lived protein and its stability is normally regulated by proteasome degradation after ubiquitination. Mutant p53 is not recognized by the ubiquitination machinery and, as a result, is stabilized and can act as a dominant negative regulator of the wild type protein [78].

Smad4 mutations are common in pancreatic cancer and are associated with poor prognosis compared with patients that harbor a wild-type Smad4 in their tumors [79]. Smad4 is part of the TGFβ signal transduction cascade. Ligation of TGFβ to its cell surface receptors TβRI and TβRII activates proteins Smad2 and Smad3 which form dimers with Smad4 and act as transcription factors [80]. PPARγ is a transcription suppression target of the TGFβ signaling pathway in diverse tissues [81, 82] and deregulation of this pathway as a result of Smad4 mutations may lead to PPARγ upregulation in pancreatic carcinomas (Figure 4). This reverse association may also explain the poor prognosis associated not only with Smad4 mutations [79] but also with PPARγ upregulation [5]. A reciprocal regulation whence PPARγ agonists inhibit TGFβ signaling is evident in some experimental systems but probably represents a PPARγ-independent effect of these ligands [83, 84]. The UPS controls TGFβ signaling by degradation of most of its protein components. HECT E3 ligases of the Nedd4 (Neural precursor cells Expressed Developmentally Downregulated 4) family including Nedd4-2, Smurf1 and 2, WWP1, and Itch/AIP4 participate in TGFβ signalling regulation [85, 86]. In addition, receptor endocytosis after TGFβ ligation, which leads to either degradation

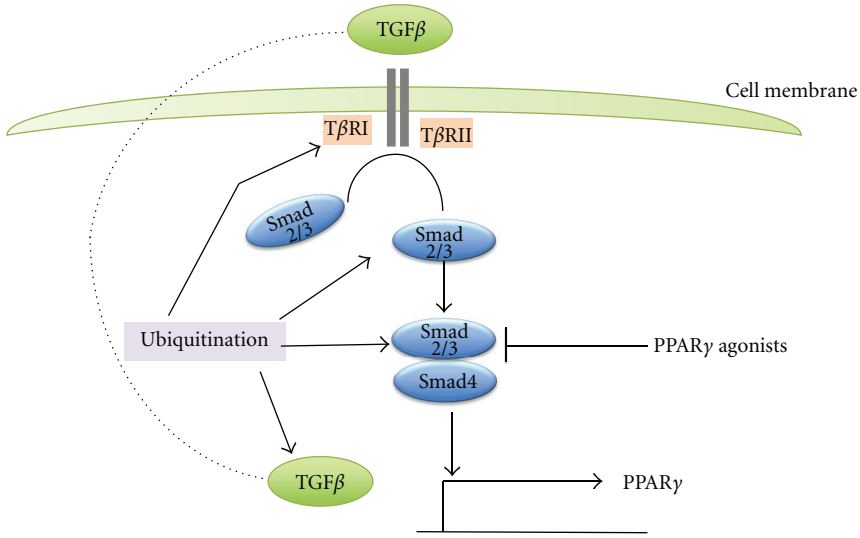

Figure 4: The TGFβ pathway and regulations by ubiquitination and PPARγ. Its deregulation by Smad4 mutations may lead to PPARγ upregulation in pancreatic carcinomas. Several components of TGFβ pathway are targets of ubiquitination with degradative and nondegradative outcomes.

in the lysosome or recycling to the cell surface, is UPS regulated [87]. Other ubiquitination modifications of TGFβ cascade proteins with a nondegradational outcome have been identified [88]. Pancreatic cancer-associated Smad4 mutant proteins are more prone to ubiquitination and subsequent proteasome degradation than the wild-type Smad4 [89].

It is concluded from the above discussion that all major pathways affected in pancreatic cancer are interconnected with PPARγ and are regulated in multiple nodes by the UPS.

6. Inflammation and Fibrosis in Pancreatic Cancer: Role of PPARγ and the UPS

There exists a relationship between chronic pancreatitis and pancreatic cancer [1]. Obesity, a condition of low-grade inflammation is also associated with pancreatic cancer [90]. Chronic inflammation leads to fibrosis (also referred to as desmoplasia or desmoplastic reaction) and to a change in the cellular microenvironment that promotes carcinogenesis. Transcription factor NF-κB is a major regulator of inflammation and is regulated by the UPS in multiple levels [65]. A major regulating point in the NF-κB pathway involves phosphorylation of inhibitor of NF-κB, IκB which is then ubiquitinated and degraded in the proteasome. In addition, NF-κB lies downstream of activated K-ras and as a result, it may be activated secondary to diverse signals in pancreatic cancer. These signals not only favor carcinogenesis but also perpetuate the inflammatory environment [72, 91]. NF-κB signaling results in phosphorylation of histone H3 in the promoter of Notch target gene and transcriptional repressor Hes (Hairy and Enhancer of Split) and through this modification cooperates with Notch in upregulation of Hes [92]. Hes suppresses, among other genes, transcription of PPARγ, neutralizing an anti-inflammatory signal in pancreatic cancer and thus promoting an inflammatory microenvironment. There exists a reciprocal antagonism of PPARγ towards NF-κB that may be relevant in pancreatic carcinoma cases with increased PPARγ expression [93] (Figure 5). Several mechanisms are proposed to contribute in PPARγ antagonism to NF-κB. First, PPARγ, as already mentioned in the previous section, induces PTEN in pancreatic cancer cells which dephosphorylates and inhibits kinase PI3K blocking the signal from activated K-ras to NF-κB [62]. This may be an important mechanism with therapeutic implications because, in addition to K-ras mutations, PTEN downregulation is frequent in pancreatic cell lines and tumor specimens [94, 95]. A second mechanism relates to a direct ligand-dependent transrepression of NF-κB target genes by PPARγ through recruitment of co-repressors [96]. A third mechanism involves the downregulation by PPARγ of cytokines and STAT transcription factors that are NF-κB activators or effectors [97].

Fibrosis is a frequent feature of pancreatic cancer and has been proposed to be a cause of drug resistance creating a protective barrier for the neoplastic cells that chemotherapeutics cannot penetrate at least at concentrations to be effective [98]. TGFβ signaling is a central player in fibrosis and in carcinogenesis. In pancreatic cancer, there is an imbalance between the canonical Smad transduction which is debilitated due to Smad4 mutations and the noncanonical MAPK pathway which, in addition to the nonaffected transduction from TGFβ receptors, receives the input from the activated K-ras [99]. This imbalance promotes TGFβ-associated fibrosis and carcinogenesis. PTEN induction by PPARγ appears to be important for an antagonistic effect of the nuclear receptor towards TGFβ signaling, similar to its role in PPARγ antagonism towards NF-κB (Figure 6). In this instance kinase p70 Ribosomal S6 Kinase-1 is inhibited downstream of PTEN and results in inhibition of transcription factor Zf9, a TGFβ1 gene inducer [100].

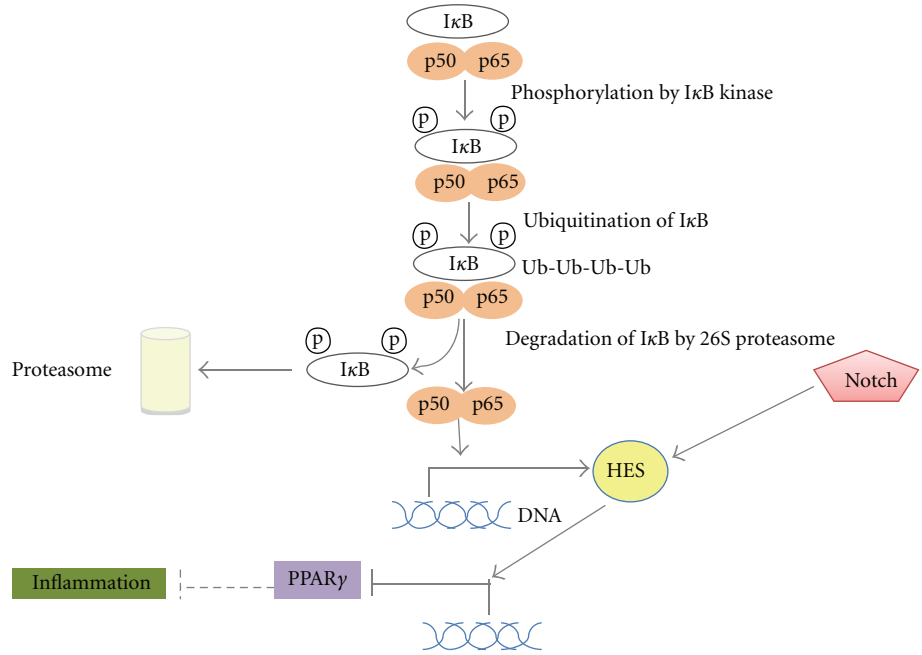

Figure 5: A schematic representation of the role of PPARγ and UPS in inflammation. UPS regulates NF-κB (here subunits p65 and p50 are depicted) in multiple points, one of which is degradation of inhibitor IκB. Activated NF-κB cooperates with Notch in the induction of Hes. Hes is a transcriptional repressor of PPARγ and thus inflammation is promoted.

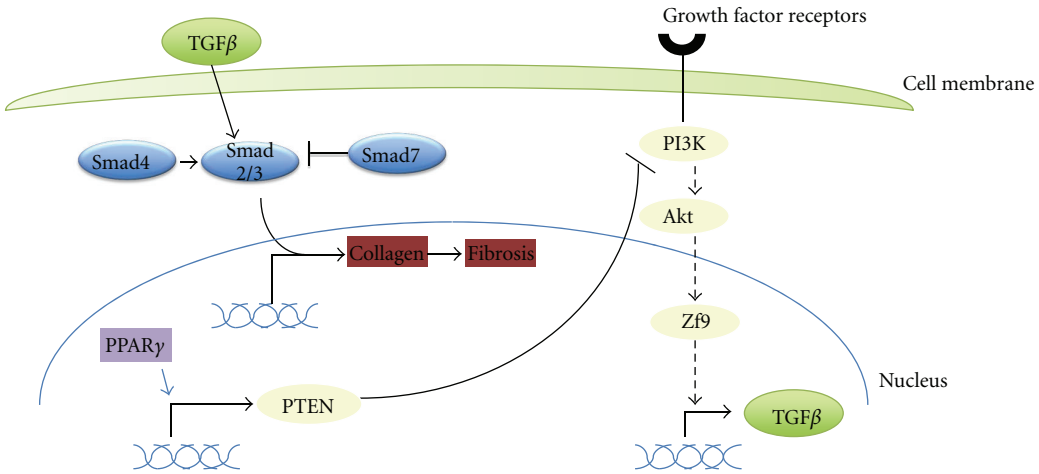

Figure 6: The role of PPARγ in the pathogenesis of fibrosis. PTEN induction by PPARγ inhibits the PI3K/Akt pathway downregulating transcription factor Zf9, an inducer of TGFβ. Resulting decrease of TGFβ signaling leads to a decrease of collagen production. UPS regulates all these pathways (not shown).

Reciprocally MAPK cascade activation antagonizes PPARγ by promoting its nuclear exclusion [101].

Pancreatic stellate cells, cells morphologically and biochemically similar to hepatic stellate (Ito) cells [102], are principal effectors in inflammation-associated pancreatic fibrosis. Physiologically, these cells are quiescent but after activation, for example, in pancreatitis, they produce increased collagen and other matrix proteins leading to fibrosis [103]. Studies in animal models have shown that stellate cells promote tumor formation when coadministered with pancreatic cancer cells [104] suggesting an experimental explanation for the link between inflammation, fibrosis, and cancer. PPARγ activation decreased collagen synthesis of pancreatic stellate cells in vitro and enhanced their differentiation to adipocytes with production of lipid metabolism-related proteins [105]. A decrease in their proliferation was also observed.

Fibrosis may also be a result of EMT (Epithelial to Mesenchymal Transition), a program of cancer cells that allows the acquisition of fibroblast-like morphology and properties by epithelial cells and promotes detachment from epithelial membranes, motility, and metastasis [106].

It is conceivable that cells having undergone EMT and acquired fibroblast properties contribute to the production of fibrotic matrix and promote drug resistance [107]. In addition, this resistance is an innate property of EMT of epithelial cells and relates to common pathways mediating EMT and the acquisition of a stem cell phenotype that accompanies it [108]. Moreover activated pancreatic stellate cells promote the stem cell phenotype of pancreatic cancer cells, expression of resistance proteins such as ABCG2, EMT in vitro, and tumorigenicity in vivo [109, 110]. The UPS is an important modulator of EMT by regulating both signal transduction pathways and transcription factors mediating it [111, 112].

PPARγ as an antagonist of TGFβ signaling, a promoter of EMT, is expected to inhibit this process. Indeed, this has been confirmed in a study of lung cancer cells [113]. Nevertheless, another study using mouse and rat intestinal epithelial cells concluded that PPARγ activation promotes EMT [114]. This effect was dependent on activation of kinases ERK1 and ERK2 of the MAPK cascade. ERK activation was a result of Rho GTPase activity in this study, a molecular event that was also observed in a study of PPARγ inhibitor T0070907 discussed in a previous section which, in contrast, has found migration inhibition by inhibiting PPARγ [59]. Discrepant effects of PPARγ on EMT replicate discrepancies that have been seen with different mouse models of colorectal carcinogenesis with some models showing cancer protection by PPARγ activation while others displaying cancer-promoting effects [115] and may be explained by differences in cellular context, expressed by quantitative and qualitative differences in activity status of other parallel pathways such as the TGFβ, the MEK/ERK, and the PI3K/Akt pathways.

Despite these issues, the bulk of the data supporting a role of PPARγ in suppression of inflammation and fibrosis also suggests a beneficial role of the nuclear receptor in carcinogenesis suppression.

7. Therapeutic Perspectives

Given the above discussed antagonism of PPARγ activation against several carcinogenesis promoting pathways but also its antagonism to inflammation and fibrosis predisposing to cancer, PPARγ is a rational pharmacologic target in pancreatic cancer. Such a target has the additional advantage that there already exist drugs in clinical use, the thiazolidinediones, with known safety profile [12]. Although safety concerns related to severe hepatotoxicity have led to the withdrawal of troglitazone from the market, this toxicity is not a class effect [116]. More recently, an increased risk of bladder cancer has been noticed in diabetic patients taking pioglitazone but not those treated with rosiglitazone [117] again arguing against a class effect but adding to the safety concerns with thiazolidinediones.

There are ample preclinical data supporting the effectiveness of thiazolidinediones in pancreatic cancer, as discussed in a previous section. In addition, combination of thiazolidinediones with commonly used chemotherapy drugs such as gemcitabine and platinum resulted in synergistic antineoplastic effects [118, 119] encouraging moving forward to clinical trials. Nevertheless, initial clinical trials of thiazolidinediones in various malignancies as monotherapy have not produced significant benefit [120].

The role of UPS in most carcinogenesis-related processes and the clinical success of its inhibition by the boronic acid derivative bortezomib in multiple myeloma have confirmed UPS as a valid anti-neoplastic target [121]. Despite this success of bortezomib in myeloma and subtypes of Non-Hodgkin lymphoma, results in solid tumors were generally disappointing. In pancreatic cancer, despite encouraging preclinical data [122], no benefit was observed in a phase I study investigating the combination of bortezomib with gemcitabine [123].

How can one reconcile these disappointing clinical results with drugs modulating apparently valid targets that have been extensively investigated preclinically? Both PPARγ and the proteasome, despite representing single targets, are involved in multiple cellular processes: the proteasome by degrading hundreds of cellular proteins and PPARγ by transcribing dozens of target genes, suppressing others and interacting with several parallel signals. Thus, the final output of both PPARγ activation and proteasome inhibition in a given neoplastic cell is highly context-dependent. As a result, there is a need for predictive markers to help delineate a priori patients that have the greatest probability of response. This quest of predictive markers is indeed a cornerstone of modern oncology and a prerequisite for the development of targeted treatments. Concerning proteasome inhibition, such markers were not necessary in myeloma possibly because myeloma cells have functions such as antibody production after recombination that makes them sensitive to this inhibition in the majority of cases. More specific pharmacologic interventions could also be a solution that could be attained by inhibition of specific ubiquitination enzymes instead of the proteasome. Such inhibitors are already in development [124, 125]. Regarding PPARγ, current activators, as mentioned, have safety concerns. In addition they have off-target effects that have been a hurdle for the preclinical study of PPARγ activation but may also be at least partially responsible for the encountered adverse events. Thus, development of more specific activators is highly desirable. Given the importance of both PPARγ and the UPS in regulating pancreatic cancer cells and their interrelation as outlined in this paper, it is worth investigating the existence of possible subsets of pancreatic cancers that would be sensitive to the combination of specific PPARγ activators with UPS inhibitors.

References

[1] H. Manuel, "Pancreatic cancer," *The New England Journal of Medicine*, vol. 362, no. 17, pp. 1605–1617, 2010.

[2] H. A. Burris III, M. J. Moore, J. Andersen et al., "Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial," *Journal of Clinical Oncology*, vol. 15, no. 6, pp. 2403–2413, 1997.

[3] T. Conroy, F. Desseigne, M. Ychou et al., "FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer,"

The New England Journal of Medicine, vol. 364, no. 19, pp. 1817–1825, 2011.

[4] I. A. Voutsadakis, "Molecular predictors of gemcitabine response in pancreatic cancer," *World Journal of Gastrointestinal Oncology*, vol. 3, no. 11, pp. 153–164, 2011.

[5] G. Kristiansen, J. Jacob, A. C. Buckendahl et al., "Peroxisome proliferator-activated receptor γ is highly expressed in pancreatic cancer and is associated with shorter overall survival times," *Clinical Cancer Research*, vol. 12, no. 21, pp. 6444–6451, 2006.

[6] C. Vigouroux, L. Fajas, E. Khallouf et al., "Human peroxisome proliferator-activated receptor-γ2: genetic mapping, identification of a variant in the coding sequence, and exclusion as the gene responsible for lipoatrophic diabetes," *Diabetes*, vol. 47, no. 3, pp. 490–492, 1998.

[7] L. Fajas, D. Auboeuf, E. Raspé et al., "The organization, promoter analysis, and expression of the human PPARγ gene," *Journal of Biological Chemistry*, vol. 272, no. 30, pp. 18779–18789, 1997.

[8] C. Huin, L. Corriveau, A. Bianchi et al., "Differential expression of peroxisome proliferator-activated receptors (PPARs) in the developing human fetal digestive tract," *Journal of Histochemistry and Cytochemistry*, vol. 48, no. 5, pp. 603–611, 2000.

[9] B. D. Abbott, C. R. Wood, A. M. Watkins, K. P. Das, and C. S. Lau, "Peroxisome proliferator-activated receptors alpha, beta, and gamma mRNA and protein expression in human fetal tissues," *PPAR Research*, vol. 2010, Article ID 690907, 19 pages, 2010.

[10] I. A. Voutsadakis, "Peroxisome proliferator-activated receptor γ (PPARγ) and colorectal carcinogenesis," *Journal of Cancer Research and Clinical Oncology*, vol. 133, no. 12, pp. 917–928, 2007.

[11] S. Mandrup and A. Bugge, "Molecular mechanisms and genome-wide aspects of PPAR subtype specific transactivation," *PPAR Research*, vol. 2010, Article ID 169506, 12 pages, 2010.

[12] H. Yki-Järvinen, "Thiazolidinediones," *The New England Journal of Medicine*, vol. 351, no. 11, pp. 1106–1118, 2004.

[13] C. Diradourian, J. Girard, and J. P. Pégorier, "Phosphorylation of PPARs: from molecular characterization to physiological relevance," *Biochimie*, vol. 87, no. 1, pp. 33–38, 2005.

[14] H. S. Camp and S. R. Tafuri, "Regulation of peroxisome proliferator-activated receptor γ activity by mitogen-activated protein kinase," *Journal of Biological Chemistry*, vol. 272, no. 16, pp. 10811–10816, 1997.

[15] D. Shao, S. M. Rangwala, S. T. Bailey, S. L. Krakow, M. J. Reginato, and M. A. Lazar, "Interdomain communication regulating ligand binding by PPAR-γ," *Nature*, vol. 396, no. 6709, pp. 377–380, 1998.

[16] I. A. Voutsadakis, "Ubiquitin, ubiquitination and the ubiquitin-proteasome system in cancer," *Atlas of Genetics and Cytogenetics in Oncology and Haematology*, vol. 14, no. 11, pp. 1088–1099, 2010.

[17] B. A. Schulman and J. W. Harper, "Ubiquitin-like protein activation by E1 enzymes: the apex for downstream signalling pathways," *Nature Reviews Molecular Cell Biology*, vol. 10, no. 5, pp. 319–331, 2009.

[18] S. J. L. van Wijk and H. T. M. Timmers, "The family of ubiquitin-conjugating enzymes (E2s): deciding between life and death of proteins," *The FASEB Journal*, vol. 24, no. 4, pp. 981–993, 2010.

[19] M. B. Metzger, V. A. Hristova, and A. M. Weissman, "HECT and RING finger families of E3 ubiquitin ligases at a glance," *Journal of Cell Science*, vol. 125, pp. 531–537, 2012.

[20] W. Li and Y. Ye, "Polyubiquitin chains: functions, structures, and mechanisms," *Cellular and Molecular Life Sciences*, vol. 65, no. 15, pp. 2397–2406, 2008.

[21] M. J. Clague, J. M. Coulson, and S. Urbé, "Cellular functions of the DUBs," *Journal of Cell Science*, vol. 125, pp. 277–286, 2012.

[22] H. Walczak, K. Iwai, and I. Dikic, "Generation and physiological roles of linear ubiquitin chains," *BMC Biology*, vol. 10, article 23, 6 pages, 2012.

[23] C. Behrends and J. W. Harper, "Constructing and decoding unconventional ubiquitin chains," *Nature Structural and Molecular Biology*, vol. 18, no. 5, pp. 520–528, 2011.

[24] I. A. Voutsadakis, "Pathogenesis of colorectal carcinoma and therapeutic implications: the roles of the ubiquitin-proteasome system and Cox-2: cancer," *Journal of Cellular and Molecular Medicine*, vol. 11, no. 2, pp. 252–285, 2007.

[25] Y. Kravtsova-Ivantsiv and A. Ciechanover, "Non-canonical ubiquitin-based signals for proteasomal degradation," *Journal of Cell Science*, vol. 125, pp. 539–548, 2012.

[26] K. Haglund and I. Dikic, "The role of ubiquitylation in receptor endocytosis and endosomal sorting," *Journal of Cell Science*, vol. 125, pp. 265–275, 2012.

[27] S. Bergink and S. Jentsch, "Principles of ubiquitin and SUMO modifications in DNA repair," *Nature*, vol. 458, no. 7237, pp. 461–467, 2009.

[28] A. Mocciaro and M. Rape, "Emerging regulatory mechanisms in ubiquitin-dependent cell cycle control," *Journal of Cell Science*, vol. 125, pp. 255–263, 2012.

[29] H. D. Ulrich, "Ubiquitin and SUMO in DNA repair at a glance," *Journal of Cell Science*, vol. 125, pp. 249–254, 2012.

[30] J. M. Winget and T. Mayor, "The diversity of ubiquitin recognition: hot spots and varied specificity," *Molecular Cell*, vol. 38, no. 5, pp. 627–635, 2010.

[31] M. Rechsteiner, "The 26S proteasome," in *Protein Degradation*, R. J. Mayer, A. Ciechanover, and M. Rechsteiner, Eds., vol. 1, pp. 220–247, Wiley-VCH, New York, NY, USA, 2005.

[32] D. H. Wolf and W. Hilt, "The proteasome: a proteolytic nanomachine of cell regulation and waste disposal," *Biochimica et Biophysica Acta*, vol. 1695, no. 1–3, pp. 19–31, 2004.

[33] J. L. Harris, P. B. Alper, J. Li, M. Rechsteiner, and B. J. Backes, "Substrate specificity of the human proteasome," *Chemistry and Biology*, vol. 8, no. 12, pp. 1131–1141, 2001.

[34] Z. Nawaz and B. W. O'Malley, "Urban renewal in the nucleus: is protein turnover by proteasomes absolutely required for nuclear receptor-regulated transcription?" *Molecular Endocrinology*, vol. 18, no. 3, pp. 493–499, 2004.

[35] I. A. Voutsadakis and C. N. Papandreou, "The ubiquitin-proteasome system in prostate cancer and its transition to castration resistance ," *Urologic Oncology*. In press.

[36] V. Perissi, A. Aggarwal, C. K. Glass, D. W. Rose, and M. G. Rosenfeld, "A corepressor/coactivator exchange complex required for transcriptional activation by nuclear receptors and other regulated transcription factors," *Cell*, vol. 116, no. 4, pp. 511–526, 2004.

[37] S. Hauser, G. Adelmant, P. Sarraf, H. M. Wright, E. Mueller, and B. M. Spiegelman, "Degradation of the peroxisome proliferator-activated receptor γ is linked to ligand-dependent activation," *Journal of Biological Chemistry*, vol. 275, no. 24, pp. 18527–18533, 2000.

[38] B. Lefebvre, Y. Benomar, A. Guédin et al., "Proteasomal degradation of retinoid X receptor α reprograms transcriptional activity of PPARγ in obese mice and humans," *Journal of Clinical Investigation*, vol. 120, no. 5, pp. 1454–1468, 2010.

[39] P. Wei, D. Pan, C. Mao, and Y.-X. Wang, "RNF34 is a cold-regulated E3 ubiquitin ligase for PGC-1α and Modulates brown fat cell metabolism," *Molecular and Cellular Biology*, vol. 32, no. 2, pp. 266–275, 2012.

[40] L. Amazit, A. Roseau, J. A. Khan et al., "Ligand-dependent degradation of SRC-1 is pivotal for progesterone receptor transcriptional activity," *Molecular Endocrinology*, vol. 25, no. 3, pp. 394–408, 2011.

[41] C. Ferry, S. Gaouar, B. Fischer et al., "Cullin 3 mediates SRC-3 ubiquitination and degradation to control the retinoic acid response," *Journal of Biological Chemistry*, vol. 108, no. 51, pp. 20603–20608, 2011.

[42] R. C. Wu, Q. Feng, D. M. Lonard, and B. W. O'Malley, "SRC-3 coactivator functional lifetime is regulated by a phosphor-dependent ubiquitin time clock," *Cell*, vol. 129, no. 6, pp. 1125–1140, 2007.

[43] G. J. K. Praefcke, K. Hofmann, and R. J. Dohmen, "SUMO playing tag with ubiquitin," *Trends in Biochemical Sciences*, vol. 37, no. 1, pp. 23–31, 2012.

[44] G. Pascual, A. L. Fong, S. Ogawa et al., "A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-γ," *Nature*, vol. 437, no. 7059, pp. 759–763, 2005.

[45] S. S. Chung, B. Y. Ahn, M. Kim et al., "Control of adipogenesis by the SUMO-specific protease SENP2," *Molecular and Cellular Biology*, vol. 30, no. 9, pp. 2135–2146, 2010.

[46] M. M. Rytinki and J. J. Palvimo, "SUMOylation attenuates the function of PGC-1α," *Journal of Biological Chemistry*, vol. 284, no. 38, pp. 26184–26193, 2009.

[47] M. Anbalagan, B. Huderson, L. Murphy, and B. G. Rowan, "Post-translational modifications of nuclear receptors and human disease," *Nuclear Receptor Signaling*, vol. 10, pp. 1–13, 2012.

[48] M. Luconi, G. Cantini, and M. Serio, "Peroxisome proliferator-activated receptor gamma (PPARγ): is the genomic activity the only answer?" *Steroids*, vol. 75, no. 8-9, pp. 585–594, 2010.

[49] M. Toyota, Y. Miyazaki, S. Kitamura et al., "Peroxisome proliferator-activated receptor γ reduces the growth rate of pancreatic cancer cells through the reduction of cyclin D1," *Life Sciences*, vol. 70, no. 13, pp. 1565–1575, 2002.

[50] S. Kawa, T. Nikaido, H. Unno, N. Usuda, K. Nakayama, and K. Kiyosawa, "Growth inhibition and differentiation of pancreatic cancer cell lines by PPARγ ligand troglitazone," *Pancreas*, vol. 24, no. 1, pp. 1–7, 2002.

[51] K. Hashimoto, B. J. Farrow, and B. M. Evers, "Activation and role of MAP Kinases in 15d-PGJ2-induced apoptosis in the human pancreatic cancer cell line MIA PaCa-2," *Pancreas*, vol. 28, no. 2, pp. 153–159, 2004.

[52] W. Motomura, M. Nagamine, S. Tanno et al., "Inhibition of cell invasion and morphological change by troglitazone in human pancreatic cancer cells," *Journal of Gastroenterology*, vol. 39, no. 5, pp. 461–468, 2004.

[53] H. Sawai, J. Liu, H. A. Reber, O. J. Hines, and G. Eibl, "Activation of peroxisome proliferator-activated receptor-γ decreases pancreatic cancer cell invasion through modulation of the plasminogen activator system," *Molecular Cancer Research*, vol. 4, no. 3, pp. 159–167, 2006.

[54] A. Galli, E. Ceni, D. W. Crabb et al., "Antidiabetic thiazolidinediones inhibit invasiveness of pancreatic cancer cells via PPARγ independent mechanisms," *Gut*, vol. 53, no. 11, pp. 1688–1697, 2004.

[55] E. Ceni, T. Mello, M. Tarocchi et al., "Antidiabetic thiazolidinediones induce ductal differentiation but not apoptosis in pancreatic cancer cells," *World Journal of Gastroenterology*, vol. 11, no. 8, pp. 1122–1130, 2005.

[56] M. Tsujie, S. Nakamori, J. Okami et al., "Thiazolidinediones inhibit growth of gastrointestinal, biliary, and pancreatic adenocarcinoma cells through activation of the peroxisome proliferator-activated receptor γ/retinoid X receptor α pathway," *Experimental Cell Research*, vol. 289, no. 1, pp. 143–151, 2003.

[57] Y. Takeuchi, M. Takahashi, K. Sakano et al., "Suppression of N-nitrosobis(2-oxopropyl)amine-induced pancreatic carcinogenesis in hamsters by pioglitazone, a ligand of peroxisome proliferator-activated receptor γ," *Carcinogenesis*, vol. 28, no. 8, pp. 1692–1696, 2007.

[58] Y. W. Dong, X. P. Wang, and K. Wu, "Suppression of pancreatic carcinoma growth by activating peroxisome proliferator-activated receptor γ involves angiogenesis inhibition," *World Journal of Gastroenterology*, vol. 15, no. 4, pp. 441–448, 2009.

[59] A. Nakajima, A. Tomimoto, K. Fujita et al., "Inhibition of peroxisome proliferator-activated receptor γ activity suppresses pancreatic cancer cell motility," *Cancer Science*, vol. 99, no. 10, pp. 1892–1900, 2008.

[60] M. A. Abramson, A. Jazag, J. A. van der Zee, and E. E. Whang, "The molecular biology of pancreatic cancer," *Gastrointestinal Cancer Research*, vol. 1, supplement 2, pp. S7–S12, 2007.

[61] A. D. Cox and C. J. Der, "Ras history: the saga continues," *Small GTPases*, vol. 1, no. 1, pp. 2–27, 2010.

[62] B. Farrow and B. M. Evers, "Activation of PPARγ increases PTEN expression in pancreatic cancer cells," *Biochemical and Biophysical Research Communications*, vol. 301, no. 1, pp. 50–53, 2003.

[63] L. Patel, I. Pass, P. Coxon, C. P. Downes, S. A. Smith, and C. H. Macphee, "Tumor suppressor and anti-inflammatory actions of PPARγ agonists are mediated via upregulation of PTEN," *Current Biology*, vol. 11, no. 10, pp. 764–768, 2001.

[64] N. R. Leslie and M. Foti, "Non-genomic loss of PTEN function in cancer: not in my genes," *Trends in Pharmacological Sciences*, vol. 32, no. 3, pp. 131–140, 2011.

[65] A. C. Schmukle and H. Walczak, "No one can whistle a symphony alone- how different ubiquitin linkages cooperate to orchestrate NF-κB activity," *Journal of Cell Science*, vol. 125, pp. 549–559, 2012.

[66] S. Manenti, C. Delmas, and J. M. Darbon, "Cell adhesion protects c-Raf-1 against ubiquitin-dependent degradation by the proteasome," *Biochemical and Biophysical Research Communications*, vol. 294, no. 5, pp. 976–980, 2002.

[67] Z. Lu, S. Xu, C. Joazeiro, M. H. Cobb, and T. Hunter, "The PHD domain of MEKK1 acts as an E3 ubiquitin ligase and mediates ubiquitination and degradation of ERK1/2," *Molecular Cell*, vol. 9, no. 5, pp. 945–956, 2002.

[68] P. Coulombe, G. Rodier, S. Pelletier, J. Pellerin, and S. Meloche, "Rapid turnover of extracellular signal-regulated kinase 3 by the ubiquitin-proteasome pathway defines a novel paradigm of mitogen-activated protein kinase regulation during cellular differentiation," *Molecular and Cellular Biology*, vol. 23, no. 13, pp. 4542–4558, 2003.

[69] H. Guenou, K. Kaabeche, C. Dufour, H. Miraoui, and P. J. Marie, "Down-regulation of ubiquitin ligase Cbl induced by twist haploinsufficiency in Saethre-Chotzen syndrome results in increased PI3K/Akt signaling and osteoblast proliferation," *American Journal of Pathology*, vol. 169, no. 4, pp. 1303–1311, 2006.

[70] M. Adachi, K. R. Katsumura, K. Fujii, S. Kobayashi, H. Aoki, and M. Matsuzaki, "Proteasome-dependent decrease in Akt by growth factors in vascular smooth muscle cells," *FEBS Letters*, vol. 554, no. 1-2, pp. 77–80, 2003.

[71] J. Li, M. J. Poi, and M. D. Tsai, "Regulatory mechanisms of tumor suppressor $P16^{INK4A}$ and their relevance to cancer," *Biochemistry*, vol. 50, no. 25, pp. 5566–5582, 2011.

[72] Z. Wang, S. Banerjee, A. Ahmad et al., "Activated K-ras and INK4a/Arf deficiency cooperate during the development of pancreatic cancer by activation of notch and NF-κB signaling pathways," *PLoS ONE*, vol. 6, no. 6, Article ID e20537, 2011.

[73] O. Barbash, E. Egan, L. L. Pontano, J. Kosak, and J. A. Diehl, "Lysine 269 is essential for cyclin D1 ubiquitylation by the SCF Fbx4/αB-crystallin ligase and subsequent proteasome-dependent degradation," *Oncogene*, vol. 28, no. 49, pp. 4317–4325, 2009.

[74] L. Fajas, V. Egler, R. Reiter, S. Miard, A. M. Lefebvre, and J. Auwerx, "PPARγ controls cell proliferation and apoptosis in an RB-dependent manner," *Oncogene*, vol. 22, no. 27, pp. 4186–4193, 2003.

[75] H. Zand, A. Rhimipour, M. Bakhshayesh, M. Shafiee, I. Nour Mohammadi, and S. Salimi, "Involvement of PPAR-γ and p53 in DHA-induced apoptosis in Reh cells," *Molecular and Cellular Biochemistry*, vol. 304, no. 1-2, pp. 71–77, 2007.

[76] C. Han, A. J. Demetris, G. K. Michalopoulos, Q. Zhan, J. H. Shelhamer, and T. Wu, "PPARγ ligands inhibit cholangiocarcinoma cell growth through p53-dependent GADD45 and p21WAF1/Cip1 pathway," *Hepatology*, vol. 38, no. 1, pp. 167–177, 2003.

[77] D. Bonofiglio, E. Cione, D. Vizza et al., "Bid as a potential target of apoptotic effects exerted by low doses of PPARγ and RXR ligands in breast cancer cells," *Cell Cycle*, vol. 10, no. 14, pp. 2344–2354, 2011.

[78] H. Solomon, S. Madar, and V. Rotter, "Mutant p53 gain of function is interwoven into the hallmarks of cancer," *Journal of Pathology*, vol. 225, no. 4, pp. 475–478, 2011.

[79] A. Blackford, O. K. Serrano, C. L. Wolfgang et al., "SMAD4 gene mutations are associated with poor prognosis in pancreatic cancer," *Clinical Cancer Research*, vol. 15, no. 14, pp. 4674–4679, 2009.

[80] J. Massagué, "TGFβ in cancer," *Cell*, vol. 134, no. 2, pp. 215–230, 2008.

[81] K. Gong, Y.-F. Chen, P. Li et al., "Transforming growth factor-β inhibits myocardial PPARβ expression in pressure overload-induced cardiac fibrosis and remodeling in mice," *Journal of Hypertension*, vol. 29, no. 9, pp. 1810–1819, 2011.

[82] V. Subramanian, J. Golledge, E. B. Heywood, D. Bruemmer, and A. Daugherty, "Regulation of peroxisome proliferator-activated receptor-γ by angiotensin II via transforming growth factor-β1-activated p38 mitogen-activated protein kinase in aortic smooth muscle cells," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 32, no. 2, pp. 397–405, 2012.

[83] A. A. Kulkarni, T. H. Thatcher, K. C. Olsen, S. B. Maggirwar, R. P. Phipps, and P. J. Sime, "PPAR-γ ligands repress TGFβ-induced myofibroblast differentiation by targeting the PI3K/Akt pathway: implications for therapy of fibrosis," *PLoS ONE*, vol. 6, no. 1, Article ID e15909, 2011.

[84] J. H. Yu, K. H. Kim, and H. Kim, "SOCS3 and PPAR-γ ligands inhibit the expression of IL-6 and TGF-β1 by regulating JAK2/STAT3 signaling in pancreas," *International Journal of Biochemistry and Cell Biology*, vol. 40, no. 4, pp. 677–688, 2008.

[85] Y. Inoue and T. Imamura, "Regulation of TGF-β family signaling by E3 ubiquitin ligases," *Cancer Science*, vol. 99, no. 11, pp. 2107–2112, 2008.

[86] F. Huang and Y.-G. Chen, "Regulation of TGF-β receptor activity," *Cell and Bioscience*, vol. 2, no. 9, pp. 1–10, 2012.

[87] S. Polo, "Signaling-mediated control of ubiquitin ligases in endocytosis," *BMC Biology*, vol. 10, article 25, 9 pages, 2012.

[88] L.-Y. Tang and Y. E. Zhang, "Non-degradative ubiquitination in Smad-dependent TGF-β signaling," *Cell and Bioscience*, vol. 1, no. 43, pp. 1–5, 2011.

[89] A. Morén, U. Hellman, Y. Inada, T. Imamura, C. H. Heldin, and A. Moustakas, "Differential ubiquitination defines the functional status of the tumor suppressor Smad4," *Journal of Biological Chemistry*, vol. 278, no. 35, pp. 33571–33582, 2003.

[90] B. Bao, Z. Wang, Y. Li et al., "The complexities of obesity and diabetes with the development and progression of pancreatic cancer," *Biochimica et Biophysica Acta*, vol. 1815, no. 2, pp. 135–146, 2011.

[91] Y. Ben-Neriah and M. Karin, "Inflammation meets cancer, with NF-κB as the matchmaker," *Nature Immunology*, vol. 12, no. 8, pp. 715–723, 2011.

[92] E. Maniati, M. Bossard, N. Cook et al., "Crosstalk between the canonical NF-κB and Notch signaling pathways inhibits Pparγ expression and promotes pancreatic cancer progression in mice," *Journal of Clinical Investigation*, vol. 121, no. 12, pp. 4685–4699, 2011.

[93] I. A. Voutsadakis, "Peroxisome Proliferator Activated Receptor-γ (PPARγ): roles in chronic inflammation and intestinal oncogenic transformation," in *From Inflammation to Cancer. Advances in Diagnosis and Therapy for Gastrointestinal and Hepatological Diseases*, C. H. Cho and J. Yu, Eds., World Scientific, River Edge, NJ, USA, 2012.

[94] T. Asano, Y. Yao, J. Zhu, D. Li, J. L. Abbruzzese, and S. A. G. Reddy, "The PI 3-kinase/Akt signaling pathway is activated due to aberrant Pten expression and targets transcription factors NF-κB and c-Myc in pancreatic cancer cells," *Oncogene*, vol. 23, no. 53, pp. 8571–8580, 2004.

[95] H. Ying, K. G. Elpek, A. Vinjamoori et al., "PTEN is a major tumor suppressor in pancreatic ductal adenocarcinoma and regulates an NF-κB-cytokine network," *Cancer Discovery*, vol. 1, no. 2, pp. 158–169, 2011.

[96] M. Ricote, A. C. Li, T. M. Willson, C. J. Kelly, and C. K. Glass, "The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation," *Nature*, vol. 391, no. 6662, pp. 79–82, 1998.

[97] C. Jiang, A. T. Ting, and B. Seed, "PPAR-γ agonists inhibit production of monocyte inflammatory cytokines," *Nature*, vol. 391, no. 6662, pp. 82–86, 1998.

[98] M. A. Shields, S. Dangi-Garimella, A. J. Redig, and H. G. Munshi, "Biochemical role of the collagen-rich tumour microenvironment in pancreatic cancer progression," *Biochemical Journal*, vol. 441, no. 2, pp. 541–552, 2012.

[99] D. A. Chapnick, L. Warner, J. Bernet, T. Rao, and X. Liu, "Partners in crime: the TGFβ and MAPK pathways in cancer progression," *Cell and Bioscience*, vol. 1, no. 42, pp. 1–8, 2011.

[100] S. J. Lee, E. K. Yang, and S. G. Kim, "Peroxisome proliferator-activated receptor-γ and retinoic acid X receptor α represses

the TGFβ1 gene via PTEN-mediated p70 ribosomal S6 kinase-1 inhibition: role for Zf9 dephosphorylation," *Molecular Pharmacology*, vol. 70, no. 1, pp. 415–425, 2006.

[101] E. Burgermeister, D. Chuderland, T. Hanoch, M. Meyer, M. Liscovitch, and R. Seger, "Interaction with MEK causes nuclear export and downregulation of peroxisome proliferator-activated receptor γ," *Molecular and Cellular Biology*, vol. 27, no. 3, pp. 803–817, 2007.

[102] R. Jaster, "Molecular regulation of pancreatic stellate cell function," *Molecular Cancer*, vol. 3, article 26, 8 pages, 2004.

[103] M. V. Apte and J. S. Wilson, "Dangerous liaisons: pancreatic stellate cells and pancreatic cancer cells," *Journal of Gastroenterology and Hepatology*, vol. 27, supplement 2, pp. 69–74, 2012.

[104] M. G. Bachem, M. Schünemann, M. Ramadani et al., "Pancreatic carcinoma cells induce fibrosis by stimulating proliferation and matrix synthesis of stellate cells," *Gastroenterology*, vol. 128, no. 4, pp. 907–921, 2005.

[105] M. L. Kruse, S. Hopf-Jensen, C. Timke et al., "Differentiation potential of pancreatic fibroblastoid cells/stellate cells: effects of peroxisome proliferator-activated receptor gamma ligands," *International Journal of Cell Biology*, vol. 2011, Article ID 816791, 11 pages, 2011.

[106] R. Kalluri and R. A. Weinberg, "The basics of epithelial-mesenchymal transition," *Journal of Clinical Investigation*, vol. 119, no. 6, pp. 1420–1428, 2009.

[107] J. M. López-Nouoa and M. A. Nieto, "Inflammation and EMT: an alliance towards organ fibrosis and cancer progression," *EMBO Molecular Medicine*, vol. 1, no. 6-7, pp. 303–314, 2009.

[108] S. A. Mani, W. Guo, M. J. Liao et al., "The epithelial-mesenchymal transition generates cells with properties of stem cells," *Cell*, vol. 133, no. 4, pp. 704–715, 2008.

[109] S. Hamada, A. Masamune, T. Takikawa et al., "Pancreatic stellate cells enhance stem cell-like phenotypes in pancreatic cancer cells," *Biochemical and Biophysical Research Communications*, vol. 421, no. 2, pp. 349–354, 2012.

[110] K. Kikuta, A. Masamune, T. Watanabe et al., "Pancreatic stellate cells promote epithelial-mesenchymal transition in pancreatic cancer cells," *Biochemical and Biophysical Research Communications*, vol. 403, no. 3-4, pp. 380–384, 2010.

[111] I. A. Voutsadakis, "Ubiquitination and the ubiquitin-proteasome system as regulators of transcription and transcription factors in epithelial mesenchymal transition of cancer," *Tumour Biology*, vol. 33, no. 4, pp. 897–910, 2012.

[112] I. A. Voutsadakis, "The ubiquitin-proteasome system and signal transduction pathways regulating Epithelial Mesenchymal transition of cancer," *Journal of Biomedical Science*, vol. 19, no. 67, pp. 1–13, 2012.

[113] A. K. Reka, H. Kurapati, V. R. Narala et al., "Peroxisome proliferator-activated receptor-γ activation inhibits tumor metastasis by antagonizing smad3-mediated epithelial-mesenchymal transition," *Molecular Cancer Therapeutics*, vol. 9, no. 12, pp. 3221–3232, 2010.

[114] L. Chen, B. M. Necela, W. Su et al., "Peroxisome proliferator-activated receptor γ promotes epithelial to mesenchymal transformation by Rho GTPase-dependent activation of ERK1/2," *Journal of Biological Chemistry*, vol. 281, no. 34, pp. 24575–24587, 2006.

[115] R. A. Gupta and R. N. DuBois, "Controversy: PPARγ a target for treatment of colorectal cancer," *American Journal of Physiology*, vol. 283, no. 2, pp. G266–G269, 2002.

[116] A. J. Scheen, "Hepatotoxicity with thiazolidinediones: is it a class effect?" *Drug Safety*, vol. 24, no. 12, pp. 873–888, 2001.

[117] L. Azoulay, H. Yin, K. B. Filion et al., "The use of pioglitazone and the risk of bladder cancer in people with type 2 diabetes: nested case-control study," *British Medical Journal*, vol. 344, Article ID e3645, 2012.

[118] H. Koga, K. Selvendiran, R. Sivakumar et al., "PPARγ potentiates anticancer effects of gemcitabine on human pancreatic cancer cells," *International Journal of Oncology*, vol. 40, no. 3, pp. 679–685, 2012.

[119] G. D. Girnun, E. Naseri, S. B. Vafai et al., "Synergy between PPARγ ligands and platinum-based drugs in cancer," *Cancer Cell*, vol. 11, no. 5, pp. 395–406, 2007.

[120] A. Galli, T. Mello, E. Ceni, E. Surrenti, and C. Surrenti, "The potential of antidiabetic thiazolidinediones for anticancer therapy," *Expert Opinion on Investigational Drugs*, vol. 15, no. 9, pp. 1039–1049, 2006.

[121] A. A. Chanan-Khan, I. Borrello, K. P. Lee, and D. E. Reece, "Development of target-specific treatments in multiple myeloma," *British Journal of Haematology*, vol. 151, no. 1, pp. 3–15, 2010.

[122] N. Awasthi, M. A. Schwarz, and R. E. Schwarz, "Proteasome inhibition enhances antitumour effects of gemcitabine in experimental pancreatic cancer," *HPB*, vol. 11, no. 7, pp. 600–605, 2009.

[123] S. R. Alberts, N. R. Foster, R. F. Morton et al., "PS-341 and gemcitabine in patients with metastatic pancreatic adenocarcinoma: a North Central Cancer Treatment Group (NCCTG) randomized phase II study," *Annals of Oncology*, vol. 16, no. 10, pp. 1654–1661, 2005.

[124] M. Wang, B. C. Medeiros, H. P. Erba, D. J. DeAngelo, F. J. Giles, and R. T. Swords, "Targeting protein neddylation: a novel therapeutic strategy for the treatment of cancer," *Expert Opinion on Therapeutic Targets*, vol. 15, no. 3, pp. 253–264, 2011.

[125] M. P. Dickens, R. Fitzgerald, and P. M. Fischer, "Small-molecule inhibitors of MDM2 as new anticancer therapeutics," *Seminars in Cancer Biology*, vol. 20, no. 1, pp. 10–18, 2010.

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Review Article

PPARs Signaling and Cancer in the Gastrointestinal System

Valerio Pazienza,[1] Manlio Vinciguerra,[2] and Gianluigi Mazzoccoli[3]

[1] *Gastroenterology Unit IRCCS "Casa Sollievo della Sofferenza" Hospital, Viale dei Cappuccini n.1, 71013 San Giovanni Rotondo, Italy*
[2] *The Institute of Hepatology 69-75 Chenies Mews London, WC1E 6HX London, UK*
[3] *Division of Internal Medecine IRCCS "Casa Sollievo della Sofferenza" Hospital, Viale dei Cappuccini n.1, 71013 San Giovanni Rotondo, Italy*

Correspondence should be addressed to Valerio Pazienza, v.pazienza@operapadrepio.it

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Nowadays, the study of the peroxisome proliferators activated receptors (PPARs) as potential targets for cancer prevention and therapy has gained a strong interest. From a biological point of view, the overall responsibility of PPARs in cancer development and progression is still controversial since several studies report both antiproliferative and tumor-promoting actions for these signaling molecules in human cancer cells and animal models. In this paper, we discuss PPARs functions in the context of different types of gastrointestinal cancer.

1. Introduction

Since the discovery of the peroxisome proliferators activated receptors (PPARs) [1] in Xenopus frogs as receptors that induce the proliferation of peroxisomes in cells [2], three main forms transcribed from three different genes have been indentified: PPARα, PPARβ/δ, and PPARγ. Despite the little divergence of homology observed, each isoform possesses distinct biological activities and is expressed in different tissues [3]. PPARα is mainly expressed in the liver, the kidney, and the heart and is primarily involved in lipid metabolism. PPARγ is a master regulator of adipogenesis and fat storage: it regulates adipocyte differentiation and insulin sensitivity in adipose tissue. PPARβ/δ is found in a broad range of tissues but markedly expressed in brain, adipose tissue, and skin and its function awaits further exploration. PPARs are key mediators of energy homeostasis, lipid, and glucose metabolism although they have also been associated with other biological processes including development, differentiation, inflammation, atherosclerosis, wound healing, and tumor formation. All PPARs heterodimerize with the retinoid X receptor (RXR) to bind successively to specific DNA regions of target genes named PPREs (peroxisome proliferator hormone response elements). Like PPARs, RXR exists as three distinct isoforms: RXRα, β, and γ, all of which are activated by the endogenous agonist 9-*cis* retinoic acid [4]. Contrasting observations confer to PPARs a double-edge sword nature in cancerogenesis, considering that either tumor suppressing or stimulating effects have been evidenced for these nuclear receptors [5].

PPARs function is modified by the specific shape of their ligand-binding domain induced by ligand binding and by a number of coactivator and corepressor proteins, the presence of which can stimulate or inhibit receptor function, respectively [6]. Endogenous ligands for the PPARs include free fatty acids and eicosanoids. PPAR isoform-specific agonists, specifically fibrates for PPARα and thiazolidinediones for PPARγ, are currently prescribed as lipid and glucose-lowering drugs, respectively [7]. Although several reports highlight antiproliferative and prodifferentiative actions of PPARγ ligands in cancer cell lines and animal models of human neoplastic disease [8], more recent studies illustrating tumor-promoting effects of PPARγ, in particular in colon and breast cancer models, raise considerable concern about the practicability and safety of PPARγ ligands as anticancer

drugs [9, 10]. In this paper we discuss PPARs functions in the context of different types of gastrointestinal cancer.

2. PPARs and Gastrointestinal Tract Cancer

Numerous studies in the last decade have focused on the effects of PPARs activity on gastrointestinal tract tumor biology, exploring mechanisms, target genes, clinical applications, and evaluating the potential therapeutic use in cancer treatment of PPARs agonists, which seemed promising as components of combination treatments in both *in vitro* and *in vivo* models of cancer [11–13]. In particular, a possible role for PPARγ as a tumor suppressor and as an inducer of differentiation of cancer stem cells has been explored, and its protein level in tumor specimens has been identified as a significant prognostic marker [14].

A recent meta-analysis has found an association between the *PPARG* polymorphism 34 C > G and colon cancer risk [15], and a *PPARG* germline mutation replacing serine 289 with cysteine in the mature protein (S289C) has been reported associated with dyslipidemia and colonic polyp formation progressing to full-blown adenocarcinoma [16]. Furthermore, studies performed in animal models challenged with procarcinogenic and anticarcinogenic agents have put in evidence that PPARγ signaling pathway is critically engaged in the antitumor activity of normal organisms [17]. Anyway, the role of PPARγ in the neoplastic diseases of the gastrointestinal tract remains controversial, as this nuclear receptor shows dissimilar growth-suppressive effects in different cancers. Moreover, PPARγ activation induces diverse growth inhibition in different cancer cell lines [18]. PPARγ inhibits tumor growth only in the presence of functional APC but not in cells with loss of APC function [19], and PPARγ agonists have been reported to have tumor-promoting effects in the $Apc^{Min/+}$ mice [10], suggesting that loss of APC may alter the normal response of intestinal epithelial tumor cells to PPARγ agonists. The latter could be one important feature that can explain the discrepancies reported in the literature about the dual role of PPARγ in gastrointestinal cancer.

In the esophagus, the evaluation of PPARγ protein and mRNA expression levels in samples of normal esophageal squamous epithelium, Barrett's esophagus, and esophageal adenocarcinoma has shown a trend toward increased expression going from normal tissue to pathological samples and a trend towards increased PPARγ expression with decreasing levels of differentiation [20]. Similarly, PPARγ expression is increased in human gastric cancer tissue [21], and immunohistochemistry has evidenced its overexpression in gastric mucosal dysplasia and gastric carcinoma compared with chronic gastritis [22]. In addition, the presence of PPARγ protein has been evidenced in surgically resected specimens from well differentiated, moderately differentiated, and poorly differentiated gastric adenocarcinoma [23]. On the other hand, PPARγ agonists show dose-dependent inhibitory effects on the proliferation of gastric cancer cell lines, and this effect is augmented by the simultaneous addition of 9-*cis* retinoic acid; flow cytometry demonstrates G1 cell cycle arrest and a significant increase of annexin V-positive cells, suggesting that induction of apoptosis together with G1 cell cycle arrest may be one of the mechanisms of the antiproliferative effect of PPARγ activation in human gastric cancer cells [23].

Regarding the large bowel, high expression of PPARγ is detected in the normal mucosa of the colon and rectum, and a deficiency in intestinal PPARγ is associated with enhanced tumorigenicity in mouse small intestine and colon. A series of evidence suggests that *PPARG* is a tumor suppressor gene in colorectal cancer: (i) loss of function point mutations has been evidenced in one allele of *PPARG* in primary colorectal patients, and the mutations impair the function of PPARγ by affecting the ligand-binding domain, which results in an inability to bind ligands and control gene regulation; (ii) polymorphism in the PPARγ gene has been found in colorectal cancer patients; (iii) expression of PPARγ in colorectal cancer is associated with a good prognosis [24]. Anyway, decreased PPARγ expression compared with adjacent normal colonic mucosa is detected in a number of colorectal cancer patients [25], and *PPARG* inactivation seems to play a role in colorectal cancer progression, although the events involved are not yet clear. In a large series of primary colorectal cancers, about 60% of tumors showed PPARγ upregulation, whereas 35% of the tumours showed lower PPARγ levels compared to the nontumorous normal mucosa. A significant association was evidenced between low PPARγ expression and distant metastases and reduced patients' survival [26].

PPARG epigenetic silencing has been found to be coordinated by ubiquitin-like with PHD and RING finger domains 1 (UHRF1), a member of a subfamily of RING-finger-type E3 ubiquitin ligases, which mediates colorectal cancer progression. This protein is encoded by the UHRF1 gene and its expression peaks at late G1 phase and continues during G2 and M phases of the cell cycle, playing a major role in the G1/S transition by regulating topoisomerase II alpha and retinoblastoma gene expression and functioning in the p53-dependent DNA damage checkpoints. UHRF1 binds to specific DNA sequences and recruits a histone deacetylase to regulate gene expression, functioning as a cofactor that coordinates the epigenetic silencing of tumor suppressor genes. UHRF1 overexpression induces *PPARG* silencing through its recruitment on the *PPARG* promoter promoting DNA methylation and histone repressive modifications, and it is associated with a higher proliferative, clonogenic, and migration potential, and with phenotypic features resembling those occurring in the epithelial-mesenchymal transition [27]. PPARγ agonists such as thiazolidinediones, also known as glitazones (rosiglitazone, troglitazone, and pioglitazone), have been shown to induce apoptosis in human colon cancer cells, and the molecular mechanism involves glycogen synthase kinase-3β (GSK-3β), a crucial activator of nuclear factor-kappa B (NF-kappaB), which plays a critical role in the mediation of survival signals in cancer cells, with inhibition of NF-kappaB activity and GSK-3β expression in a dose-dependent manner. Glitazone treatment inhibits colon cancer cell growth, and cells are arrested in G(0)/G(1) phase followed by the induction of apoptosis with concomitant

decrease in the expression of the G(0)/G(1) phase regulatory proteins Cdk2, Cdk4, cyclin B1, D1, and E, decrease in the antiapoptotic protein Bcl-2, and increase in the expression of the proapoptotic-associated proteins caspase-3, caspase-9, and Bax [28]. Similarly to the phenomenon evidenced in gastric cancer lines [23], the effect is augmented by the simultaneous addition of the RXRα ligand 9-*cis* retinoic acid [29].

On the other hand, inhibiting PPARγ prevents proliferation of human colon cancer HT-29 cells, as evidenced by challenge with cyclic phosphatidic acid (cPA), a structural analog of lysophosphatidic acid (LPA), and a specific, high-affinity PPARγ antagonist [30]. Moreover, synthetic and physiological agonists of PPARγ and PPARβ/δ induce expression of vascular endothelial growth factor (VEGF) in the colorectal tumor cell lines SW480 and HT29 [31]. Interestingly, PPARβ/δ is a promising drug target since its agonists promote terminal differentiation, but there are reports showing either pro- or anticarcinogenic effects of PPARβ/δ in cancer models [32]. Expression of PPARβ/δ mRNA and protein is lower in human and Apc (+/Min-FCCC) mouse colon tumors in respect of matched normal tissue, and stable overexpression of PPARβ/δ in human HT29 colon cancer cell lines enhances ligand activation of PPARβ/δ and inhibition of clonogenicity [33]. The role of PPARβ/δ in the pathogenesis of colorectal cancer has been evaluated in studies performed *in vivo* on rectal cancer patients and *in vitro* on colon cancer cell lines with different metastatic potentials. The intensity of PPARβ/δ expression has been found increased in human rectal cancer tissue compared to adjacent or distant normal mucosa [34], in rectal cancers with better differentiation than in those with poor differentiation, and in early-stage tumors than in advanced ones [35]. Besides, PPARβ knockdown *in vitro* has evidenced that PPARβ/δ may facilitate differentiation and inhibit the cell-fibronectin adhesion of colon cancer cell lines [35].

Anyway, some colorectal cancer cell lines are resistant to PPARγ agonists, because elevated PPARδ expression and/or activation of PPARδ antagonize the ability of PPARγ to induce colorectal carcinoma cell death, as a result of opposing effects of PPARδ and PPARγ in regulating programmed cell death mediated by survivin and caspase-3: activation of PPARγ results in decreased survivin expression and increased caspase-3 activity, whereas activation of PPARδ counteracts these effects [36]. In addition, the concomitant expression of PPAR β/δ and cyclooxygenase (COX)-2 in tumor tissues is associated with a higher incidence of liver metastasis and consequent poor prognosis in colorectal cancer patients [37].

PPARγ activation induces expression of Krüppel-like factor (KLF) 4, known also as gut-enriched Krüppel-like factor (GKLF), which acts as a transcriptional activator or repressor depending on the promoter context and/or cooperation with other transcription factors. KLF4 is a nodal player in the network of PPARγ-regulated genes, and treatment of colon cancer cells with PPARγ agonists influences KLF4 target genes, whose expression is decreased (cyclin D1) or increased (GPA33, encoding the glycoprotein A33 that is a colon cancer antigen, p21WAF1/Cip1, and keratin 19), respectively [38].

Epigenetic silencing of *PPARG* in colorectal cancer may be a significant prognostic marker of tumor progression, and methylation on a specific region of the promoter is strongly correlated with PPARγ lack of expression in primary colorectal cancers and with patients' poor prognosis [26]. The same methylation pattern is found in PPARγ negative colorectal cancer cell lines. Transcriptional silencing is due to the recruitment of methyl CpG binding protein 2 (MeCP2), histone deacetylase 1 (HDAC1), and histone-lysine N-methyltransferase (EZH2) that impart repressive chromatin signatures determining an increased cell proliferative and invasive potential [26].

As reported in this section, many clinical and experimental data support the critical role played by PPARs in gastrointestinal tumorigenesis and neoplastic gut disease behavior, but the molecular mechanisms involved are still a matter of debate. Furthermore, the results of many studies are conflicting and lead to the conclusion that PPARs may have both tumor suppressor and procarcinogenic activity. These controversies may arise from methodological differences among the study protocols, anyway some evidence suggests that ligand-related PPARs activation induces growth arrest in cancer cells and tumor growth inhibition deriving from antiproliferative or proapoptotic effects. On the other hand, PPARs have been found to stimulate tumor cell proliferation and induce neo-angiogenesis, favoring cancer growth and spreading. PPARs agonists provoke several physiological modifications influencing lipid metabolism, glucose homeostasis, and inflammation signaling cascade, and considering that among the major risk factors for colorectal cancer are comprised obesity, metabolic derangement, and chronic inflammatory bowel disease, PPARs modulation could be a valuable tool in the prevention and treatment of colorectal cancer. A mandatory and preliminary condition is represented by the full understanding of the complex mechanisms involved in the regulation of PPARs transcriptional activity and unveiling of the intricacy of PPAR-dependent and PPAR-independent effects stimulated by the different ligands. The same PPAR is able to modulate different target genes and cooperate with other nuclear receptors and signalling molecules involved in cell proliferation and cell death, increasing the difficulty to dissect the role of the single players that take part in this physiologically basic but really intricate network.

3. PPARs and Liver Cancer

Hepatocellular carcinoma (HCC) is the most common type of liver cancer. HCC often arises from viral hepatitis infection (hepatitis B or C), cirrhosis, alcohol consumption being its most common cause. HCC has recently been linked to nonalcoholic fatty liver disease (NAFLD), the hepatic manifestation of obesity and metabolic syndrome. HCC presents with an aberrant lipid metabolism as revealed by quantitative profiling in patient plasma by using ultraperformance liquid chromatography coupled to mass spectrometry approaches

[46]. Compared to other cancers, HCC is quite a rare tumor and, in countries where hepatitis is not endemic, most malignant cancers in the liver are not primary HCC but metastasis (spread) of cancer from elsewhere in the body, for example, colorectal cancer. A great bulk of evidence suggests a role for lipid-sensing nuclear receptors in the pathogenesis of NAFLD and HCC. Lipid sensing nuclear receptors, including PPARs, are the master transcriptional regulators of lipid and carbohydrate metabolism and inflammatory responses, thus standing as suitable therapeutic targets for both NAFLD and HCC [47, 48]. In the leptin-deficient ob/ob mouse model of metabolic syndrome, PPARγ is critical for the development of hepatic steatosis, through modulation of its target protein fat-specific protein 27 (Fsp27) [49]. Hepatic transcriptional effects of PPARα, PPARγ, and PPARδ are multiple and recent hypothesis-driven and unbiased genomewide high-throughput approaches in hepatocytes are continuously uncovering new target genes involved in lipid metabolism or confirming established ones, as *ACSL3, ACOX1, SULT2A1, ACADL, CD36, IGFBP1,* and *G0S2* [50]. PPARs, as other nuclear receptors, can be activated in the liver by several hundreds of environmental chemicals and contaminants, and this has been demonstrated to contribute to the process of hepatocarcinogenesis as observed in *in vitro* and *in vivo* rodent models by large screening studies: however, the biological differences between rodents and humans and the distinct mode of actions make it difficult to extrapolate useful information for the clinics and to determine human carcinogenic risk upon exposure to environmental chemicals [51]. PPARα plays a dominant role in hepatocarcinogenesis induced by trichloroethylene (TCE), an industrial solvent and a widespread environmental contaminant [52]. Beinga central regulator of triglyceride homeostasis and mediating hepatocarcinogenesis in rodents, not surprisingly PPARα, contributes to steatosis and HCC induced by hepatitis C virus (HCV) in rodent models [53, 54]. In human hepatocarcinoma cells, PPARα is chiefly related to apoptosis as evidenced by determination of BAD, myc, and protein phosphatase 2A protein content and PPARγ is instead chiefly related to cell proliferation, evidenced by decreased cell number and increased number of cells in the G0/G1 phase of the cycle [55]. Mice lacking one allele of *PPARG* were more susceptible to liver cancer in a diethylnitrosamine (DEN)-induced HCC model: PPARγ suppressed tumor cell growth through reducing cell proliferation and inducing G(2)/M phase arrest, apoptosis, and upregulating growth differentiation factor-15 [56]. Consistently, troglitazone, a PPARγ ligand, inhibited growth and induced apoptosis of HepG2 cells in a dose-dependent manner [57]. Moreover, in the partial hepatectomy rat model of liver regeneration, it was shown that PPARγ signaling is a key negative regulator of hepatocyte proliferation and may be responsible for the inhibition of liver growth during regeneration [58]. PPARs actively crosstalk with other signaling mediators implicated in lipid metabolism and hepatocyte malignancy; for instance, AMP-activated protein kinase (AMPK), an energy sensing enzyme implicated in the transition from NAFLD to HCC [59], and whose activation has been reported to be lipid lowering and antitumoral in mice and in hepatoma cells [60, 61]. In HCC cells, AMPK activators AICAR and metformin inhibit directly transcriptional activities of PPARα and PPARγ to modulate energy generation through fatty acid oxidation process [62]. Mice with a combination of genetic inactivations for hepatic growth hormone and glucocorticoid receptor signaling effectors displayed upregulation of prolipogenic PPARγ and downstream transcription factor SREBP-1c, demonstrating a crosstalk between these molecular networks [63]. Mice with specific inactivation of the NF-kappaB essential modulator gene (NEMO (L-KO) mice) exposed to a high-fat diet display a worsened liver steatosis as a consequence of PPARα and increased PPARγ expression [64]. From a therapeutic perspective, PPARγ agonists, such as antidiabetic thiazolidinediones (TZD), have *in vitro* antiproliferative effect, have been associated with lower risk and a better prognosis in HCC, not only related to anti-NAFLD but also to antiviral hepatitis effects [65]. The effective anticancer properties and the underlying molecular mechanisms of these drugs *in vivo* remain unclear because the primary target of TZD is PPARγ, which is upregulated in HCC and seems to provide tumor-promoting responses. Reconciling this discrepancy, it may be that these established PPARs agonists exert a hypolipidemic and antitumoral action in liver cells through PPAR-independent pathways [66, 67].

As mentioned, when the liver is infected with hepatic viruses, this can ultimately result in liver cancer, and hepatitis viruses are one of the leading causes of chronic liver disease [68]. Hepatitis viruses are a global health problem if we consider approximately 200 million patients carrying a chronic HCV infection and about 350 million chronically infected with HBV [69].

PPARs were suggested as new therapeutic targets in the traditional treatment of HCV-induced liver injury when two studies found that PPARα drastically decreased in HCV-infected patients [70] together with its target gene carnitine palmitoyl acyl-CoA transferase 1A (CPT1A) [71]. The impaired PPARα expression was due to HCV core protein expression [71]. Successively, we and others have recently uncovered a role for PPARγ in HCV infection [42, 72, 73]. Granted that HCV is classified in six different major genotypes and that mechanisms involved in pathobiology of disease are genotype dependent [39, 73], from a biological point of view, reduced PPARγ levels found in *in vitro* models of HCV expressing the core protein genotype 3a are associated with increased fat accumulation and impaired insulin signaling [72, 73]. The latter impairs the sustained response rate to peg-interferon plus ribavirin in chronic hepatitis C patients [40]. PPARγ degrades IRS1 protein through suppressor of cytokine signaling protein 7 (SOCS-7) whose expression could be pharmacologically controlled by agonist and antagonist of PPARγ [41]. PPARγ agonists have already been suggested as an adjuvant therapy in chronic hepatitis C [74, 75]. In fact, there is the belief that correcting insulin resistance is a rational option in chronic hepatitis C patients [76]. However, new modalities of this correction have to be explored based on the mechanisms inducing insulin resistance, as insulin-sensitizing therapy

should be tailored according to the infecting HCV genotype, as suggested [76].

Steatosis is a common histological feature of chronic infection between hepatitis C and B virus. Another common feature is the ability of both viruses in modulating PPARα and PPARγ activity/expression which are related to steatosis. As for HBV, *in vitro* studies using hepatoma cell lines and studies on transgenic mouse models for HBV have provided indication for a role of PPARs in HBV-related diseases and in controlling viral transcription and replication. Kim et al. [77] demonstrated that SREBP-1 and PPARγ were transcriptionally induced by HBV X protein (HBx) in order to provoke hepatic steatosis in HepG2-HBx stable cells and HBx-transgenic mice.

Moreover thiazolidinediones (TZD, class of PPARγ ligands) have been suggested as useful drugs for HCC chemoprevention and treatment as TZD administration in hepatitis B virus (HBV)-transgenic mice reduced tumor incidence in the liver, inhibiting hepatocyte proliferation and increasing apoptosis, probably through inhibition of nucleophosmin (NPM) protein and mRNA expression [68]. Furthermore it was also reported a role for PPARs in regulating HBV transcription and regulation *in vivo* [78] and *in vitro* [79]. Guidotti et al. [78] demonstrated that HBV transgenic mice treated with two synthetic PPARα ligands (Wy-14,643 and clofibric acid) resulted in an increased HBV transcription rates suggesting that in patients receiving these drugs who are also infected with HBV viral replication may be activated, and this could have potentially detrimental effects on the outcome of the viral infection. Conversely, Wakui et al. [79] demonstrated that the PPARα ligand bezafibrate had no effect on HBV replication within HepG2 cells whilst a PPARγ ligand, rosiglitazone, reduced the amount of HBV DNA, hepatitis B *surface* antigen (HBsAg), and hepatitis B *e* antigen (HBeAg) in the culture supernatant, suggesting that the combination therapy of rosiglitazone and nucleot(s)ide analogues or interferon could be a therapeutic rational option also for chronic HBV infection.

4. PPARs and Pancreatic Cancer

Pancreatic cancer (PC) is one of the most lethal malignant diseases with a really terrible prognosis and is ranked as the fourth leading cause of cancer-related deaths worldwide [80]. PC is referred to as a "silent killer" because early pancreatic cancer often does not cause symptoms and the later symptoms are usually nonspecific and varied. Despite many advances in modern medicine, the available therapeutic strategies based on surgery and conventional chemotherapy are still largely unsatisfactory in patients with pancreatic cancer. When patients present locally advanced or metastatic tumors (which render them ineligible for surgical resection), they are treated with the gold standard chemotherapy which is based on gemcitabine, an S-phase nucleoside cytidine analogue. The overall survival is unacceptably small, and novel therapeutic approaches to overcome the resistance of PC to conventional anticancer therapies are urgently needed. Scientists are also looking for an ideal combination partner in therapeutic settings that require the inhibition of tumor-protecting mechanisms/proteins to overcome treatment resistance.

PPARγ is commonly upregulated in pancreatic ductal adenocarcinoma and might be considered a prognostic marker in this disease [81].

To date several research groups have demonstrated the ability of thiazolidinedione (TZD, class of PPARγ ligands) to attenuate the growth of pancreatic cancer cells *in vitro*, which was associated to G1 cell cycle arrest and cell differentiation and to increased apoptotic cell death [43]. Moreover, Hashimoto et al. [82] suggest a double beneficial effect of TZD showing the dual advantage of inhibiting pancreatic cancer cell growth while reducing the invasiveness of the tumor cells. Moreover, TZD attenuated pancreatic cancer cell migration and invasion by modulation of actin organization and expression of matrix metalloproteinase-2 and plasminogen activator inhibitor-1, respectively [83, 84]. An increasing number of studies have implicated STAT activation, particularly STAT3, in transformation and tumor progression. Direct targeting of STAT3 in malignant tumors may represent another important therapeutic tool as STAT proteins are emerging as ideal targets for cancer therapy [44]. Vitale et al. [85] showed that, in pancreatic cancer cells, PPAR-γ agonist (troglitazone, TGZ) counteracts STAT3 protein potentiating the anticancer effects of IFN-β through the induction of cell cycle perturbations and the occurrence of autophagy cell death in pancreatic cancer cells. Co-incubation of pancreatic cancer cells with IFN-β and TGZ suppresses STAT3 activation and delays G0/G1-S phase progression that occurred together with an increase in p21 and p27 protein expression that was more evident after 24 hours of treatment with the pharmacological combination.

Even though we did not observe a PPARγ altered expression in 30 matched pairs of tumour and adjacent normal tissue samples collected from patients undergoing pancreatic resection [45], a recent study supports a role of PPARγ as an ideal partner of the standard therapy based on gemcitabine since the anticancer effect of gemcitabine can be enhanced by ligands for PPARγ such as pioglitazone (Pio) and rosiglitazone [86]. The authors demonstrate that Pio significantly inhibits the NF-κB transcriptional activity and potentiates the gemcitabine effect on the apoptosis rate in three different pancreatic cancer cell lines as demonstrated by cotreatment with Pio and Gem on caspase-3 and caspase-7 cleavage. The authors conclude that since Pio is widely used in the treatment of diabetes mellitus, it may become a possible partner of Gem-based chemotherapy. Considered the adverse effects associated with TZDs, such as weight gain, macular edema, bone loss, and heart failure in at-risk individuals [87, 88], scientists must press on investigating new analogs of PPARγ agonists in order to potentiate the beneficial effect while reducing the side effects (Figure 1 and Table 1).

5. Conclusion

A potential role for PPARs agonists in the adjuvant treatment of digestive system cancers is advisable, but further studies

Table 1: Differential patterns of PPARs expression in gastrointestinal system disease.

Organ	PPARs expression		Author and reference
Esophagus	PPARγ ↑	PPARγ overexpression influences the development of Barrett's esophagus and esophageal adenocarcinoma	Wang et al. [20]
Stomach	PPARγ ↑	Crucial role of PPARγ in the pathogenesis of gastric carcinoma	Ma et al. [21]
	PPARγ ↑	PPARγ is upregulated in gastric adenocarcinoma	Yao et al. [22]
	PPARγ ↑	PPARγ protein evidenced in gastric adenocarcinoma specimens and PPARγ agonists show dose-dependent inhibitory effects on the proliferation of gastric cancer cell lines	Sato et al. [23]
Colon-rectum	PPARγ ↑	PPARγ expression in colorectal cancer is associated with a good prognosis	Dai and Wang [24]
	PPARγ ↓	PPARγ underexpression is detected in a number of colorectal cancer patients, and epigenetic silencing of PPARγ is a biomarker for colorectal cancer progression and adverse patients' outcome	Pancione et al. [26]
	PPARγ ↓	PPARγ epigenetic silencing is coordinated by UHRF1 mediating colorectal cancer progression, and a significant low PPARγ expression is associated with distant metastases and reduced patients' survival	Sabatino et al. [27]
Liver	PPARα and PPARγ ↓	HCV decreases PPARs in order to induce triglycerides accumulation	Ripoli and Pazienza [39] Romero-Gómez et al. [40] Pazienza et al. [41]
	PPARγ ↑	HBx enhances C/EBPα that in turn induces PPARγ expression and activation	Dharancy et al. [42] Tsujie et al. [43]
Pancreas	PPARγ ↑	PPARγ is highly expressed in pancreatic cancer and is associated with shorter overall survival times	Yu and Jove [44]
	PPARγ —	PPARγ is unaltered in PC but expression levels between PPARγ and DNMT1 and between DNMT1 and DNMT3B are highly correlated	Pazienza et al. [45]

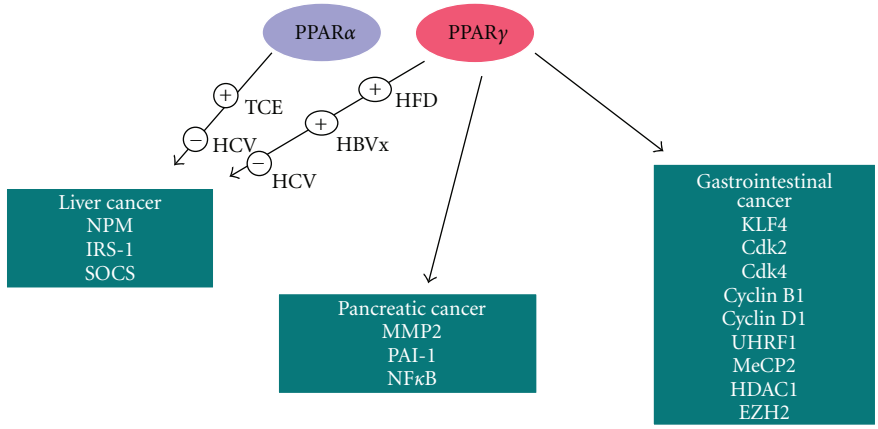

Figure 1: Schematic representation of the PPARs signaling operating in cancer. Krueppel-like factor 4 (KLF4); cyclin-dependent kinase (2, 4); cyclin B1, D1; ubiquitin-like, containing PHD and RING finger domains, 1; methyl CpG binding protein 2 (MeCP2); Histone deacetylase 1(HDAC1); histone-lysine N-methyltransferase (EZH2); matrix metallopeptidase 2 (MMP2); plasminogen activator inhibitor 1 (PAI-1) nuclear factor-kappaB (NFκB); nucleophosmin (NPM); insulin receptor substrate 1 (IRS-1); suppressor of cytokine signal (SOCS). For further explanations, please refer to the text.

are warranted in order to better clarify the role of PPARs in gastrointestinal cancerogenesis. PPARs could have prognostic and/or therapeutic roles, but there is urgent need to shed light on the favorable potential or harmful risk of their modulators.

Conflict of Interests

The authors declare that they have no competing interests.

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References

[1] I. Issemann and S. Green, "Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators," *Nature*, vol. 347, no. 6294, pp. 645–650, 1990.

[2] C. Dreyer, G. Krey, H. Keller, F. Givel, G. Helftenbein, and W. Wahli, "Control of the peroxisomal β-oxidation pathway by a novel family of nuclear hormone receptors," *Cell*, vol. 68, no. 5, pp. 879–887, 1992.

[3] P. Escher and W. Wahli, "Peroxisome proliferator-activated receptors: Insight into multiple cellular functions," *Mutation Research*, vol. 448, no. 2, pp. 121–138, 2000.

[4] D. J. Mangelsdorf, U. Borgmeyer, R. A. Heyman et al., "Characterization of three RXR genes that mediate the action of 9-cis retinoic acid," *Genes and Development*, vol. 6, no. 3, pp. 329–344, 1992.

[5] D. Panigrahy, A. Kaipainen, M. W. Kieran, and S. Huang, "PPARs: a double-edged sword in cancer yherapy?" *PPAR Research*, vol. 2008, Article ID 350351, 2 pages, 2008.

[6] S. Yu and J. K. Reddy, "Transcription coactivators for peroxisome proliferator-activated receptors," *Biochimica et Biophysica Acta*, vol. 1771, no. 8, pp. 936–951, 2007.

[7] W. Xu and P. Charoensuksai, "PPARs in rhythmic metabolic regulation and implications in health and disease," *PPAR Research*, vol. 2010, Article ID 243643, 9 pages, 2010.

[8] G. Eibl, "The role of PPAR-γ and its interaction with COX-2 in pancreatic cancer," *PPAR Research*, vol. 2008, Article ID 326915, 6 pages, 2008.

[9] K. Yang, K. H. Fan, S. A. Lamprecht et al., "Peroxisome proliferator-activated receptor γ agonist troglitazone induces colon tumors in normal C57BL/6J mice and enhances colonic carcinogenesis in Apc1638 N/+ Mlh1+/− double mutant mice," *International Journal of Cancer*, vol. 116, no. 4, pp. 495–499, 2005.

[10] M. V. Pino, M. F. Kelley, and Z. Jayyosi, "Promotion of colon tumors in C57BL/6J-APCmin/+ mice by thiazolidinedione PPARγ agonists and a structurally unrelated PPARγ agonist," *Toxicologic Pathology*, vol. 32, no. 1, pp. 58–63, 2004.

[11] I. A. Voutsadakis, "Peroxisome proliferator-activated receptor γ (PPARγ) and colorectal carcinogenesis," *Journal of Cancer Research and Clinical Oncology*, vol. 133, no. 12, pp. 917–928, 2007.

[12] J. Bassaganya-Riera, A. B. Carter, S. A. Misyak, and R. Hontecillas, "Dietary modulation of inflammation-induced colorectal cancer through PPARγ," *PPAR Research*, vol. 2009, Article ID 498352, 9 pages, 2009.

[13] J. M. Peters, Y. M. Shah, and F. J. Gonzalez, "The role of peroxisome proliferator-activated receptors in carcinogenesis and chemoprevention," *Nature Reviews Cancer*, vol. 12, no. 3, pp. 181–195, 2012.

[14] G. T. Robbins and D. Nie, "PPAR gamma, bioactive lipids, and cancer progression," *Frontiers in Bioscience*, vol. 17, pp. 1816–1834, 2012.

[15] Y. L. Lu, G. L. Li, H. L. Huang, J. Zhong, and L. C. Dai, "Peroxisome proliferator-activated receptor-γ 34C>G polymorphism and colorectal cancer risk: a meta-analysis," *World Journal of Gastroenterology*, vol. 16, no. 17, pp. 2170–2175, 2010.

[16] D. Capaccio, A. Ciccodicola, L. Sabatino et al., "A novel germline mutation in peroxisome proliferator-activated receptor γ gene associated with large intestine polyp formation and dyslipidemia," *Biochimica et Biophysica Acta*, vol. 1802, no. 6, pp. 572–581, 2010.

[17] Y. Dai, L. Qiao, W. C. Kwok et al., "Peroxisome proliferator-activated receptor-γ contributes to the inhibitory effects of embelin on colon carcinogenesis," *Cancer Research*, vol. 69, no. 11, pp. 4776–4783, 2009.

[18] L. Qiao, B. Zou, and B. C. Y. Wong, "Current understanding of the role of PPAR γ in gastrointestinal cancers," *PPAR Research*, vol. 2009, Article ID 816957, 8 pages, 2009.

[19] G. D. Girnun, W. M. Smith, S. Drori et al., "APC-dependent suppression of colon carcinogenesis by PPARγ," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 21, pp. 13771–13776, 2002.

[20] W. Wang, R. Wang, Z. Zhang, D. Li, and Y. Yu, "Enhanced PPAR-γ expression may correlate with the development of Barrett's esophagus and esophageal adenocarcinoma," *Oncology Research*, vol. 19, no. 3-4, pp. 141–147, 2011.

[21] X. M. Ma, H. Yu, and N. Huai, "Peroxisome proliferator-activated receptor-γ is essential in the pathogenesis of gastric carcinoma," *World Journal of Gastroenterology*, vol. 15, no. 31, pp. 3874–3883, 2009.

[22] L. Yao, F. Liu, L. Sun et al., "Upregulation of PPARγ in tissue with gastric carcinoma," *Hybridoma*, vol. 29, no. 4, pp. 341–343, 2010.

[23] H. Sato, S. Ishihara, K. Kawashima et al., "Expression of peroxisome proliferator-activated receptor (PPAR)γ in gastric cancer and inhibitory effects of PPARγ agonists," *British Journal of Cancer*, vol. 83, no. 10, pp. 1394–1400, 2000.

[24] Y. Dai and W. H. Wang, "Peroxisome proliferator-activated receptor γ and colorectal cancer," *The World Journal of Gastrointestinal Oncology*, vol. 2, no. 3, pp. 159–164, 2010.

[25] P. A. Konstantinopoulos, G. P. Vandoros, G. Sotiropoulou-Bonikou, A. Kominea, and A. G. Papavassiliou, "NF-κB/PPARγ and/or AP-1/PPARγ 'on/off' switches and induction of CBP in colon adenocarcinomas: correlation with COX-2 expression," *International Journal of Colorectal Disease*, vol. 22, no. 1, pp. 57–68, 2007.

[26] M. Pancione, L. Sabatino, A. Fucci et al., "Epigenetic silencing of peroxisome proliferator- activated receptor γ is a biomarker for colorectal cancer progression and adverse patients' outcome," *PLoS One*, vol. 5, no. 12, Article ID e14229, 2010.

[27] L. Sabatino, A. Fucci, M. Pancione et al., "UHRF1 coordinates peroxisome proliferator activated receptor gamma (PPARG)

epigenetic silencing and mediates colorectal cancer progression," *Oncogene*. In press.

[28] J. O. Ban, D. H. Kwak, J. H. Oh et al., "Suppression of NF-κB and GSK-3β is involved in colon cancer cell growth inhibition by the PPAR agonist troglitazone," *Chemico-Biological Interactions*, vol. 188, no. 1, pp. 75–85, 2010.

[29] A. Papi, P. Rocchi, A. M. Ferreri, and M. Orlandi, "RXRγ and PPARγ ligands in combination to inhibit proliferation and invasiveness in colon cancer cells," *Cancer Letters*, vol. 297, no. 1, pp. 65–74, 2010.

[30] T. Tsukahara, S. Hanazawa, T. Kobayashi, Y. Iwamoto, and K. Murakami-Murofushi, "Cyclic phosphatidic acid decreases proliferation and survival of colon cancer cells by inhibiting peroxisome proliferator-activated receptor γ," *Prostaglandins and Other Lipid Mediators*, vol. 93, no. 3-4, pp. 126–133, 2010.

[31] C. Röhrl, U. Kaindl, I. Koneczny et al., "Peroxisome-proliferator-activated receptors γ and β/δ mediate vascular endothelial growth factor production in colorectal tumor cells," *Journal of Cancer Research and Clinical Oncology*, vol. 137, no. 1, pp. 29–39, 2011.

[32] J. M. Peters, J. E. Foreman, and F. J. Gonzalez, "Dissecting the role of peroxisome proliferator-activated receptor-β/δ (PPARβ/δ) in colon, breast, and lung carcinogenesis," *Cancer and Metastasis Reviews*, vol. 30, no. 3-4, pp. 619–640, 2011.

[33] J. E. Foreman, W. C. L. Chang, P. S. Palkar et al., "Functional characterization of peroxisome proliferator-activated receptor-β/δ expression in colon cancer," *Molecular Carcinogenesis*, vol. 50, no. 11, pp. 884–900, 2011.

[34] L. Yang, H. Zhang, Z. G. Zhou, H. Yan, G. Adell, and X. F. Sun, "Biological function and prognostic significance of peroxisome proliferator-activated receptor δ in rectal cancer," *Clinical Cancer Research*, vol. 17, no. 11, pp. 3760–3770, 2011.

[35] L. Yang, B. Olsson, D. Pfeifer et al., "Knockdown of peroxisome proliferator-activated receptor-β induces less differentiation and enhances cell-fibronectin adhesion of colon cancer cells," *Oncogene*, vol. 29, no. 4, pp. 516–526, 2010.

[36] D. Wang, W. Ning, D. Xie, L. Guo, and R. N. DuBois, "Peroxisome proliferator-activated receptor δ confers resistance to peroxisome proliferator-activated receptor γ-induced apoptosis in colorectal cancer cells," *Oncogene*, vol. 31, no. 8, pp. 1013–1023, 2012.

[37] M. Yoshinaga, K. Taki, S. Somada et al., "The expression of both peroxisome proliferator-activated receptor delta and cyclooxygenase-2 in tissues is associated with poor prognosis in colorectal cancer patients," *Digestive Diseases and Sciences*, vol. 56, no. 4, pp. 1194–1200, 2011.

[38] J. Rageul, S. Mottier, A. Jarry et al., "KLF4-dependent, PPARγ-induced expression of GPA33 in colon cancer cell lines," *International Journal of Cancer*, vol. 125, no. 12, pp. 2802–2809, 2009.

[39] M. Ripoli and V. Pazienza, "Impact of HCV genetic differences on pathobiology of disease," *Expert Review of Anti-Infective Therapy*, vol. 9, no. 9, pp. 747–759, 2011.

[40] M. Romero-Gómez, M. Del Mar Viloria, R. J. Andrade et al., "Insulin resistance impairs sustained response rate to peginterferon plus ribavirin in chronic hepatitis C patients," *Gastroenterology*, vol. 128, no. 3, pp. 636–641, 2005.

[41] V. Pazienza, M. Vinciguerra, A. Andriulli, and A. Mangia, "Hepatitis C virus core protein genotype 3a increases SOCS-7 expression through PPAR-γ in Huh-7 cells," *Journal of General Virology*, vol. 91, no. 7, pp. 1678–1686, 2010.

[42] S. Dharancy, M. Lemoine, P. Mathurin, L. Serfaty, and L. Dubuquoy, "Peroxisome proliferator-activated receptors in HCV-related infection," *PPAR Research*, vol. 2009, Article ID 357204, 5 pages, 2009.

[43] M. Tsujie, S. Nakamori, J. Okami et al., "Thiazolidinediones inhibit growth of gastrointestinal, biliary, and pancreatic adenocarcinoma cells through activation of the peroxisome proliferator-activated receptor gamma/retinoid X receptor alpha pathway," *Experimental Cell Research*, vol. 289, no. 1, pp. 143–151, 2003.

[44] H. Yu and R. Jove, "The stats of cancer—new molecular targets come of age," *Nature Reviews Cancer*, vol. 4, no. 2, pp. 97–105, 2004.

[45] V. Pazienza, F. Tavano, G. Benegiamo et al., "Correlations among PPARγ, DNMT1, and DNMT3B expression levels and pancreatic cancer," *PPAR Research*, vol. 2012, Article ID 461784, 7 pages, 2012.

[46] A. D. Patterson, O. Maurhofer, D. Beyoglu et al., "Aberrant lipid metabolism in hepatocellular carcinoma revealed by plasma metabolomics and lipid profiling," *Cancer Research*, vol. 71, no. 21, pp. 6590–6600, 2011.

[47] M. Vacca, C. Degirolamo, R. Mariani-Costantini, G. Palasciano, and A. Moschetta, "Lipid-sensing nuclear receptors in the pathophysiology and treatment of the metabolic syndrome," *Wiley Interdisciplinary Reviews: Systems Biology and Medicine*, vol. 3, no. 5, pp. 562–587, 2011.

[48] G. Li and G. L. Guo, "Role of class II nuclear receptors in liver carcinogenesis," *Anti-Cancer Agents in Medicinal Chemistry*, vol. 11, no. 6, pp. 529–542, 2011.

[49] K. Matsusue, T. Kusakabe, T. Noguchi et al., "Hepatic steatosis in leptin-deficient mice is promoted by the PPARγ target gene Fsp27," *Cell Metabolism*, vol. 7, no. 4, pp. 302–311, 2008.

[50] D. L. M. van der Meer, T. Degenhardt, S. Väisänen et al., "Profiling of promoter occupancy by PPARα in human hepatoma cells via ChIP-chip analysis," *Nucleic Acids Research*, vol. 38, no. 9, Article ID gkq012, pp. 2839–2850, 2010.

[51] K. Z. Guyton, W. A. Chiu, T. F. Bateson et al., "A reexamination of the PPAR-α activation mode of action as a basis for assessing human cancer risks of environmental contaminants," *Environmental Health Perspectives*, vol. 117, no. 11, pp. 1664–1672, 2009.

[52] J. C. Corton, "Evaluation of the role of peroxisome proliferator-activated receptor α (PPARα in mouse liver tumor induction by trichloroethylene and metabolites," *Critical Reviews in Toxicology*, vol. 38, no. 10, pp. 857–875, 2008.

[53] N. Tanaka, K. Moriya, K. Kiyosawa, K. Koike, F. J. Gonzalez, and T. Aoyama, "PPARα activation is essential for HCV core protein-induced hepatic steatosis and hepatocellular carcinoma in mice," *Journal of Clinical Investigation*, vol. 118, no. 2, pp. 683–694, 2008.

[54] N. Tanaka, K. Moriya, K. Kiyosawa, K. Koike, and T. Aoyama, "Hepatitis C virus core protein induces spontaneous and persistent activation of peroxisome proliferator-activated receptor α in transgenic mice: Implications for HCV-associated hepatocarcinogenesis," *International Journal of Cancer*, vol. 122, no. 1, pp. 124–131, 2008.

[55] M. Maggiora, M. Oraldi, G. Muzio, and R. A. Canuto, "Involvement of PPARα and PPARγ in apoptosis and proliferation of human hepatocarcinoma HepG2 cells," *Cell Biochemistry and Function*, vol. 28, no. 7, pp. 571–577, 2010.

[56] J. Yu, B. Shen, E. S. H. Chu et al., "Inhibitory role of peroxisome proliferator-activated receptor gamma in hepatocarcinogenesis in mice and in vitro," *Hepatology*, vol. 51, no. 6, pp. 2008–2019, 2010.

[57] Y. M. Zhou, Y. H. Wen, X. Y. Kang, H. H. Qian, J. M. Yang, and Z. F. Yin, "Troglitazone, a peroxisome proliferator-activated receptor γ ligand, induces growth inhibition and apoptosis of HepG2 human liver cancer cells," *World Journal of Gastroenterology*, vol. 14, no. 14, pp. 2168–2173, 2008.

[58] Y. Yamamoto, T. Ono, D. K. Dhar et al., "Role of peroxisome proliferator-activated receptor-gamma (PPARγ) during liver regeneration in rats," *Journal of Gastroenterology and Hepatology*, vol. 23, no. 6, pp. 930–937, 2008.

[59] N. MartíNez-LóPez, M. Varela-Rey, D. FernáNdez-Ramos et al., "Activation of LKB1-Akt pathway independent of phosphoinositide 3-kinase plays a critical role in the proliferation of hepatocellular carcinoma from nonalcoholic steatohepatitis," *Hepatology*, vol. 52, no. 5, pp. 1621–1631, 2010.

[60] D. Vara, M. Salazar, N. Olea-Herrero, M. Guzmán, G. Velasco, and I. Díaz-Laviada, "Anti-tumoral action of cannabinoids on hepatocellular carcinoma: role of AMPK-dependent activation of autophagy," *Cell Death and Differentiation*, vol. 18, no. 7, pp. 1099–1111, 2011.

[61] K. Bhalla, B. J. Hwang, R. E. Dewi et al., "Metformin prevents liver tumorigenesis by inhibiting pathways driving hepatic lipogenesis," *Cancer Prevention Research*, vol. 5, no. 4, pp. 544–552, 2012.

[62] M. S. Sozio, C. Lu, Y. Zeng, S. Liangpunsakul, and D. W. Crabb, "Activated AMPK inhibits PPAR-α and PPAR-γ transcriptional activity in hepatoma cells," *American Journal of Physiology. Gastrointestinal and Liver Physiology*, vol. 301, no. 4, pp. G739–G747, 2011.

[63] K. M. Mueller, J. W. Kornfeld, K. Friedbichler et al., "Impairment of hepatic growth hormone and glucocorticoid receptor signaling causes steatosis and hepatocellular carcinoma in mice," *Hepatology*, vol. 54, no. 4, pp. 1398–1409, 2011.

[64] F. T. Wunderlich, T. Luedde, S. Singer et al., "Hepatic NF-κB essential modulator deficiency prevents obesity-induced insulin resistance but synergizes with high-fat feeding in tumorigenesis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 4, pp. 1297–1302, 2008.

[65] J. Shim, B. H. Kim, Y. I. Kim et al., "The peroxisome proliferator-activated receptor γ ligands, pioglitazone and 15-deoxy-Δ12,14-prostaglandin J2, have antineoplastic effects against hepatitis B virus-associated hepatocellular carcinoma cells," *International Journal of Oncology*, vol. 36, no. 1, pp. 223–231, 2010.

[66] D. Yamasaki, N. Kawabe, H. Nakamura et al., "Fenofibrate suppresses growth of the human hepatocellular carcinoma cell via PPARα-independent mechanisms," *European Journal of Cell Biology*, vol. 90, no. 8, pp. 657–664, 2011.

[67] A. Galli, E. Ceni, T. Mello et al., "Thiazolidinediones inhibit hepatocarcinogenesis in hepatitis B virus-transgenic mice by peroxisome proliferator-activated receptor γ-independent regulation of nucleophosmin," *Hepatology*, vol. 52, no. 2, pp. 493–505, 2010.

[68] I. Saito, T. Miyamura, A. Ohbayashi et al., "Hepatitis C virus infection is associated with the development of hepatocellular carcinoma," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 87, no. 17, pp. 6547–6549, 1990.

[69] S. Dharancy, M. Lemoine, P. Mathurin, L. Serfaty, and L. Dubuquoy, "Peroxisome proliferator-activated receptors in HCV-related infection," *PPAR Research*, vol. 2009, Article ID 357204, 5 pages, 2009.

[70] Y. Cheng, S. Dharancy, M. Malapel, and P. Desreumaux, "Hepatitis C virus infection down-regulates the expression of peroxisome proliferator-activated receptor α and carnitine palmitoyl acyl-CoA transferase 1A," *World Journal of Gastroenterology*, vol. 11, no. 48, pp. 7591–7596, 2005.

[71] S. Dharancy, M. Malapel, G. Perlemuter et al., "Impaired expression of the peroxisome proliferator-activated receptor alpha during hepatitis C virus infection," *Gastroenterology*, vol. 128, no. 2, pp. 334–342, 2005.

[72] A. De Gottardi, V. Pazienza, P. Pugnale et al., "Peroxisome proliferator-activated receptor-α and -γ mRNA levels are reduced in chronic hepatitis C with steatosis and genotype 3 infection," *Alimentary Pharmacology and Therapeutics*, vol. 23, no. 1, pp. 107–114, 2006.

[73] V. Pazienza, S. Clément, P. Pugnale et al., "The hepatitis C virus core protein of genotypes 3a and 1b downregulates insulin receptor substrate 1 through genotype-specific mechanisms," *Hepatology*, vol. 45, no. 5, pp. 1164–1171, 2007.

[74] H. M. Elgouhari, K. B. Cesario, R. Lopez, and N. N. Zein, "Pioglitazone improves early virologic kinetic response to PEG IFN/RBV combination therapy in hepatitis C genotype 1 naive pts," *Hepatology*, vol. 48, article 383, 2008.

[75] K. Overbeck, D. Genné, A. Golay, and F. Negro, "Pioglitazone in chronic hepatitis C not responding to pegylated interferon-α and ribavirin," *Journal of Hepatology*, vol. 49, no. 2, pp. 295–298, 2008.

[76] F. Negro, "Correction of insulin resistance in chronic hepatitis C patients not responding to the standard of care: more questions than answers," *Journal of Hepatology*, vol. 50, no. 6, pp. 1271–1272, 2009.

[77] K. H. Kim, H. J. Shin, K. Kim et al., "Hepatitis B Virus X Protein Induces Hepatic Steatosis Via Transcriptional Activation of SREBP1 and PPARγ," *Gastroenterology*, vol. 132, no. 5, pp. 1955–1967, 2007.

[78] L. G. Guidotti, C. M. Eggers, A. K. Raney et al., "In vivo regulation of hepatitis B virus replication by peroxisome proliferators," *Journal of Virology*, vol. 73, no. 12, pp. 10377–10386, 1999.

[79] Y. Wakui, J. Inoue, Y. Ueno et al., "Inhibitory effect on hepatitis B virus in vitro by a peroxisome proliferator-activated receptor-β ligand, rosiglitazone," *Biochemical and Biophysical Research Communications*, vol. 396, no. 2, pp. 508–514, 2010.

[80] A. Jemal, R. Siegel, E. Ward, Y. Hao, J. Xu, and M. J. Thun, "Cancer statistics, 2009," *CA Cancer Journal for Clinicians*, vol. 59, no. 4, pp. 225–249, 2009.

[81] G. Kristiansen, J. Jacob, A. C. Buckendahl et al., "Peroxisome proliferator-activated receptor γ is highly expressed in pancreatic cancer and is associated with shorter overall survival times," *Clinical Cancer Research*, vol. 12, no. 21, pp. 6444–6451, 2006.

[82] K. Hashimoto, B. J. Farrow, and B. M. Evers, "Activation and Role of MAP Kinases in 15d-PGJ2-Induced Apoptosis in the Human Pancreatic Cancer Cell Line MIA PaCa-2," *Pancreas*, vol. 28, no. 2, pp. 153–159, 2004.

[83] A. Galli, E. Ceni, D. W. Crabb et al., "Antidiabetic thiazolidinediones inhibit invasiveness of pancreatic cancer cells via PPARγ independent mechanisms," *Gut*, vol. 53, no. 11, pp. 1688–1697, 2004.

[84] W. Motomura, M. Nagamine, S. Tanno et al., "Inhibition of cell invasion and morphological change by troglitazone in human pancreatic cancer cells," *Journal of Gastroenterology*, vol. 39, no. 5, pp. 461–468, 2004.

[85] G. Vitale, S. Zappavigna, M. Marra et al., "The PPAR-γ agonist troglitazone antagonizes survival pathways induced by STAT-3 in recombinant interferon-β treated pancreatic cancer cells," *Biotechnology Advances*, vol. 30, no. 1, pp. 169–184, 2012.

[86] H. Koga, K. Selvendiran, R. Sivakumar et al., "PPARγ potentiates anticancer effects of gemcitabine on human pancreatic cancer cells," *International Journal of Oncology*, vol. 40, no. 3, pp. 679–685, 2012.

[87] P. Shah and S. Mudaliar, "Pioglitazone: side effect and safety profile," *Expert Opinion on Drug Safety*, vol. 9, no. 2, pp. 347–354, 2010.

[88] C. V. Rizos, M. S. Elisaf, D. P. Mikhailidis, and E. N. Liberopoulos, "How safe is the use of thiazolidinediones in clinical practice?" *Expert Opinion on Drug Safety*, vol. 8, no. 1, pp. 15–32, 2009.

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Review Article

PPARs in Liver Diseases and Cancer: Epigenetic Regulation by MicroRNAs

Marion Peyrou, Pierluigi Ramadori, Lucie Bourgoin, and Michelangelo Foti

Department of Cell Physiology and Metabolism, Faculty of Medicine, Centre Médical Universiatire (CMU), 1206 Geneva, Switzerland

Correspondence should be addressed to Michelangelo Foti, michelangelo.foti@unige.ch

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Peroxisome-proliferator-activated receptors (PPARs) are ligand-activated nuclear receptors that exert in the liver a transcriptional activity regulating a whole spectrum of physiological functions, including cholesterol and bile acid homeostasis, lipid/glucose metabolism, inflammatory responses, regenerative mechanisms, and cell differentiation/proliferation. Dysregulations of the expression, or activity, of specific PPAR isoforms in the liver are therefore believed to represent critical mechanisms contributing to the development of hepatic metabolic diseases, disorders induced by hepatic viral infections, and hepatocellular adenoma and carcinoma. In this regard, specific PPAR agonists have proven to be useful to treat these metabolic diseases, but for cancer therapies, the use of PPAR agonists is still debated. Interestingly, in addition to previously described mechanisms regulating PPARs expression and activity, microRNAs are emerging as new important regulators of PPAR expression and activity in pathophysiological conditions and therefore may represent future therapeutic targets to treat hepatic metabolic disorders and cancers. Here, we reviewed the current knowledge about the general roles of the different PPAR isoforms in common chronic metabolic and infectious liver diseases, as well as in the development of hepatic cancers. Recent works highlighting the regulation of PPARs by microRNAs in both physiological and pathological situations with a focus on the liver are also discussed.

1. Introduction

Obesity, the metabolic syndrome (*MetS*), diabetes, hepatitis viruses (*HBV/HCV*), and abusive alcohol consumption are currently the principal etiological factors favoring the occurrence of hepatocellular adenoma and carcinoma worldwide [1–8]. With obesity, MetS, and diabetes, the liver can develop a wide spectrum of disorders, occurring in individuals in absence of excessive alcohol consumption, or HBV/HCV infections, and ranging from insulin resistance (*IR*), nonalcoholic fatty liver diseases (*NAFLD, including steatosis and insulin resistance*), to nonalcoholic steatohepatitis (*NASH, inflammation, and fibrosis associated with steatosis*), and to cirrhosis (*characterized by replacement of liver tissue by extracellular matrix and regenerative nodules*) [9, 10]. Hepatocellular adenoma (*HCA*) and carcinoma (*HCC*) can then occur as the ultimate stage of these diseases [11–13]. Since obesity and metabolic diseases have reached pandemic proportions worldwide, the incidence of IR/NAFLD/NASH/cirrhosis and HCA/HCC is expected to dramatically increase in the future, likely becoming the most common hepatic diseases worldwide [1, 6]. With hepatitis viruses (*HBV/HCV*) and abusive alcohol consumption, a very similar spectrum of histological abnormalities is observed in the liver (*ranging from IR to hepatic steatosis, steatohepatitis, fibrosis, and cirrhosis*) and also precedes HCA/HCC development. However, the molecular mechanisms triggering IR, steatosis, inflammation, fibrosis and cirrhosis are significantly different depending on the etiologies of these hepatic diseases.

Hepatic steatosis consists in the excessive accumulation of neutral lipids (*mainly triglycerides and cholesterol esters*) in cytoplasmic lipid droplets of the hepatocytes. This abnormal accumulation of cytoplasmic lipid droplets can result from (i) an excessive importation of free fatty acids (*FFAs*) released by adipocytes, or coming from the diet; (ii) from a diminished hepatic export of FFA (*altered synthesis or secretion of VLDL*); (iii) an increased *de novo* lipogenesis; (iv) an impaired β-oxidation of FFA [14, 15]. Steatosis and hepatic IR are closely associated but it is still poorly understood whether it is steatosis, which causes IR, or *vice*

versa. It is clear however that steatosis and IR usually precede inflammation, fibrosis, and cirrhosis of the liver. Recent evidence indicates that progression toward inflammation, fibrosis, and cirrhosis is likely due to the appearance of additional multiple dysregulated mechanisms, including the production of lipotoxic intermediates, oxidative stress (e.g., *through alterations of the mitochondrial activity or lipid peroxidation*), mitochondrial/peroxisomal abnormality, altered nuclear receptors signaling, deregulated cytokines signalling, gut microbial signalling, hepatocyte apoptosis, and leptin resistance, all of them contributing to various extent to the progression towards inflammation and fibrosis/cirrhosis [14–18]. These are thus multifactorial diseases, for which the precise orchestration of their development and progression still remains unclear. In addition, since not all patients with steatosis develop substantial liver injuries, this varying susceptibility of individuals to progress towards inflammation/fibrosis and cirrhosis further indicates that these disorders involve multifaceted processes also highly dependent on environmental factors and genetic predisposition [16–18].

Steatosis, inflammation, fibrosis, and cirrhosis can also be regarded as preneoplastic states of the liver, since HCA/HCC might occur as an end stage of these diseases [13]. HCC is a cancer with a very poor prognosis and its clinical management has been the object of intensive research efforts. Surgical resection of the tumour and liver transplantation are currently the only treatments with curative potential. However, only few patients are eligible for surgery and critical issues are associated with tumour resection and the efficiency of hepatic regeneration. Recent evidence indicates that physical inactivity and fat-enriched diets, both becoming major health emergencies in our western society, have a significant impact not only on HCC occurrence and progression, but also on the liver regeneration process. Based on these findings, a major challenge today is to understand how specific nutrients, in particular fats, and an abnormal hepatic lipid metabolism, affect major signalling pathways promoting carcinogenesis.

Peroxisome-proliferator-activated receptors (PPARs) are ligand-activated nuclear receptors that exert a transcriptional activity regulating energy homeostasis and other basic cellular processes in multiple metabolically active organs. These receptors are classically ligand activated, with the best characterized ligands being fatty acids and their derivatives. Three major isoforms of PPARs (*PPARα, PPARβ/δ, and PPARγ*) have been characterized to date [19]. Specific isoforms of these PPARs are expressed in most of the highly metabolically active tissues such as skeletal muscles, heart, adipose tissue, and liver. In the liver specifically, PPARs regulate a whole spectrum of physiological functions including cholesterol and bile acid homeostasis, lipid and glucose metabolism, inflammatory responses, regenerative mechanisms, cell differentiation, and cell cycle [20, 21]. Dysregulations of the expression, or activity, of specific PPAR isoforms are now also well accepted to represent critical mechanisms contributing to the development of a wide range of liver diseases. Indeed, PPARs have been recently implicated in the development of widely spread diseases affecting the liver such as IR, NAFLD/NASH, alcoholic liver diseases (*ALD*), HBV/HCV infection, and HCA/HCC.

2. Role of Specific PPAR Isoforms in Liver Diseases

2.1. PPARs in Hepatic Insulin Resistance and Steatosis. The role of specific PPARs isoforms in the occurrence and development of hepatic IR and steatosis is still controversial, as well as the potential benefits of specific PPARs agonists to treat these diseases [22]. It is however well accepted that PPARs are tightly implicated in processes regulating the accumulation and storage of triglycerides, lipid oxidation, and insulin sensitivity of hepatocytes [23].

2.1.1. PPARγ. PPARγ is generally increased in steatotic livers of both animal models of obesity and human obese patients [24, 25]. *In vivo* studies demonstrated that hepatocytes- and macrophage-specific PPARγ knockout mice were protected against high-fat (*HF*) diet-induced steatosis development [26]. As well, knockdown of PPARγ using *in vivo* injection of adenoviruses could also improve fatty liver and inflammation in mice fed a high saturated fat diet [27]. However, *in vitro* or *in vivo* studies with PPARγ agonists have led to contradictory results regarding steatosis and IR development. For example, thiazolidinediones induce steatosis in human primary hepatocytes and hepatoma cells [28], but reduce *in vivo* hepatic steatosis potentially through upregulation of adiponectin production and adiponectin receptors expression in the liver and adipose tissue [29–32]. On the other hand, SKLB102 (*a barbituric acid-based derivative PPARγ agonist*) was shown to activate lipogenesis and to exacerbate steatosis in mice fed a high-fat/high-calorie diet, although this agonist appears to improve the general outcome of NAFLD/NASH, likely by stimulating insulin sensitivity in both mice or hepatoma cells [30]. In human several clinical studies have reported that thiazolidinediones (rosiglitazone and pioglitazone) lead to a decreased hepatic steatosis and a regression of lobular inflammation [33–35]. It is worthy to note that in human, genetic variants of PPARγ (*C161T, Pro12Ala*) or dominant-negative mutations are specifically associated with NAFLD and progression of NAFLD towards inflammation and fibrosis [36–41].

2.1.2. PPARα. As opposed to PPARγ, the role of PPARα to prevent hepatic steatosis, likely by favoring fatty acid oxidation, is supported by a number of studies. Indeed, PPARα expression is decreased in the liver of rodents with NAFLD [42] and PPARα knockout mice display an increased steatosis, oxidative stress, and inflammation when fed an HF Western diet [43, 44]. Fenofibrates, a class of PPARα agonists, were also shown to improve hepatic steatosis in a mouse model of hereditary fatty liver in absence of obesity or diabetes [45] and in OLETF rats, which spontaneously develop NAFLD [32]. Other PPARα agonists (e.g., *Wy 14 643*) administered to mice fed an HF diet failed to prevent liver inflammation, but could improve other metabolic parameters such as hyperglycemia and hepatic steatosis [46].

Interestingly, the protective effects of fish oil (*n-3 polyunsaturated fatty acids*) dietary supplementation in mice fed a choline/methionine-deficient diet, or of aldose reductase inhibition in the diabetic db/db mouse model, on steatosis development were strongly correlated with a significant increase in the expression of PPARα [47, 48]. Finally, as for PPARγ, a human genetic variant of PPARα (*Val227Ala*) was specifically associated with NAFLD [49]. Although PPARα agonists are used in clinic to treat mixed dyslipidemia and hypertriglyceridemia, few studies have investigated the outcomes of these treatments for NAFLD. Fenofibrate in conjunction with dietary guidelines led to an improved steatosis and liver enzymes in 42% of patients included in the study ($n = 62$) [50]. On the contrary, in another pilot study by Fernandez-Miranda et al., administration of fenofibrate could partially improve liver enzyme profiles and hepatocellular ballooning in patients with biopsies-confirmed NAFLD, but not steatosis, inflammation, and fibrosis [51]. Therefore, from the current available data, the therapeutic potential of PPARα agonists to treat IR and NAFLD in human remains to be evaluated.

2.1.3. PPARβ/δ. Recent evidence indicates that PPARβ/δ can exert a protecting activity against IR. *In vitro* studies showed that PPARβ/δ expression in HepG2 hepatoma cells could decrease steatosis and IR induced by oleic acid likely by up-regulating PTEN expression and activity [52]. Consistent with these data, PPARβ/δ knockout mice develop glucose intolerance in part by decreasing the hepatic carbohydrate catabolism [53], but PPARβ/δ-deficient mice seem to have no defect in liver triglyceride and glycogen accumulation [54]. Whether PPARβ/δ positively or negatively regulates lipid metabolism and steatosis development remains to date still controversial. Indeed, expression of PPARβ/δ in the liver of mice through adenoviral infection led to either amelioration of hepatic steatosis in obese db/db mice [55] or exacerbated accumulation of lipids in hepatocytes from HF-fed mice [56]. Several other studies with specific agonists of PPARβ/δ reported also a beneficial effect of this nuclear receptor activation on hepatic IR and steatosis development. For example, the PPARβ/δ agonist GW501516 could prevent cytokine-induced IR in human hepatoma cells and in mice liver cells [57] or could improve IR in db/db mice by suppressing hepatic glucose output [53]. GW501516 also alleviated liver steatosis by increasing fatty acid oxidation in mice fed an HF diet [58] or in mice fed a methionine/choline-deficient diet [59]. Another agonist, NNC61-5920, was able to attenuate hepatic IR and to improve the expression profile of genes involved in the lipid metabolism both in mice and rats fed an HF diet. However, whereas systemic insulin sensitivity was improved in mice fed a HF diet, rats under the same conditions displayed systemic IR, suggesting that PPARβ/δ agonists induced specie-specific metabolic effects [60]. Pharmacological activation of PPARβ/δ by L-165041 in mice rendered obese and diabetic by HF feeding could also decrease IR and hepatic steatosis [61], whereas steatosis and inflammation were improved in LDLR$-/-$ mice fed a Western diet and treated with L-165041 [62]. Clinical trials with PPARβ/δ agonists have recently provided also encouraging perspectives to treat NAFLD. Indeed, healthy volunteers treated with GW501516 displayed reduced plasmatic triglycerides after two weeks [63], whereas, in overweighted subjects, GW501516 led to reduced plasmatic triglycerides and LDL levels and a decreased fat content in the liver [64]. Finally, MBX-8025, a novel PPARβ/δ agonist, decreases liver enzymes, hypercholesterolemia and hypertriglyceridemia in dyslipidemic overweighted patients [65].

2.2. PPARs in Liver Inflammation and Fibrosis. Evidence indicates that defects in signalling associated with all three isoforms of PPARs could further contribute to the progression of hepatic IR and steatosis towards more severe stages of liver diseases.

2.2.1. PPARγ. Several studies indicated that PPARγ deficiency in hepatic stellate cells is associated with a transdifferentiation and activation of these cells leading to excessive formation of fibrotic tissue in the liver [66–68]. Surprisingly however, a recent study by Yamazaki and colleagues concluded that downregulation of PPARγ2 in the liver through adenovirus injection improves inflammation and steatosis in mice fed a high-saturated-fat diet [27]. Available data with PPARγ agonists consistently suggest that activation of PPARγ signalling protects the liver against fibrosis and inflammation in mice and rats. Indeed administration of rosiglitazone to mice or rats fed a choline/methionine-deficient diet prevented fibrosis development [69, 70]. In another study, rosiglitazone was also reported to stimulate antioxidant gene expression, β-oxidation of fatty acids and to suppress inflammation and fibrosis in mouse models of NASH [31]. Finally, in human with NASH, pioglitazone significantly improved the biochemical and histological feature of NASH and discontinuation of the treatment led to a rebound of the diseases [71].

2.2.2. PPARα. Consistent with a protective role of PPARα for IR and steatosis development, PPARα-null mice fed a Western diet develop more steatosis, oxidative stress, and inflammation in the liver than wild-type mice [44]. Deletion of PPARα in apoE2-KI mice fed a Western diet also aggravates liver steatosis and inflammation development. However, agonists of PPARα administered to foz/foz mice (*rendered diabetic by feeding with a HF diet*) failed to resolve liver inflammation although other histological parameters, including steatosis, hepatocytes ballooning and neutrophils/macrophages recruitment, were improved [46]. Intriguingly, the beneficial effects of vitamin E on NASH in human were correlated with a decreased, and not an increased, expression of PPARα [72].

2.2.3. PPARβ/δ. PPARβ/δ-deficient mice appear to be more sensitive to chemical hepatotoxic agents. For example, PPARβ/δ knockout mice treated with CCL_4 developed more liver necrosis, displayed more elevated ALT enzymes, inflammation and profibrotic genes expression than control mice, therefore suggesting that PPARβ/δ protects the liver

from inflammation and fibrosis [73]. Consistent with this study, transcriptional genomic analysis with the liver of PPARβ/δ-deficient mice suggested that PPARβ/δ could have an anti-inflammatory action in the liver [54] and the PPARβ/δ agonist GW501516 was shown to improve hepatic inflammation in mice fed a methionine/choline-deficient diet (*a widely used model for NASH*) [59].

Interestingly, PPARβ/δ seems to be required in Kupffer cells to support the switch between the proinflammatory macrophage M1 to the anti-inflammatory macrophage M2 [74], whereas in stellate cells PPARβ/δ is highly up-regulated following activation [75]. It is thus likely that PPARβ/δ has distinct roles in parenchymal and nonparenchymal hepatic cells, which affect, in a still poorly understood manner, the paracrine dialog between these cells and the development of inflammation and fibrosis.

2.3. PPARs in Alcoholic Liver Diseases and Hepatitis Virus Infections. Although studies focusing on the role of PPARs in alcoholic liver diseases (ALD) and HBV/HCV infections might be sometimes scarce (e.g., *regarding the role of PPARβ/δ in these diseases*), evidence accumulates suggesting that an unbalanced expression/activation of distinct PPAR isoforms may also contribute to the wide spectrum of liver disorders induced either by excessive alcohol consumption or HBV/HCV.

2.3.1. PPARγ. In the liver of HCV-infected patients, PPARγ expression was found to be highly up-regulated and to contribute to the development of HCV-associated steatosis [25, 76, 77]. Two viral factors, the core protein and the NS5A protein, were suggested to mediate PPARγ upregulation either through transactivation of LXRα [76–78] or by inducing SOCS7 expression [79]. PPARγ is also increased in the liver of HBV-infected patients [80], likely through HBV X protein-dependent mechanisms [81, 82]. However, whether PPARγ confers or not a replicative advantage to HBV still remains a controversial issue [83–85].

2.3.2. PPARα. With excessive alcohol consumption, PPARα gene transcription is inhibited through still unknown mechanisms [86] and may contribute, through an increased oxidative stress and inflammatory response, to the wide spectrum of liver disorders observed with alcoholism [87]. PPARα was also reported to be down-regulated by HCV in the liver of infected patients [88, 89]. Since PPARα blocks the replication and production of infectious viral particles, its downregulation likely confers a replicative advantage to the virus in spite of the resulting metabolic disorders for the host cells [90, 91].

2.3.3. PPARβ/δ. Although virtually none is known about the potential role of PPARβ/δ in HBV/HCV infection, only one study has outlined the potential benefit of PPARβ/δ activation in liver injuries related to alcohol consumption. Indeed, pharmacological activation of PPARβ/δ by L-165041 in rats chronically fed with ethanol attenuated the severity of liver injury by diminishing oxidative stress, lipid peroxidation and by restoring hepatic insulin responsiveness [92].

2.4. PPARs in Liver Cancer. As previously mentioned, *HCA* and *HCC* represent the ultimate stages of liver diseases induced by obesity (*NAFLD/NASH*), alcohol consumption, or hepatitis viruses (*HBV/HCV*) [11–13]. It is therefore expected that PPARs signalling can either favor, or refrain, carcinogenic processes in the liver. However, data have revealed that PPARs signalling might have a different outcome on carcinogenesis as compared to other metabolic diseases of the liver.

2.4.1. PPARγ. Although the role of PPARγ in the development of liver metabolic diseases with different etiologies has led in some cases to controversial results (*see previous sections*), there is a general consensus about the fact that PPARγ activity can counteract the occurrence and progression of cancer in the liver. In *in vitro* studies, PPARγ overexpression induced apoptosis in HCC cell lines [93]. On the other hand, PPARγ agonists administration to HCC cell lines promoted cell cycle arrest, apoptosis, and anoikis [94, 95], sensitized the cells to 5-fluorouracil antitumoural activity [96], and inhibited migration of these cells [97]. Interestingly, in addition to prevent HBV replication *in vitro* [85], PPARγ agonists triggered also antineoplastic effects on HBV-associated HCC cells [98]. *In vivo* studies corroborated data obtained *in vitro*. Indeed, PPARγ-null mice display a higher susceptibility to the development of HCC induced by the carcinogen diethylnitrosamine (*DEN*). The administration PPARγ agonists (*rosiglitazone*) also reduced HCC development induced by DEN in rats or by hepatoma cell xenograft in mice [99–101]. Together, these studies indicate that PPARγ may act as a tumour suppressor and that specific agonists could be used in cancer prevention.

2.4.2. PPARα. As opposed to PPARγ, activation of PPARα in the liver leads to carcinogenesis in rodents [102–104]. Indeed, chronic administration of the PPARα agonists Wy-14, 643 or bezafibrate in mice induced HCC with time, an effect that was not observed in PPARα-null mice [102, 105, 106]. Interestingly, the PPARα carcinogenic effect on the liver seems to be age dependent in rodent, suggesting that with ageing the liver become more sensitive to the carcinogenic affects of PPARα transcriptional activity [107]. An aberrant expression of PPARα also contributes to the hepatocarcinogenic events induced by the solvent trichloroethylene (*TCE*) in rodents [108] and PPARα activation was necessary to induce steatosis and HCC in a model of transgenic mice expressing the HCV core protein [109, 110]. Surprisingly, the carcinogenic effects of PPARα agonists were not confirmed in human patients, suggesting species differences in PPARα signalling [103]. Consistent with this study, mice expressing a human PPARα were less susceptible to develop cancer with agonist administration [111, 112]. These differential susceptibilities to PPARα-induced HCC in rodent versus humans could be related to the inhibition by PPARα,

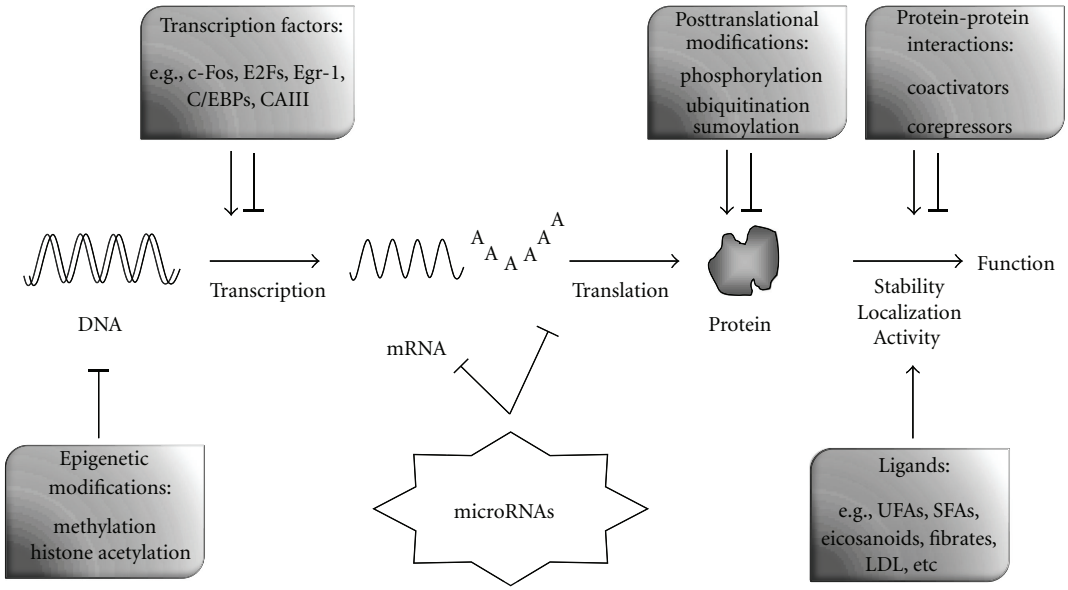

Figure 1: *Regulation of PPARs expression and activity.* The scheme presents distinct mechanisms reported to regulate the expression and activity of specific PPARs isoforms. Epigenetic mechanisms, including DNA methylation and histone acetylation, may restrict the PPARs promoter activity and several transcription factors have been described to modulate either positively or negatively the transcription of distinct PPARs. As well, posttranslational modifications at the protein level (*phosphorylation, ubiquitination, and sumoylation*) and interactions with coactivators (e.g., *CBP/p300, SRC-1, PGC1α*) or corepressors (e.g., *RIP-140α, SMRT α*) regulate the transcriptional activity, localization, and stability of PPARs isoforms. miRNAs represent a new recently described class of regulators of PPARs transcriptional activity by exerting a control on PPARs mRNA degradation and translation.

in rodents but not in human, of the expression of the let-7c miRNA, which targets the Myc oncogene [102].

2.4.3. PPARβ/δ. The role of PPARβ/δ in cancer is currently the subject of intense investigations that have led to controversial hypothesis. Indeed, different studies support a pro-carcinogenic role for PPARβ/δ as a differentiation and anti-inflammatory factor [104]. Regarding liver carcinogenesis, only few studies have provided insights into the role of PPARβ/δ in this process. PPARβ/δ agonists, such as GW501516, were reported to significantly enhance the growth of various hepatoma cells, whereas inhibition of PPARβ/δ expression by siRNAs had the opposite effect [113]. PPARβ/δ transcriptional activity was also involved in the IL-6-induced proliferation of cultured chicken hepatocytes [114]. It thus remains to investigate more in detail how PPARβ/δ activity impacts on liver cancer.

3. MicroRNAs

In addition to PPARs activation triggered by fatty acids and derivatives specific ligands, the transcriptional activity of the various PPARs isoforms in physiological and pathological situations is regulated by other numerous and complex epigenetic, transcriptional, and posttranscriptional mechanisms (Figure 1). These include epigenetic inhibitory mechanisms (e.g., *methylation of the PPARs promoter and histone acetylation*) [115, 116], positive and negative regulation of PPARs mRNA transcription by various transcription factors [117–120], posttranslational modifications of the proteins, interaction with other cellular factors, which may affect their transcriptional activities, intracellular localization, and stability of the PPARs proteins [19, 121–125]. Interestingly, a further level of complexity in these regulatory mechanisms has recently emerged and involves an epigenetic regulation of PPAR isoforms by microRNAs, which can either degrade or repress PPAR mRNAs at the translational level.

miRNAs are small noncoding RNAs of about 20 nucleotides that bind to conserved 3′UTR sequences of their target mRNA, therefore inducing either inhibition of their translation or their degradation [126]. First described in *C. elegans* [127], 1921 miRNAs have been discovered and registered to date (*according to miRBase microRNA database,* http://www.mirbase.org/) and more than 30% of proteins-coding genes are supposed to be targeted by miRNAs [128]. Most of the miRNAs are intergenics and can be expressed independently, but a few of them are also located in introns of known protein-coding genes and are co-transcribed with these genes [129]. The biogenesis of miRNAs is a well-conserved mechanism. The RNA polymerase II transcribes most of microRNAs as a long primary transcript enclosing a stem-loop structure [130]. This process can be regulated by several transcription factors that bind directly to the miRNA promoter elements and control their expression [131]. miRNAs maturation occurs in the nucleus where the RNase III

Drosha and other cofactors cleave the pri-miRNA bearing the hairpin structure to generate a pre-miRNA product [132]. The precursor pre-miRNA of about 60–70 nucleotides is then exported from the nucleus to the cytoplasm by the exportin-5 [133, 134], a process that is regulated by the Drosha activity [135, 136]. In the cytoplasm, the RNase III Dicer cleaves the miRNA stem loop to generate a mature miRNA of 20–22 nucleotides long [137]. Mature miRNA targeting a mRNA finally necessitates the RNA-induced silencing complex RISC, in which the Argonaute protein (*Ago2 for mammals*) is the catalytic center [138, 139]. Of note, Drosha, Dicer, and Ago2 are components of miRNAs processing that are essential for life since specific knockout for one of these proteins in mice induces severe developmental defects or death [140].

Finding the targets of specific miRNA currently remains a challenge. Bioinformatic predictions and proteomic analyses are performed to estimate potential targets for a given miRNA [141]. However, there is a great redundancy in miRNAs capable to target a specific mRNA and each miRNA can also target hundreds of different mRNAs [142, 143] thereby increasing tremendously the complexity of this type of global analyses. In addition, miRNAs usually induces only a maximum of twofold downregulation of their target protein, thereby exerting only a fine-tuning control of protein expression. Despite these difficulties to investigate the physiological and pathological potential roles of miRNAs, accumulating evidence indicates that miRNAs play an important role in multiple cellular processes, as well as in the development of several diseases, including the MetS, diabetes, neurodegeneration, cardiovascular and immune diseases [144–148]. Numerous studies also recently outlined miRNAs as critical regulatory factors promoting or inhibiting cancer development [149, 150]. More particularly in the liver, several miRNAs (*including miR-21, miR-29, miR-122, miR-132, miR-155, miR-192, and miR-322*) were reported to exert a control on hepatocyte differentiation, glucose and lipid metabolism, fatty liver diseases, fibrosis, and HBV/HCV infection [151–160]. In liver cancer, for example, HCC, "omics" analyses have revealed that numerous miRNAs are dysregulated. Importantly, while miR-122 is down-regulated in HCC thereby potentially impairing cell differentiation, many others are up-regulated, including miR-96, miR-221, miR-224, and miR-21, which have all been reported to regulate cell proliferation and apoptosis [161, 162].

4. MicroRNA-Dependent PPARs Regulation

The epigenetic regulation of PPARs expression and activity by miRNAs is a new field of research still in its infancy. However, accumulating evidence now suggests that alterations of the expression/activity of PPARs isoforms by distinct miRNAs could represent critical molecular mechanisms involved in the physiopathology of each organ undergoing a PPARs-dependent control. In this regard, bioinformatics predictions of miRNAs potentially targeting the different PPARs isoforms reveal that hundreds of different miRNAs could participate to the regulation of PPARs expression/activity in different cells/tissues (see Table 1). Currently, most of the studies investigating the role of miRNAs in the regulation of distinct PPARs isoforms have been performed in cultured adipocytes or in the adipose tissue. But recent works have also highlighted miRNAs-dependent regulation of PPARs in the liver and have implicated these regulatory processes in the development of hepatic diseases.

MicroRNA-Dependent PPARs Regulation in the Liver. At least 5 distinct miRNAs have been reported to directly, or indirectly, affect PPAR expression in liver cells, mostly in pathological situations, in particular NAFLD (see Table 2). For example, Zheng and coworkers performed miRNA microarrays with human hepatocyte cell line L02 exposed or not to high concentration of fatty acids. They could find more than 30 miRNA either up- or down-regulated in cells challenged with excess of fatty acids. Among them, miR-10b was up-regulated. Further analyses revealed that miR-10b induces steatosis in these cells by directly targeting PPARα and preventing its expression [163]. PPARα was also reported to be specifically regulated by two other miRNAs, miR-21 and miR-27b, in the liver. Indeed, expression of precursors or antisense nucleotides for miR-21, or miR-27b, in Huh-7 hepatoma cells could significantly modulate the expression of PPARα protein, but not its mRNA, suggesting a blockade of PPAR mRNA translation by miR-21/-27b. Interestingly, no correlations were found between PPARα protein and mRNA expression in human livers ($n = 24$). However, in the same human samples, PPARα protein expression was inversely correlated with miR-21 expression [164]. A potential role for miR-21-dependent regulation of PPARs was further supported by our own observations indicating that PPARα protein expression is up-regulated in primary hepatocytes isolated from miR-21 knockout mice, whereas PPARγ is down-regulated in the liver of mice overexpressing miR-21 (*our unpublished data*). miR-122, the most highly expressed miRNA in the liver, was also reported to specifically target PPARβ/δ in the liver of mice. Interestingly miR-122 expression appears to be circadian thereby providing an interesting link between miR-122, PPARβ/δ, and the well-known circadian metabolic control occurring in the liver [165]. Finally, PPARγ activity appears also to be under the indirect control of miR-132. Indeed, Mann and coworkers could demonstrate that, in stellate cells of mice treated with CCl4, downregulation of the miR-132 promoted the expression of MeCP2 (*a target of miR-132*), which in turn binds to PPARγ and promotes the formation of an epigenetic repressor complex inhibiting PPARγ expression and therefore promoting liver fibrosis in this mice model [116].

MicroRNA-Dependent PPARs Regulation in Other Tissues. Numerous other reports have implicated an miRNA-dependent regulation of distinct PPARs isoforms in non-hepatic tissues that affects various cellular processes including lipid metabolism, inflammation, cell differentiation, carcinogenesis, or wound skin repair.

In the adipose tissue, two studies have unveiled a physiopathologically relevant regulation of PPARγ expression by the miR-27a/b. Indeed, Karbiener et al. reported a significant downregulation of miR-27b, which directly targets

Table 1: miRNAs potentially targeting PPARγ, PPARα and PPARβ/δ. Four different databases, that is, miRanda (http://www.microRNA.org/), PicTar (http://www.pictar.org/), TargetScanHuman (http://www.targetscan.org/) and MicroCosm Targets (http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets) were scanned to find all predicted miRNAs that possibly regulate PPARγ, PPARα and PPARβ/δ expression by binding to specific seed sequences in the transcribed 3′UTR regions of different PPARs isoforms.

(a)

PPARα				
miRanda		TargetScan	MicroCosm targets	PicTar
miR-25	miR-7	miR-200	miR-892	miR-19
miR-28	miR-9	miR-203		
miR-32	miR-10	miR-204		
miR-92	miR-17	miR-211		
miR-145	miR-18	miR-214		
miR-155	miR-19	miR-219		
miR-181	miR-20	miR-223		
miR-200	miR-21	miR-291		
miR-216	miR-22	miR-294		
miR-217	miR-23	miR-295		
miR-218	miR-24	miR-302		
miR-224	miR-27	miR-338		
miR-342	miR-34	miR-351		
miR-363	miR-93	miR-372		
miR-367	miR-101	miR-373		
miR-374	miR-105	miR-427		
miR-376	miR-106	miR-428		
miR-421	miR-124	miR-429		
miR-425	miR-125	miR-449		
miR-429	miR-128	miR-506		
miR-431	miR-138	miR-508		
miR-491	miR-141	miR-518		
miR-543	miR-142	miR-519		
miR-590	miR-144	miR-520		
miR-615	miR-150	miR-548		
miR-708	miR-181	miR-590		
miR-873	miR-182	miR-670		
		miR-761		
		miR-1378		
		miR-1420		
		miR-3619		
		miR-4262		
		miR-4319		
		miR-4735		
		miR-4782		
		miR-5127		

(b)

PPARγ				
miRanda		Target scan	MicroCosm targets	Pictar
miR-1	miR-328	miR-27	miR-27	miR-27
miR-9	miR-329	miR-128	miR-30	miR-128
miR-18	miR-338	miR-130	miR-33	miR-130
miR-24	miR-340	miR-301	miR-34	miR-301
miR-25	miR-361	miR-454	miR-128	
miR-26	miR-362	miR-721	miR-130	
miR-27	miR-363	miR-3666	miR-142	

(b) Continued.

PPARγ				
miRanda		Target scan	MicroCosm targets	Pictar
miR-32	miR-367	miR-4295	miR-144	
miR-33	miR-370		miR-193	
miR-34	miR-371		miR-301	
miR-92	miR-376		miR-338	
miR-96	miR-411		miR-409	
miR-101	miR-421		miR-431	
miR-122	miR-431		miR-449	
miR-128	miR-448		miR-454	
miR-130	miR-449		miR-513	
miR-133	miR-454		miR-520	
miR-137	miR-485		miR-526	
miR-142	miR-488		miR-545	
miR-144	miR-490		miR-548	
miR-145	miR-505		miR-559	
miR-148	miR-590		miR-574	
miR-150	miR-613			
miR-152	miR-653			
miR-153	miR-758			
miR-181	miR-1271			
miR-182	miR-1297			
miR-185				
miR-194				
miR-199				
miR-204				
miR-206				
miR-211				
miR-224				
miR-301				
miR-324				

(c)

PPARβ/δ			
miRanda	Target scan	MicroCosm targets	Pictar
miR-19	miR-9	miR-17	none
miR-24	miR-17	miR-20	
miR-129	miR-20	miR-24	
miR-133	miR-29	miR-29	
miR-138	miR-34	miR-33	
miR-140	miR-93	miR-93	
miR-149	miR-106	miR-106	
miR-185	miR-129	miR-136	
miR-196	miR-138	miR-138	
miR-218	miR-148	miR-143	
miR-223	miR-150	miR-149	
miR-326	miR-214	miR-219	
miR-330	miR-223	miR-220	
miR-342	miR-427	miR-222	
miR-487	miR-449	miR-302	
miR-590	miR-518	miR-373	
miR-599	miR-519	miR-378	
miR-653	miR-761	miR-483	

(c) Continued.

PPARβ/δ			
miRanda	Target scan	MicroCosm targets	Pictar
miR-874	miR-3619	miR-492	
	miR-5127	miR-512	
		miR-513	
		miR-519	
		miR-520	
		miR-542	
		miR-544	
		miR-550	
		miR-564	
		miR-576	
		miR-631	
		miR-640	
		miR-657	
		miR-874	
		miR-885	
		miR-921	

Table 2: miRNAs reported to regulate PPARγ, PPARα, and PPARβ/δ expression and activity in the liver.

microRNA	Implication	PPAR targeted	Interaction	Reference
miR-10b	Steatosis	PPARα	Direct	[163]
miR-21	Steatosis	PPARα	Direct	[164]
miR-122	Cholesterol and lipid metabolism	PPARβ/δ	Direct	[165]
miR-27b	Steatosis	PPARα	Indirect	[164]
miR-132	Fibrosis	PPARγ	Indirect	[116]

PPARγ, during adipogenesis of human multipotent adipose-derived stem (*hMADS*) cells. They further demonstrated that inhibiting induction of PPARγ in this process by overexpressing miR-27b represses adipogenic marker gene expression and triglyceride accumulation in hMADS [166]. In a second study, levels of miR-27a were inversely correlated with those of PPARγ in mature adipocyte and miR-27a expression in the adipose tissue was down-regulated in obese mice as compared to lean mice. *In vitro* studies with 3T3-L1 preadipocytes further showed that miR-27a ectopic expression prevents adipocyte differentiation by inhibiting PPARγ through a direct binding to its 3′-UTR sequence [167]. Finally, differentiation of human preadipocytes into adipocytes was also shown to be highly dependent of miR-130 expression. Indeed, miR-130 was shown to directly target PPARγ and thereby to enhance adipogenesis. Consistent with this, expression of miR-130 in the adipose tissue of obese women was lower compared to lean women [168]. Together, these studies suggest that induction of PPARγ by alterations in miR-27a/b and/or miR-130 expressions might be linked to the development of obesity in rodents and humans. miRNAs microarray analyses of the subcutaneous adipose tissue in nondiabetic but severely obese adults also identified miR-519d as an overexpressed miRNAs that targets PPARα. The miR-519d-dependent decrease in PPARα translation was further shown to increase lipid accumulation during pre-adipocyte differentiation suggesting that PPARα loss through miR-519d overexpression importantly contributed to adipocytes hypertrophy observed with obesity [169].

Finally, recent evidence also indicates that alterations of PPARγ and PPARα expression by specific miRNAs occur in other tissues and can contribute to the development of specific disorders. For example, downregulation of PPARγ by an aberrant expression of miR-27b in cardiomyocytes was associated with cardiac hypertrophy in mice [170], whereas miR-27b-dependent PPARγ downregulation in macrophages favors the inflammatory response to LPS [171]. In human osteoarthritic chondrocytes, aberrant expression of miR-22 specifically down-regulates PPARα expression, which in turn promotes IL1-dependent inflammatory processes [172]. Then in endothelial cells of cultured human umbilical vein under oscillatory shear stress, levels of miR-21 were found to be increased and to induce an inhibition of PPARα translation, which favor monocytes adhesion and atherosclerosis formation [173]. Finally, PPARα was reported to be also a target of miR-506, which is increased in human colon cancers and contribute to chemotherapy resistance by down-regulating PPARα expression [174].

5. Conclusion

Although the therapeutic potential of PPARβ/δ for metabolic diseases such as insulin resistance, dyslipidemia, and other associated liver pathologies deserves further investigations,

agonists of PPARγ and PPARα have been developed as relevant drugs to treat these disorders. For cancer therapies, the use of PPAR agonists is more debated, in particular for PPARα, which induces liver and bladder cancers in mice, but apparently not in humans. However, the chronic administration of PPAR agonists in human for the treatment of metabolic diseases may importantly increase the risk of developing cardiovascular diseases (e.g., *for PPARγ agonists*) and specific cancers (e.g., *for PPARα agonists*). For these reasons, recommendations have been issued for a restricted and invigilated clinical use of PPAR agonists and some of them (e.g., *PPARγ agonists*) have even been withdrawn from the market in some countries. An attractive therapeutic alternative to systemic administration of PPAR agonists to treat metabolic diseases and/or cancers would be to exert more fine tuning of specific PPAR isoforms expressions/activities in the organ of interest, thereby minimizing the deleterious side effects of chronic systemic administration of these agonists. In this regard, miRNAs represent an interesting class of molecules that could be pharmacologically modulated, for example, with antagomirs, to prevent pathological alterations of PPARs expressions and activities. There might be several advantages associated with this type of therapeutic approaches. First, the aberrant expression of specific miRNAs, for example, inhibiting the expression of PPARs in diseases, is often tissue specific. Therefore, the administration of miRNA-targeting drugs should affect principally the injured organs and secondary systemic effects could be minimized as compared to systemic administration of PPAR agonists. Secondly, as mentioned previously, miRNAs can only modestly modulate (*more or less two fold*) the expression of their target mRNAs. However, preventing this pathological miRNA-mediated dysregulations of PPARs expression should help to recover a physiological PPAR transcriptional activity in contrast to the ectopic overactivation of PPARs induced by administration of agonists. Finally, based on bioinformatics predictions (see Table 1), there is a high number of miRNAs predicted to be able to affect the expression of PPARs in pathological situations. It is likely that many other miRNAs would be identified in the future as important PPARs regulators in metabolic diseases and cancer, thereby multiplying the miRNA-based therapeutic targets available to treat these diseases. Based on these considerations, additional studies are now required to further assess the pertinence of miRNA-based therapies to enhance specifically the activity of PPAR isoforms as therapeutic weapons in metabolic diseases and cancer.

Abbreviations

ALD: Alcoholic liver diseases
FFAs: Free fatty acids
IR: Insulin resistance
HCA: Hepatocellular adenoma
HBV: Hepatitis virus B
HCV: Hepatitis virus C
HCC: Hepatocellular carcinoma
MetS: Metabolic syndrome
PPARs: Peroxisome-proliferator-activated receptors
NAFLD: Nonalcoholic fatty liver disease
NASH: Nonalcoholic steatohepatitis
miRNA: MicroRNA.

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References

[1] R. Williams, "Global challenges in liver disease," *Hepatology*, vol. 44, no. 3, pp. 521–526, 2006.

[2] W. W. Yip and A. D. Burt, "Alcoholic liver disease," *Seminars in Diagnostic Pathology*, vol. 23, no. 3-4, pp. 149–160, 2006.

[3] G. Marchesini and M. Babini, "Nonalcoholic fatty liver disease and the metabolic syndrome," *Minerva Cardioangiologica*, vol. 54, no. 2, pp. 229–239, 2006.

[4] H. B. El-Serag, "Epidemiology of viral hepatitis and hepatocellular carcinoma," *Gastroenterology*, vol. 142, no. 6, pp. 1264.e1–1273.e1, 2012.

[5] A. M. Shire and L. R. Roberts, "Prevention of hepatocellular carcinoma: progress and challenges," *Minerva Gastroenterologica e Dietologica*, vol. 58, no. 1, pp. 49–64, 2012.

[6] K. A. McGlynn and W. T. London, "The global epidemiology of hepatocellular carcinoma: present and future," *Clinics in Liver Disease*, vol. 15, no. 2, pp. 223–243, 2011.

[7] C. Wang, X. Wang, G. Gong et al., "Increased risk of hepatocellular carcinoma in patients with diabetes mellitus: a systematic review and meta-analysis of cohort studies," *International Journal of Cancer*, vol. 130, no. 7, pp. 1639–1648, 2012.

[8] H. Cornellà, C. Alsinet, and A. Villanueva, "Molecular pathogenesis of hepatocellular carcinoma," *Alcoholism*, vol. 35, no. 5, pp. 821–825, 2011.

[9] M. J. Contos, J. Choudhury, A. S. Mills, and A. J. Sanyal, "The histologic spectrum of nonalcoholic fatty liver disease," *Clinics in Liver Disease*, vol. 8, no. 3, pp. 481–500, 2004.

[10] L. A. Adams and P. Angulo, "Recent concepts in non-alcoholic fatty liver disease," *Diabetic Medicine*, vol. 22, no. 9, pp. 1129–1133, 2005.

[11] S. H. Caldwell, D. M. Crespo, H. S. Kang, and A. M. S. Al-Osaimi, "Obesity and hepatocellular carcinoma," *Gastroenterology*, vol. 127, supplement 1, no. 5, pp. S97–S103, 2004.

[12] H. B. El-Serag, H. Hampel, and F. Javadi, "The association between diabetes and hepatocellular carcinoma: a systematic review of epidemiologic evidence," *Clinical Gastroenterology and Hepatology*, vol. 4, no. 3, pp. 369–380, 2006.

[13] P. A. Farazi and R. A. DePinho, "Hepatocellular carcinoma pathogenesis: from genes to environment," *Nature Reviews Cancer*, vol. 6, no. 9, pp. 674–687, 2006.

[14] E. Vanni, E. Bugianesi, A. Kotronen, S. De Minicis, H. Yki-Järvinen, and G. Svegliati-Baroni, "From the metabolic syndrome to NAFLD or vice versa?" *Digestive and Liver Disease*, vol. 42, no. 5, pp. 320–330, 2010.

[15] C. Z. Larter, S. Chitturi, D. Heydet, and G. C. Farrell, "A fresh look at NASH pathogenesis. Part 1: the metabolic movers,"

Journal of Gastroenterology and Hepatology, vol. 25, no. 4, pp. 672–690, 2010.

[16] M. Malaguarnera, M. Di Rosa, F. Nicoletti, and L. Malaguarnera, "Molecular mechanisms involved in NAFLD progression," *Journal of Molecular Medicine*, vol. 87, no. 7, pp. 679–695, 2009.

[17] J. R. Lewis and S. R. Mohanty, "Nonalcoholic fatty liver disease: a review and update," *Digestive Diseases and Sciences*, vol. 55, no. 3, pp. 560–578, 2010.

[18] K. Rombouts and F. Marra, "Molecular mechanisms of hepatic fibrosis in non-alcoholic steatohepatitis," *Digestive Diseases*, vol. 28, no. 1, pp. 229–235, 2010.

[19] L. Michalik and W. Wahli, "PPARs mediate lipid signaling in inflammation and cancer," *PPAR Research*, vol. 23, no. 7, Article ID 134059, pp. 351–363, 2008.

[20] G. Li and G. L. Guo, "Role of class II nuclear receptors in liver carcinogenesis," *Anti-Cancer Agents in Medicinal Chemistry*, vol. 11, no. 6, pp. 529–542, 2011.

[21] S. Dharancy, A. Louvet, A. Hollebecque, P. Desreumaux, P. Mathurin, and L. Dubuquoy, "Nuclear receptor PPAR and hepatology: pathophysiological and therapeutical aspects," *Gastroenterologie Clinique et Biologique*, vol. 32, no. 3, pp. 339–350, 2008.

[22] A. Tailleux, K. Wouters, and B. Staels, "Roles of PPARs in NAFLD: potential therapeutic targets," *Biochimica et Biophysica Acta*, vol. 1821, no. 5, pp. 809–818, 2012.

[23] E. R. Kallwitz, A. McLachlan, and S. J. Cotler, "Role of peroxisome proliferators-activated receptors in the pathogenesis and treatment of nonalcoholic fatty liver disease," *World Journal of Gastroenterology*, vol. 14, no. 1, pp. 22–28, 2008.

[24] P. Pettinelli and L. A. Videla, "Up-regulation of PPAR-γ mRNA expression in the liver of obese patients: an additional reinforcing lipogenic mechanism to SREBP-1c induction," *Journal of Clinical Endocrinology and Metabolism*, vol. 96, no. 5, pp. 1424–1430, 2011.

[25] E. Lima-Cabello, M. V. García-Mediavilla, M. E. Miquilena-Colina et al., "Enhanced expression of pro-inflammatory mediators and liver X-receptor-regulated lipogenic genes in non-alcoholic fatty liver disease and hepatitis C," *Clinical Science*, vol. 120, no. 6, pp. 239–250, 2011.

[26] E. Morán-Salvador, M. López-Parra, V. García-Alonso et al., "Role for PPARγ in obesity-induced hepatic steatosis as determined by hepatocyte- and macrophage-specific conditional knockouts," *The FASEB Journal*, vol. 25, no. 8, pp. 2538–2550, 2011.

[27] T. Yamazaki, S. Shiraishi, K. Kishimoto, S. Miura, and O. Ezaki, "An increase in liver PPARγ2 is an initial event to induce fatty liver in response to a diet high in butter: PPARγ2 knockdown improves fatty liver induced by high-saturated fat," *Journal of Nutritional Biochemistry*, vol. 22, no. 6, pp. 543–553, 2011.

[28] M. Moya, M. José Gómez-Lechón, J. V. Castell, and R. Jover, "Enhanced steatosis by nuclear receptor ligands: a study in cultured human hepatocytes and hepatoma cells with a characterized nuclear receptor expression profile," *Chemico-Biological Interactions*, vol. 184, no. 3, pp. 376–387, 2010.

[29] S. Liu, H. J. Wu, Z. Q. Zhang et al., "The ameliorating effect of rosiglitazone on experimental nonalcoholic steatohepatitis is associated with regulating adiponectin receptor expression in rats," *European Journal of Pharmacology*, vol. 650, no. 1, pp. 384–389, 2011.

[30] H. Zheng, S. Li, L. Ma et al., "A novel agonist of PPAR-γ based on barbituric acid alleviates the development of non-alcoholic fatty liver disease by regulating adipocytokine expression and preventing insulin resistance," *European Journal of Pharmacology*, vol. 659, no. 2-3, pp. 244–251, 2011.

[31] A. A. Gupte, J. Z. Liu, Y. Ren et al., "Rosiglitazone attenuates age- and diet-associated nonalcoholic steatohepatitis in male low-density lipoprotein receptor knockout mice," *Hepatology*, vol. 52, no. 6, pp. 2001–2011, 2010.

[32] Y. S. Seo, J. H. Kim, N. Y. Jo et al., "PPAR agonists treatment is effective in a nonalcoholic fatty liver disease animal model by modulating fatty-acid metabolic enzymes," *Journal of Gastroenterology and Hepatology*, vol. 23, no. 1, pp. 102–109, 2008.

[33] G. Lutchman, K. Promrat, D. E. Kleiner et al., "Changes in serum adipokine levels during pioglitazone treatment for nonalcoholic steatohepatitis: relationship to histological improvement," *Clinical Gastroenterology and Hepatology*, vol. 4, no. 8, pp. 1048–1052, 2006.

[34] V. Ratziu, P. Giral, S. Jacqueminet et al., "Rosiglitazone for nonalcoholic steatohepatitis: one-year results of the randomized placebo-controlled Fatty Liver Improvement with Rosiglitazone Therapy (FLIRT) Trial," *Gastroenterology*, vol. 135, no. 1, pp. 100–110, 2008.

[35] A. J. Sanyal, N. Chalasani, K. V. Kowdley et al., "Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis," *The New England Journal of Medicine*, vol. 362, no. 18, pp. 1675–1685, 2010.

[36] D. B. Savage, G. D. Tan, C. L. Acerini et al., "Human metabolic syndrome resulting from dominant-negative mutations in the nuclear receptor peroxisome proliferator-activated receptor-γ," *Diabetes*, vol. 52, no. 4, pp. 910–917, 2003.

[37] Y. Hui, L. Yu-yuan, N. Yu-qiang et al., "Effect of peroxisome proliferator-activated receptors-γ and co-activator-1α genetic polymorphisms on plasma adiponectin levels and susceptibility of non-alcoholic fatty liver disease in Chinese people," *Liver International*, vol. 28, no. 3, pp. 385–392, 2008.

[38] J. W. Rey, A. Noetel, A. Hardt et al., "Pro12Ala polymorphism of the peroxisome proliferatoractivated receptor γ2 in patients with fatty liver diseases," *World Journal of Gastroenterology*, vol. 16, no. 46, pp. 5830–5837, 2010.

[39] S. Gawrieh, S. Gawrieh, M. C. Marion et al., "Genetic variation in the peroxisome proliferator activated receptor-gamma gene is associated with histologically advanced NAFLD," *Digestive Diseases and Sciences*, vol. 57, no. 4, pp. 952–957, 2012.

[40] Z. Yang, J. Wen, Q. Li et al., "PPARG gene Pro12Ala variant contributes to the development of non-alcoholic fatty liver in middle-aged and older Chinese population," *Molecular and Cellular Endocrinology*, vol. 348, no. 1, pp. 255–259, 2012.

[41] A. C. Gupta, A. K. Chaudhory, Sukriti et al., "Peroxisome proliferators-activated receptor γ2 Pro12Ala variant is associated with body mass index in non-alcoholic fatty liver disease patients," *Hepatology International*, vol. 5, no. 1, pp. 575–580, 2011.

[42] E. Y. Jong, M. C. Kyung, H. B. Sei et al., "Reduced expression of peroxisome proliferator-activated receptor-α may have an important role in the development of non-alcoholic fatty liver disease," *Journal of Gastroenterology and Hepatology*, vol. 19, no. 7, pp. 799–804, 2004.

[43] F. Lalloyer, K. Wouters, M. Baron et al., "Peroxisome proliferator-activated receptor-α gene level differently affects lipid metabolism and inflammation in apolipoprotein E2 knock-in mice," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 31, no. 7, pp. 1573–1579, 2011.

[44] M. A. Abdelmegeed, S. H. Yoo, L. E. Henderson, F. J. Gonzalez, K. J. Woodcroft, and B. J. Song, "PPARα expression

protects male mice from high fat-induced nonalcoholic fatty liver," *Journal of Nutrition*, vol. 141, no. 4, pp. 603–610, 2011.

[45] Y. Harano, K. Yasui, T. Toyama et al., "Fenofibrate, a peroxisome proliferator-activated receptor α agonist, reduces hepatic steatosis and lipid peroxidation in fatty liver Shionogi mice with hereditary fatty liver," *Liver International*, vol. 26, no. 5, pp. 613–620, 2006.

[46] C. Z. Larter, M. M. Yeh, D. M. van Rooyen, J. Brooling, K. Ghatora, and G. C. Farrell, "Peroxisome proliferator-activated receptor-α agonist, Wy 14643, improves metabolic indices, steatosis and ballooning in diabetic mice with non-alcoholic steatohepatitis," *Journal of Gastroenterology and Hepatology*, vol. 27, no. 2, pp. 341–350, 2012.

[47] L. Qiu, J. Lin, F. Xu et al., "Inhibition of aldose reductase activates hepatic peroxisome proliferator-activated receptor-α and ameliorates hepatosteatosis in diabetic db/db mice," *Experimental Diabetes Research*, vol. 2012, Article ID 789730, 2012.

[48] C. Z. Larter, M. M. Yeh, J. Cheng et al., "Activation of peroxisome proliferator-activated receptor α by dietary fish oil attenuates steatosis, but does not prevent experimental steatohepatitis because of hepatic lipoperoxide accumulation," *Journal of Gastroenterology and Hepatology*, vol. 23, no. 2, pp. 267–275, 2008.

[49] S. Chen, Y. Li, S. Li, and C. Yu, "A Val227Ala substitution in the peroxisome proliferator activated receptor alpha (PPAR alpha) gene associated with non-alcoholic fatty liver disease and decreased waist circumference and waist-to-hip ratio," *Journal of Gastroenterology and Hepatology*, vol. 23, no. 9, pp. 1415–1418, 2008.

[50] V. G. Athyros, D. P. Mikhailidis, T. P. Didangelos et al., "Effect of multifactorial treatment on non-alcoholic fatty liver disease in metabolic syndrome: a randomised study," *Current Medical Research and Opinion*, vol. 22, no. 5, pp. 873–883, 2006.

[51] C. Fernández-Miranda, M. Pérez-Carreras, F. Colina, G. López-Alonso, C. Vargas, and J. A. Solís-Herruzo, "A pilot trial of fenofibrate for the treatment of non-alcoholic fatty liver disease," *Digestive and Liver Disease*, vol. 40, no. 3, pp. 200–205, 2008.

[52] H. T. Wu, W. Chen, K. C. Cheng, P. M. Ku, C. H. Yeh, and J. T. Cheng, "Oleic acid activates peroxisome proliferator-activated receptor delta to compensate insulin resistance in steatotic cells ," *The Journal of nutritional biochemistry*. In press.

[53] C. H. Lee, P. Olson, A. Hevener et al., "PPARδ regulates glucose metabolism and insulin sensitivity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 9, pp. 3444–3449, 2006.

[54] L. M. Sanderson, M. V. Boekschoten, B. Desvergne, M. Müller, and S. Kersten, "Transcriptional profiling reveals divergent roles of PPARα and PPARβ/δ in regulation of gene expression in mouse liver," *Physiological Genomics*, vol. 41, no. 1, pp. 42–52, 2010.

[55] R. Sharma and P. Torka, "Peroxisome proliferator-activated receptor-δ induces insulin-induced gene-1 and suppresses hepatic lipogenesis in obese diabetic mice," *Hepatology*, vol. 48, no. 6, pp. 432–441, 2008.

[56] S. Liu, B. Hatano, M. Zhao et al., "Role of peroxisome proliferator-activated receptor δ/β in hepatic metabolic regulation," *Journal of Biological Chemistry*, vol. 286, no. 2, pp. 1237–1247, 2011.

[57] L. Serrano-Marco, E. Barroso, I. El Kochairi et al., "The peroxisome proliferator-activated receptor (PPAR) beta/delta agonist GW501516 inhibits IL-6-induced signal transducer and activator of transcription 3 (STAT3) activation and insulin resistance in human liver cells," *Diabetologia*, vol. 55, no. 3, pp. 743–751, 2012.

[58] E. Barroso, R. Rodríguez-Calvo, L. Serrano-Marco et al., "The PPARβ/δ activator GW501516 prevents the down-regulation of AMPK caused by a high-fat diet in liver and amplifies the PGC-1α-lipin 1-PPARα pathway leading to increased fatty acid oxidation," *Endocrinology*, vol. 152, no. 5, pp. 1848–1859, 2011.

[59] T. Nagasawa, Y. Inada, S. Nakano et al., "Effects of bezafibrate, PPAR pan-agonist, and GW501516, PPARδ agonist, on development of steatohepatitis in mice fed a methionine- and choline-deficient diet," *European Journal of Pharmacology*, vol. 536, no. 1-2, pp. 182–191, 2006.

[60] J. M. Ye, J. Tid-Ang, N. Turner et al., "PPARδ agonists have opposing effects on insulin resistance in high fat-fed rats and mice due to different metabolic responses in muscle," *British Journal of Pharmacology*, vol. 163, no. 3, pp. 556–566, 2011.

[61] H. T. Wu, C. T. Chen, K. C. Cheng, Y. X. Li, C. H. Yeh, and J. T. Cheng, "Pharmacological activation of peroxisome proliferator-activated receptor delta improves insulin resistance and hepatic steatosis in high fat diet-induced diabetic mice," *Hormone and Metabolic Research*, vol. 43, no. 9, pp. 631–635, 2011.

[62] H. J. Lim, J. H. Park, S. Lee, H. E. Choi, K. S. Lee, and H. Y. Park, "PPARδ ligand L-165041 ameliorates Western diet-induced hepatic lipid accumulation and inflammation in LDLR$^{-/-}$ mice," *European Journal of Pharmacology*, vol. 622, no. 1–3, pp. 45–51, 2009.

[63] D. L. Sprecher, C. Massien, G. Pearce et al., "Triglyceride: high-density lipoprotein cholesterol effects in healthy subjects administered a peroxisome proliferator activated receptor δ agonist," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 27, no. 2, pp. 359–365, 2007.

[64] U. Risérus, D. Sprecher, T. Johnson et al., "Activation of peroxisome proliferator-activated receptor (PPAR)δ promotes reversal of multiple metabolic abnormalities, reduces oxidative stress, and increases fatty acid oxidation in moderately obese men," *Diabetes*, vol. 57, no. 2, pp. 332–339, 2008.

[65] H. E. Bays, S. Schwartz, T. Littlejohn III et al., "MBX-8025, a novel peroxisome proliferator receptor-delta agonist: lipid and other metabolic effects in dyslipidemic overweight patients treated with and without atorvastatin," *Journal of Clinical Endocrinology and Metabolism*, vol. 96, no. 9, pp. 2889–2897, 2011.

[66] T. Miyahara, L. Schrum, R. Rippe et al., "Peroxisome proliferator-activated receptors and hepatic stellate cell activation," *Journal of Biological Chemistry*, vol. 275, no. 46, pp. 35715–35722, 2000.

[67] F. Marra, E. Efsen, R. G. Romanelli et al., "Ligands of peroxisome proliferator-activated receptor γ modulate profibrogenic and proinflammatory actions in hepatic stellate cells," *Gastroenterology*, vol. 119, no. 2, pp. 466–478, 2000.

[68] F. Zhang, Y. Lu, and S. Zheng, "Peroxisome proliferator-activated receptor-gamma cross-regulation of signaling events implicated in liver fibrogenesis," *Cell Signal*, vol. 24, no. 3, pp. 596–605, 2012.

[69] Y. M. Nan, N. Fu, W. J. Wu et al., "Rosiglitazone prevents nutritional fibrosis and steatohepatitis in mice," *Scandinavian Journal of Gastroenterology*, vol. 44, no. 3, pp. 358–365, 2009.

[70] V. Tahan, F. Eren, E. Avsar et al., "Rosiglitazone attenuates liver inflammation in a rat model of nonalcoholic steatohepatitis," *Digestive Diseases and Sciences*, vol. 52, no. 12, pp. 3465–3472, 2007.

[71] G. Lutchman, A. Modi, D. E. Kleiner et al., "The effects of discontinuing pioglitazone in patients with nonalcoholic steatohepatitis," *Hepatology*, vol. 46, no. 2, pp. 424–429, 2007.

[72] F. Yakaryilmaz, S. Guliter, B. Savas et al., "Effects of vitamin e treatment on peroxisome proliferator-activated receptor-α expression and insulin resistance in patients with non-alcoholic steatohepatitis: results of a pilot study," *Internal Medicine Journal*, vol. 37, no. 4, pp. 229–235, 2007.

[73] W. Shan, C. J. Nicol, S. Ito et al., "Peroxisome proliferator-activated receptor-β/δ protects against chemically induced liver toxicity in mice," *Hepatology*, vol. 47, no. 1, pp. 225–235, 2008.

[74] K. Kang, S. M. Reilly, V. Karabacak et al., "Adipocyte-derived Th2 cytokines and myeloid PPARdelta regulate macrophage polarization and insulin sensitivity," *Cell Metabolism*, vol. 7, no. 6, pp. 485–495, 2008.

[75] K. Hellemans, L. Michalik, A. Dittie et al., "Peroxisome proliferator-activated receptor-β signaling contributes to enhanced proliferation of hepatic stellate cells," *Gastroenterology*, vol. 124, no. 1, pp. 184–201, 2003.

[76] K. Kim, K. H. Kim, E. Ha, J. Y. Park, N. Sakamoto, and J. Cheong, "Hepatitis C virus NS5A protein increases hepatic lipid accumulation via induction of activation and expression of PPARgamma," *FEBS Letters*, vol. 583, no. 17, pp. 2720–2726, 2009.

[77] K. H. Kim, S. P. Hong, K. Kim, M. J. Park, K. J. Kim, and J. Cheong, "HCV core protein induces hepatic lipid accumulation by activating SREBP1 and PPARγ," *Biochemical and Biophysical Research Communications*, vol. 355, no. 4, pp. 883–888, 2007.

[78] M. V. García-Mediavilla, S. Pisonero-Vaquero, E. Lima-Cabello et al., "Liver X receptor alpha-mediated regulation of lipogenesis by core and NS5A proteins contributes to HCV-induced liver steatosis and HCV replication," *Laboratory Investigation*, vol. 92, no. 8, pp. 1191–1202, 2012.

[79] V. Pazienza, M. Vinciguerra, A. Andriulli, and A. Mangia, "Hepatitis C virus core protein genotype 3a increases SOCS-7 expression through PPAR-γ in Huh-7 cells," *Journal of General Virology*, vol. 91, pp. 1678–1686, 2010.

[80] Y. H. Niu, R. T. Yi, H. L. Liu, T. Y. Chen, S. L. Zhang, and Y. R. Zhao, "Expression of COX-2 and PPARg in the livers of patients with acute on chronic HBV-related liver failure and their relationship with clinic parameters," *Zhonghua Gan Zang Bing Za Zhi*, vol. 19, no. 7, pp. 511–516, 2011.

[81] K. H. Kim, H. J. Shin, K. Kim et al., "Hepatitis B virus X protein induces hepatic steatosis via transcriptional activation of SREBP1 and PPARgamma," *Gastroenterology*, vol. 132, no. 5, pp. 1955–1967, 2007.

[82] Y. H. Choi, H. I. Kim, J. K. Seong et al., "Hepatitis B virus X protein modulates peroxisome proliferator-activated receptor γ through protein-protein interaction," *FEBS Letters*, vol. 557, no. 1–3, pp. 73–80, 2004.

[83] C. R. Ondracek and A. McLachlan, "Role of peroxisome proliferator-activated receptor gamma coactivator 1alpha in AKT/PKB-mediated inhibition of hepatitis B virus biosynthesis," *Journal of Virology*, vol. 85, no. 22, pp. 11891–11900, 2011.

[84] S. Yoon, J. Jung, T. Kim et al., "Adiponectin, a downstream target gene of peroxisome proliferator-activated receptor γ, controls hepatitis B virus replication," *Virology*, vol. 409, no. 2, pp. 290–298, 2011.

[85] Y. Wakui, J. Inoue, Y. Ueno et al., "Inhibitory effect on hepatitis B virus in vitro by a peroxisome proliferator-activated receptor-γ ligand, rosiglitazone," *Biochemical and Biophysical Research Communications*, vol. 396, no. 2, pp. 508–514, 2010.

[86] D. W. Crabb, A. Galli, M. Fischer, and M. You, "Molecular mechanisms of alcoholic fatty liver: role of peroxisome proliferator-activated receptor alpha," *Alcohol*, vol. 34, no. 1, pp. 35–38, 2004.

[87] T. Moriya, H. Naito, Y. Ito, and T. Nakajima, "'Hypothesis of seven balances': molecular mechanisms behind alcoholic liver diseases and association with PPARα," *Journal of Occupational Health*, vol. 51, no. 5, pp. 391–403, 2009.

[88] C. Wu, R. Gilroy, R. Taylor et al., "Alteration of hepatic nuclear receptor-mediated signaling pathways in hepatitis C virus patients with and without a history of alcohol drinking," *Hepatology*, vol. 54, no. 6, pp. 1966–1974, 2011.

[89] S. Dharancy, M. Malapel, G. Perlemuter et al., "Impaired expression of the peroxisome proliferator-activated receptor alpha during hepatitis C virus infection," *Gastroenterology*, vol. 128, no. 2, pp. 334–342, 2005.

[90] J. Goldwasser, P. Y. Cohen, W. Lin et al., "Naringenin inhibits the assembly and long-term production of infectious hepatitis C virus particles through a PPAR-mediated mechanism," *Journal of Hepatology*, vol. 55, no. 5, pp. 963–971, 2011.

[91] B. Rakic, S. M. Sagan, M. Noestheden et al., "Peroxisome proliferator-activated receptor α antagonism inhibits hepatitis C virus replication," *Chemistry and Biology*, vol. 13, no. 1, pp. 23–30, 2006.

[92] M. Pang, S. M. de la Monte, L. Longato et al., "PPARδ agonist attenuates alcohol-induced hepatic insulin resistance and improves liver injury and repair," *Journal of Hepatology*, vol. 50, no. 6, pp. 1192–1201, 2009.

[93] J. Yu, B. Shen, E. S. H. Chu et al., "Inhibitory role of peroxisome proliferator-activated receptor gamma in hepatocarcinogenesis in mice and in vitro," *Hepatology*, vol. 51, no. 6, pp. 2008–2019, 2010.

[94] K. L. Schaefer, K. Wada, H. Takahashi et al., "Peroxisome proliferator-activated receptor γ inhibition prevents adhesion to the extracellular matrix and induces anoikis in hepatocellular carcinoma cells," *Cancer Research*, vol. 65, no. 6, pp. 2251–2259, 2005.

[95] K. Yoshizawa, D. P. Cioca, S. Kawa, E. Tanaka, and K. Kiyosawa, "Peroxisome proliferator-activated receptor γ ligand troglitazone induces cell cycle arrest and apoptosis of hepatocellular carcinoma cell lines," *Cancer*, vol. 95, no. 10, pp. 2243–2251, 2002.

[96] L. Q. Cao, X. L. Wang, Q. Wang et al., "Rosiglitazone sensitizes hepatocellular carcinoma cell lines to 5-fluorouracil antitumor activity through activation of the PPARγ signaling pathway," *Acta Pharmacologica Sinica*, vol. 30, no. 9, pp. 1316–1322, 2009.

[97] H. J. Lee, Y. Su, P. H. Yin, H. C. Lee, and C. W. Chi, "PPARγ/PGC-1α pathway in E-cadherin expression and motility of HepG2 cells," *Anticancer Research*, vol. 29, no. 12, pp. 5057–5063, 2009.

[98] J. Shim, B. H. Kim, Y. I. Kim et al., "The peroxisome proliferator-activated receptor γ ligands, pioglitazone and 15-deoxy-Δ(12,14)-prostaglandin J(2), have antineoplastic effects against hepatitis B virus-associated hepatocellular

carcinoma cells," *International Journal of Oncology*, vol. 36, no. 1, pp. 223–231, 2010.

[99] I. Borbath, I. Leclercq, P. Moulin, C. Sempoux, and Y. Horsmans, "The PPARgamma agonist pioglitazone inhibits early neoplastic occurrence in the rat liver," *European Journal of Cancer*, vol. 43, no. 11, pp. 1755–1763, 2007.

[100] J. Yu, L. Qiao, L. Zimmermann et al., "Troglitazone inhibits tumor growth in hepatocellular carcinoma in vitro and in vivo," *Hepatology*, vol. 43, no. 1, pp. 134–143, 2006.

[101] M. C. Hsu, C. C. Huang, H. C. Chang, T. H. Hu, and W. C. Hung, "Overexpression of Jab1 in hepatocellular carcinoma and its inhibition by peroxisome proliferator-activated receptorγ ligands in vitro and in vivo," *Clinical Cancer Research*, vol. 14, no. 13, pp. 4045–4052, 2008.

[102] Y. M. Shah, K. Morimura, Q. Yang, T. Tanabe, M. Takagi, and F. J. Gonzalez, "Peroxisome proliferator-activated receptor α regulates a microRNA-mediated signaling cascade responsible for hepatocellular proliferation," *Molecular and Cellular Biology*, vol. 27, no. 12, pp. 4238–4247, 2007.

[103] F. J. Gonzalez and Y. M. Shah, "PPARα: mechanism of species differences and hepatocarcinogenesis of peroxisome proliferators," *Toxicology*, vol. 246, no. 1, pp. 2–8, 2008.

[104] J. M. Peters, Y. M. Shah, and F. J. Gonzalez, "The role of peroxisome proliferator-activated receptors in carcinogenesis and chemoprevention," *Nature Reviews*, vol. 12, no. 3, pp. 181–195, 2012.

[105] J. M. Peters, R. C. Cattley, and F. J. Gonzalez, "Role of PPARα in the mechanism of action of the nongenotoxic carcinogen and peroxisome proliferator Wy-14,643," *Carcinogenesis*, vol. 18, no. 11, pp. 2029–2033, 1997.

[106] T. Hays, I. Rusyn, A. M. Burns et al., "Role of peroxisome proliferator-activated receptor-α (PPARα) in bezafibrate-induced hepatocarcinogenesis and cholestasis," *Carcinogenesis*, vol. 26, no. 1, pp. 219–227, 2005.

[107] J. Youssef and M. Badr, "Enhanced hepatocarcinogenicity due to agonists of peroxisome proliferator-activated receptors in senescent rats: role of peroxisome proliferation, cell proliferation, and apoptosis," *TheScientificWorldJournal*, vol. 2, pp. 1491–1500, 2002.

[108] J. C. Corton, "Evaluation of the role of peroxisome proliferator-activated receptor α (PPARα) in mouse liver tumor induction by trichloroethylene and metabolites," *Critical Reviews in Toxicology*, vol. 38, no. 10, pp. 857–875, 2008.

[109] N. Tanaka, K. Moriya, K. Kiyosawa, K. Koike, and T. Aoyama, "Hepatitis C virus core protein induces spontaneous and persistent activation of peroxisome proliferator-activated receptor α in transgenic mice: implications for HCV-associated hepatocarcinogenesis," *International Journal of Cancer*, vol. 122, no. 1, pp. 124–131, 2008.

[110] N. Tanaka, K. Moriya, K. Kiyosawa, K. Koike, F. J. Gonzalez, and T. Aoyama, "PPARα activation is essential for HCV core protein-induced hepatic steatosis and hepatocellular carcinoma in mice," *Journal of Clinical Investigation*, vol. 118, no. 2, pp. 683–694, 2008.

[111] K. Morimura, C. Cheung, J. M. Ward, J. K. Reddy, and F. J. Gonzalez, "Differential susceptibility of mice humanized for peroxisome proliferator-activated receptor α to Wy-14,643-induced liver tumorigenesis," *Carcinogenesis*, vol. 27, no. 5, pp. 1074–1080, 2006.

[112] C. Cheung, T. E. Akiyama, J. M. Ward et al., "Diminished hepatocellular proliferation in mice humanized for the nuclear receptor peroxisome proliferator-activated receptor α," *Cancer Research*, vol. 64, no. 11, pp. 3849–3854, 2004.

[113] L. Xu, C. Han, K. Lim, and T. Wu, "Cross-talk between peroxisome proliferator-activated receptor δ and cytosolic phospholipase A(2)α/cyclooxygenase-2/prostaglandin E(2) signaling pathways in human hepatocellular carcinoma cells," *Cancer Research*, vol. 66, no. 24, pp. 11859–11868, 2006.

[114] H. N. Suh, S. H. Lee, M. Y. Lee, Y. J. Lee, J. H. Lee, and H. J. Han, "Role of interleukin-6 in the control of DNA synthesis of hepatocytes: involvement of PKC, p44/42 MAPKs, and PPARδ," *Cellular Physiology and Biochemistry*, vol. 22, no. 5-6, pp. 673–684, 2008.

[115] J. E. Leader, C. Wang, M. Fu, and R. G. Pestell, "Epigenetic regulation of nuclear steroid receptors," *Biochemical Pharmacology*, vol. 72, no. 11, pp. 1589–1596, 2006.

[116] J. Mann, D. C. K. Chu, A. Maxwell et al., "MeCP2 controls an epigenetic pathway that promotes myofibroblast transdifferentiation and fibrosis," *Gastroenterology*, vol. 138, no. 2, pp. 705.e4–714.e4, 2010.

[117] Y. Zhou, X. Jia, M. Zhou, and J. Liu, "Egr-1 is involved in the inhibitory effect of leptin on PPARγ expression in hepatic stellate cell in vitro," *Life Sciences*, vol. 84, no. 15-16, pp. 544–551, 2009.

[118] H. Xiao, S. E. LeBlanc, Q. Wu et al., "Chromatin accessibility and transcription factor binding at the PPARγ2 promoter during adipogenesis is protein kinase A-dependent," *Journal of Cellular Physiology*, vol. 226, no. 1, pp. 86–93, 2011.

[119] M. C. Mitterberger, G. Kim, U. Rostek, R. L. Levine, and W. Zwerschke, "Carbonic anhydrase III regulates peroxisome proliferator-activated receptor-gamma2," *Experimental Cell Research*, vol. 318, no. 8, pp. 877–886, 2012.

[120] S. R. Farmer, "Transcriptional control of adipocyte formation," *Cell Metabolism*, vol. 4, no. 4, pp. 263–273, 2006.

[121] G. P. Lobo, J. Amengual, H. N. M. Li et al., "β,β-carotene decreases peroxisome proliferator receptor γ activity and reduces lipid storage capacity of adipocytes in a β,β-carotene oxygenase 1-dependent manner," *Journal of Biological Chemistry*, vol. 285, no. 36, pp. 27891–27899, 2010.

[122] K. M. Wadosky and M. S. Willis, "The story so far: post-translational regulation of peroxisome proliferator-activated receptors by ubiquitination and SUMOylation," *American Journal of Physiology*, vol. 302, no. 3, pp. H515–H526, 2012.

[123] C. E. Juge-Aubry, E. Hammar, C. Siegrist-Kaiser et al., "Regulation of the transcriptional activity of the peroxisome proliferator-activated receptor by phosphorylation of a ligand-independent trans-activating domain," *Journal of Biological Chemistry*, vol. 274, no. 15, pp. 10505–10510, 1999.

[124] A. J. Gilde and M. Van Bilsen, "Peroxisome proliferator-activated receptors (PPARS): regulators of gene expression in heart and skeletal muscle," *Acta Physiologica Scandinavica*, vol. 178, no. 4, pp. 425–434, 2003.

[125] S. Yu and J. K. Reddy, "Transcription coactivators for peroxisome proliferator-activated receptors," *Biochimica et Biophysica Acta*, vol. 1771, no. 8, pp. 936–951, 2007.

[126] A. Grimson, K. K. H. Farh, W. K. Johnston, P. Garrett-Engele, L. P. Lim, and D. P. Bartel, "MicroRNA targeting specificity in mammals: determinants beyond seed pairing," *Molecular Cell*, vol. 27, no. 1, pp. 91–105, 2007.

[127] R. C. Lee, R. L. Feinbaum, and V. Ambros, "The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14," *Cell*, vol. 75, no. 5, pp. 843–854, 1993.

[128] W. Filipowicz, S. N. Bhattacharyya, and N. Sonenberg, "Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight?" *Nature Reviews Genetics*, vol. 9, no. 2, pp. 102–114, 2008.

[129] H. K. Saini, S. Griffiths-Jones, and A. J. Enright, "Genomic analysis of human microRNA transcripts," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 45, pp. 17719–17724, 2007.

[130] Y. Lee, M. Kim, J. Han et al., "MicroRNA genes are transcribed by RNA polymerase II," *The EMBO Journal*, vol. 23, no. 20, pp. 4051–4060, 2004.

[131] K. Breving and A. Esquela-Kerscher, "The complexities of microRNA regulation: mirandering around the rules," *International Journal of Biochemistry and Cell Biology*, vol. 42, no. 8, pp. 1316–1329, 2010.

[132] Y. Zeng and B. R. Cullen, "Efficient processing of primary microRNA hairpins by Drosha requires flanking nonstructured RNA sequences," *Journal of Biological Chemistry*, vol. 280, no. 30, pp. 27595–27603, 2005.

[133] D. P. Bartel, "MicroRNAs: genomics, biogenesis, mechanism, and function," *Cell*, vol. 116, no. 2, pp. 281–297, 2004.

[134] R. Yi, Y. Qin, I. G. Macara, and B. R. Cullen, "Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs," *Genes and Development*, vol. 17, no. 24, pp. 3011–3016, 2003.

[135] E. Lund, S. Güttinger, A. Calado, J. E. Dahlberg, and U. Kutay, "Nuclear export of microRNA precursors," *Science*, vol. 303, no. 5654, pp. 95–98, 2004.

[136] S. A. Melo and M. Esteller, "A precursor microRNA in a cancer cell nucleus: get me out of here!," *Cell Cycle*, vol. 10, no. 6, pp. 922–925, 2011.

[137] H. Zhang, F. A. Kolb, L. Jaskiewicz, E. Westhof, and W. Filipowicz, "Single processing center models for human Dicer and bacterial RNase III," *Cell*, vol. 118, no. 1, pp. 57–68, 2004.

[138] R. I. Gregory, T. P. Chendrimada, N. Cooch, and R. Shiekhattar, "Human RISC couples microRNA biogenesis and posttranscriptional gene silencing," *Cell*, vol. 123, no. 4, pp. 631–640, 2005.

[139] T. Kawamata, H. Seitz, and Y. Tomari, "Structural determinants of miRNAs for RISC loading and slicer-independent unwinding," *Nature Structural and Molecular Biology*, vol. 16, no. 9, pp. 953–960, 2009.

[140] C. Y. Park, Y. S. Choi, and M. T. McManus, "Analysis of microRNA knockouts in mice," *Human Molecular Genetics*, vol. 19, no. R2, pp. R169–R175, 2010.

[141] D. Baek, J. Villén, C. Shin, F. D. Camargo, S. P. Gygi, and D. P. Bartel, "The impact of microRNAs on protein output," *Nature*, vol. 455, no. 7209, pp. 64–71, 2008.

[142] P. Sood, A. Krek, M. Zavolan, G. Macino, and N. Rajewsky, "Cell-type-specific signatures of microRNAs on target mRNA expression," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 8, pp. 2746–2751, 2006.

[143] Y. Gao, J. Schug, L. B. McKenna, J. Le Lay, K. H. Kaestner, and L. E. Greenbaum, "Tissue-specific regulation of mouse microRNA genes in endoderm-derived tissues," *Nucleic Acids Research*, vol. 39, no. 2, pp. 454–463, 2011.

[144] T. Thum, "MicroRNA therapeutics in cardiovascular medicine," *EMBO Molecular Medicine*, vol. 4, no. 1, pp. 3–14, 2012.

[145] P. Kantharidis, B. Wang, R. M. Carew, and H. Y. Lan, "Diabetes complications: the microRNA perspective," *Diabetes*, vol. 60, no. 7, pp. 1832–1837, 2011.

[146] V. Rottiers and A. M. Naar, "MicroRNAs in metabolism and metabolic disorders," *Nature Reviews Molecular Cell Biology*, vol. 13, no. 4, pp. 239–250, 2012.

[147] K. C. Sonntag, "MicroRNAs and deregulated gene expression networks in neurodegeneration," *Brain Research*, vol. 1338, pp. 48–57, 2010.

[148] R. M. O'Connell, D. S. Rao, A. A. Chaudhuri, and D. Baltimore, "Physiological and pathological roles for microRNAs in the immune system," *Nature Reviews Immunology*, vol. 10, no. 2, pp. 111–122, 2010.

[149] P. P. Medina, M. Nolde, and F. J. Slack, "OncomiR addiction in an in vivo model of microRNA-21-induced pre-B-cell lymphoma," *Nature*, vol. 467, no. 7311, pp. 86–90, 2010.

[150] S. Costinean, N. Zanesi, Y. Pekarsky et al., "Pre-B cell proliferation and lymphoblastic leukemia/high-grade lymphoma in Eμ-miR155 transgenic mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 18, pp. 7024–7029, 2006.

[151] H. Xu, J. H. He, Z. D. Xiao et al., "Liver-enriched transcription factors regulate microRNA-122 that targets CUTL1 during liver development," *Hepatology*, vol. 52, no. 4, pp. 1431–1442, 2010.

[152] J. Krützfeldt, N. Rajewsky, R. Braich et al., "Silencing of microRNAs in vivo with "antagomirs"," *Nature*, vol. 438, no. 7068, pp. 685–689, 2005.

[153] M. Castoldi, M. V. Spasic, S. Altamura et al., "The liver-specific microRNA miR-122 controls systemic iron homeostasis in mice," *Journal of Clinical Investigation*, vol. 121, no. 4, pp. 1386–1396, 2011.

[154] D. Ferland-McCollough, S. E. Ozanne, K. Siddle, A. E. Willisand, and M. Bushell, "The involvement of microRNAs in type 2 diabetes," *Biochemical Society Transactions*, vol. 38, no. 6, pp. 1565–1570, 2010.

[155] J. Haybaeck, N. Zeller, and M. Heikenwalder, "The parallel universe: microRNAs and their role in chronic hepatitis, liver tissue damage and hepatocarcinogenesis," *Swiss Medical Weekly*, vol. 141, Article ID w13287, 2011.

[156] S. Bala and G. Szabo, "MicroRNA signature in alcoholic liver disease," *International Journal of Hepatology*, vol. 2012, Article ID 498232, 2012.

[157] O. Waidmann, V. Bihrer, T. Pleli et al., "Serum microRNA-122 levels in different groups of patients with chronic hepatitis B virus infection," *Journal of Viral Hepatitis*, vol. 19, no. 2, pp. e58–e65, 2012.

[158] J. I. Henke, D. Goergen, J. Zheng et al., "MicroRNA-122 stimulates translation of hepatitis C virus RNA," *The EMBO Journal*, vol. 27, no. 24, pp. 3300–3310, 2008.

[159] X. Jiang, E. Tsitsiou, S. E. Herrick, and M. A. Lindsay, "MicroRNAs and the regulation of fibrosis," *FEBS Journal*, vol. 277, no. 9, pp. 2015–2021, 2010.

[160] S. Putta, L. Lanting, G. Sun, G. Lawson, M. Kato, and R. Natarajan, "Inhibiting microRNA-192 ameliorates renal fibrosis in diabetic nephropathy," *Journal of the American Society of Nephrology*, vol. 23, no. 3, pp. 458–469, 2012.

[161] Y. Ladeiro, G. Couchy, C. Balabaud et al., "MicroRNA profiling in hepatocellular tumors is associated with clinical features and oncogene/tumor suppressor gene mutations," *Hepatology*, vol. 47, no. 6, pp. 1955–1963, 2008.

[162] Y. Wang, A. T. C. Lee, J. Z. I. Ma et al., "Profiling microRNA expression in hepatocellular carcinoma reveals microRNA-224 up-regulation and apoptosis inhibitor-5 as a microRNA-224-specific target," *Journal of Biological Chemistry*, vol. 283, no. 19, pp. 13205–13215, 2008.

[163] L. Zheng, G. C. Lv, J. Sheng, and Y. D. Yang, "Effect of miRNA-10b in regulating cellular steatosis level by targeting PPAR-α expression, a novel mechanism for the pathogenesis of NAFLD," *Journal of Gastroenterology and Hepatology*, vol. 25, no. 1, pp. 156–163, 2010.

[164] K. Kida, M. Nakajima, T. Mohri et al., "PPARalpha is regulated by miR-21 and miR-27b in human liver," *Pharmaceutical Research*, vol. 28, no. 10, pp. 2467–2476, 2011.

[165] D. Gatfield, G. Le Martelot, C. E. Vejnar et al., "Integration of microRNA miR-122 in hepatic circadian gene expression," *Genes and Development*, vol. 23, no. 11, pp. 1313–1326, 2009.

[166] M. Karbiener, C. Fischer, S. Nowitsch et al., "MicroRNA miR-27b impairs human adipocyte differentiation and targets PPARγ," *Biochemical and Biophysical Research Communications*, vol. 390, no. 2, pp. 247–251, 2009.

[167] S. Y. Kim, A. Y. Kim, H. W. Lee et al., "MiR-27a is a negative regulator of adipocyte differentiation via suppressing PPARgamma expression," *Biochemical and Biophysical Research Communications*, vol. 392, no. 3, pp. 323–328, 2010.

[168] E. K. Lee, M. J. Lee, K. Abdelmohsen et al., "MiR-130 suppresses adipogenesis by inhibiting peroxisome proliferator-activated receptor γ expression," *Molecular and Cellular Biology*, vol. 31, no. 4, pp. 626–638, 2011.

[169] R. Martinelli, C. Nardelli, V. Pilone et al., "MiR-519d overexpression is associated with human obesity," *Obesity*, vol. 18, no. 11, pp. 2170–2176, 2010.

[170] J. Wang, Y. Song, Y. Zhang et al., "Cardiomyocyte overexpression of miR-27b induces cardiac hypertrophy and dysfunction in mice," *Cell Research*, vol. 22, no. 3, pp. 516–527, 2012.

[171] C. Jennewein, A. Von Knethen, T. Schmid, and B. Brüne, "MicroRNA-27b contributes to lipopolysaccharide-mediated peroxisome proliferator-activated receptor γ (PPARγ) mRNA destabilization," *Journal of Biological Chemistry*, vol. 285, no. 16, pp. 11846–11853, 2010.

[172] D. Iliopoulos, K. N. Malizos, P. Oikonomou, and A. Tsezou, "Integrative MicroRNA and proteomic approaches identify novel osteoarthritis genes and their collaborative metabolic and inflammatory networks," *PLoS ONE*, vol. 3, no. 11, Article ID e3740, 2008.

[173] J. Zhou, K. C. Wang, W. Wu et al., "MicroRNA-21 targets peroxisome proliferators-activated receptor-α in an autoregulatory loop to modulate flow-induced endothelial inflammation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 25, pp. 10355–10360, 2011.

[174] J. L. Tong, C. P. Zhang, F. Nie et al., "MicroRNA 506 regulates expression of PPAR alpha in hydroxycamptothecin-resistant human colon cancer cells," *FEBS Letters*, vol. 585, no. 22, pp. 3560–3568, 2011.

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Review Article

The Role of Peroxisome Proliferator-Activated Receptors in Colorectal Cancer

Joo-In Park and Jong-Young Kwak

Department of Biochemistry, College of Medicine, Dong-A University, 3 Ga 1, Dongdaesin-Dong, Seo-Gu, Busan 602-714, Republic of Korea

Correspondence should be addressed to Joo-In Park, jipark@dau.ac.kr

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Colorectal cancer is one of the most common cancers in the world. Dietary fat intake is a major risk factor for colorectal cancer. Some nuclear hormone receptors play an important role in regulating nutrient metabolism and energy homeostasis. Among these receptors, special attention has been focused on the role of peroxisome proliferator-activated receptors (PPARs) in colorectal cancer, because PPARs are involved in regulation of lipid and carbohydrate metabolism. PPARs are ligand-activated intracellular transcription factors. The PPAR subfamily consists of three subtypes encoded by distinct genes named PPARα, PPARβ/δ, and PPARγ. PPARγ is the most extensively studied subtype of PPARs. Even though many investigators have studied the expression and clinical implications of PPARs in colorectal cancer, there are still many controversies about the role of PPARs in colorectal cancer. In this paper, the recent progresses in understanding the role of PPARs in colorectal cancer are summarized.

1. Introduction

Colorectal cancer is one of the most common cancers in the world. Its incidence appears to be increasing, particularly in developed countries [1–3]. Colorectal carcinogenesis results from the loss of the normal regulatory pathways involved in cell proliferation and cell death. Especially, molecular alterations of multiple pathways including Wnt (Wingless type)/adenomatous polyposis coli (APC), cyclooxygenase-2 (COX-2), and Ras are known to play important roles in progression of colorectal cancer. Recent progresses in the development of new chemotherapeutic agents have improved the prognosis of colorectal cancer patients [4]. However, for most patients with advanced colorectal cancer, it is still difficult to achieve a complete remission, especially with surgery or chemotherapy. Therefore, significant effort has been exerted to identify novel drug targets for both the prevention and treatment of colorectal cancer.

The peroxisome proliferator-activated receptors (PPARs) belong to members of the nuclear hormone receptor superfamily including receptors for steroid, retinoid, vitamin D, and thyroid hormones [5]. PPARs have received the attention of investigators interested in studying about the intracellular pathways that control signal transduction and gene transcription since their discovery in 1990. The name of PPARs was derived from its property to proliferate peroxisomes in rodent liver, where PPARα plays the major role. However, none of the PPARs could be contributed to peroxisome proliferation in humans [6]. PPARs are metabolic regulators involved in the regulation of glucose and lipid homeostasis. Ligand-activated PPAR forms heterodimer with the retinoid X receptor (RXR) and binds to a PPAR response element (PPRE) to regulate the transcription of numerous target genes [7, 8]. The target genes are involved in cell differentiation, proliferation, immune/inflammation response, and lipid metabolism. PPAR subfamily consists of three members such as PPARα, PPARβ/δ, and PPARγ. PPAR isoforms consist of activation domain (A/B), DNA-binding domain (C), hinge region (D), and ligand-binding domain (E). Each subtype has different characteristics as summarized in Figure 1. PPARα is expressed in brown adipose tissue, liver, kidney, heart, skeletal muscle, and enterocyte. Ligands

for PPARα are fibrates, leukotriene B4, and so on. PPARα is involved in peroxisome proliferation, lipid catabolism, lipid-lowering effect, anti-inflammation, keratinocyte differentiation and proliferation, and skin wound healing. PPARβ/δ is ubiquitously expressed and is involved in reverse cholesterol transport, cell proliferation, apoptosis, and so on. PPARγ is expressed in adipose tissue, colon, immune system, hematopoietic cells, and retina. PPARγ is involved in lipid anabolism, adipocyte differentiation, control of inflammation, macrophage maturation, embryo implantation, and molecular targets of antidiabetic thiazolidinediones (Reviewed in [9]). Of the three PPARs identified to now, PPARγ represents the most promising target in view of the many reports implicating this molecule in cancer cell growth.

2. The Role of PPARα in Colorectal Cancer

Although the procarcinogenic effects of PPARα in rodent hepatocarcinoma are evident, less is known about the role of PPARα in human colorectal cancer. Previous studies showed that activation of PPARα by exogenous agonists causes inhibition of tumor cell growth in cell lines derived from colorectal cancer [10]. However, there is no evidence showing that PPARα expression is elevated in human cancers. Recent studies have shown that aspirin and other nonsteroidal anti-inflammatory drugs reduce the relative risk of developing colorectal cancers [11, 12]. The products of COX activity are known to be involved in carcinogenesis [13–15]. COX-2 is not expressed in most normal tissues but is induced upon stimulation by inflammatory agents, and also by oncogenes, growth factors, carcinogens, and tumor promoters [16–21]. Overexpression of COX-2 contributes to colorectal carcinogenesis by promoting the invasiveness of malignant cells, inhibiting apoptosis, and supporting angiogenesis [22–24]. Furthermore, human colorectal carcinoma patients with COX-2 positive tumors show a significantly poorer prognosis than those with tumors negative for COX-2 [25]. It was recently demonstrated that bile acids, particularly secondary bile acids such as lithocholic acid and chenodeoxycholic acid, can stimulate cell proliferation [26] and act as tumor promoters in colon carcinogenesis [27, 28]. Previous reports have suggested that endogenous bile acids are ligands for nuclear receptors such as farnesoid X receptor (FXR), pregnane X receptor (PXR), and vitamin D receptor (VDR) [29–32]. A recent study reported that bile acids also induce the expression of the PPARα gene via activation of FXR and leads to expression of COX-2 contributing to colorectal carcinogenesis [33]. These data suggest that PPARα has the tumor-promoting activity.

There is a growing importance of chemotherapy for malignant colon cancers. However, resistance to anticancer drugs is still a major obstacle in the failure of chemotherapy in colorectal cancer patients. Tong et al. demonstrated that decreased expression of PPARα confers resistance to hydroxycamptothecin, an inhibitor of topoisomerase I [34]. Thus, they suggest that increased expression of PPARα is necessary to overcome hydroxycamptothecin resistance even though its reason is not clarified.

3. The Role of PPARγ in Colorectal Cancer

The PPARγ is a ligand-activated transcription factor of the nuclear receptor superfamily [35, 36] and is expressed in a variety of malignant tissues including prostate, breast, and colon [37–41]. Upon activation, PPARγ forms heterodimer with RXR and mediate transcriptional activation by binding to the PPRE [7, 8]. In the inactive state, association of various corepressor molecules with PPARγ (e.g., nuclear receptor corepressor or silencing mediator for retinoid receptor and thyroid hormone receptors) prevents this complex from binding to DNA. For transcriptional transactivation of PPARγ, recruitment of coactivators (e.g., CCAAT/enhancer-binding protein, cyclic adenosine monophosphate response-element-binding protein, steroid receptor coactivator-1, receptor-interacting protein 140, PPARγ coactivator-1, and PPARγ binding protein) is required which replace co-repressors from the heterodimer complex. Transcriptional transrepression occurs through a genome independent mechanism and is mediated via physical association of the heterodimer with other activated transcription factors (STAT, NF-κB, and AP-1) thereby blocking their functions (reviewed in [42]). PPARγ has been known to be related to inflammation, immune response, and pathogenesis of some disorders including obesity, atherosclerosis, cancer, and so on [43]. There are natural ligands for PPARγ, including long-chain polyunsaturated fatty acids, eicosanoids, components of oxidized low density lipoproteins (oxLDL) [44], and oxidized alkyl phospholipids. The prostaglandin J_2 derivative, 15d-PGJ_2 is the most potent endogenous ligand for the PPARγ receptor. The antidiabetic thiazolidinedione (TZD) class of drugs including troglitazone, rosiglitazone, pioglitazone and ciglitazone are synthetic ligands for PPARγ [44]. Recent studies have focused on the effect of PPARγ ligands as anticancer agents. However, there are still controversies about the antitumor activity of PPARγ agonists. Thus, this paper describes the role of PPARγ in colorectal cancer and its detailed mechanisms clarified until now.

3.1. The Role of PPARγ as a Tumor Suppressor in Colorectal Cancer. Several studies have focused on the putative association between the various polymorphisms and mutations of the PPARγ gene and the occurrence of cancer. It was described that 4 somatic PPARγ gene mutations resulting in reducing its function occurred in 55 sporadic colon cancers [45]. However, Ikezoe et al. [46] analyzed 397 clinical samples and cell lines including colon, breast, and lung cancers for mutations of PPARγ gene and showed the absence of PPARγ gene mutations. These data suggest that PPARγ mutations may occur in cancers but they are rare.

There has been substantial accumulation of experimental data supporting that synthetic PPARγ ligands as well as 15d-PGJ_2 induce apoptosis in several types of cancer cells [41, 43]. Although increasing evidence has established that PPARγ agonist induces growth arrest in cancer cells, the molecular mechanism of the growth inhibition by PPARγ agonist is not well understood and complicated. This paper describes some of the molecular mechanisms for anticancer activity of PPARγ (Table 1 and Figure 2) as follows.

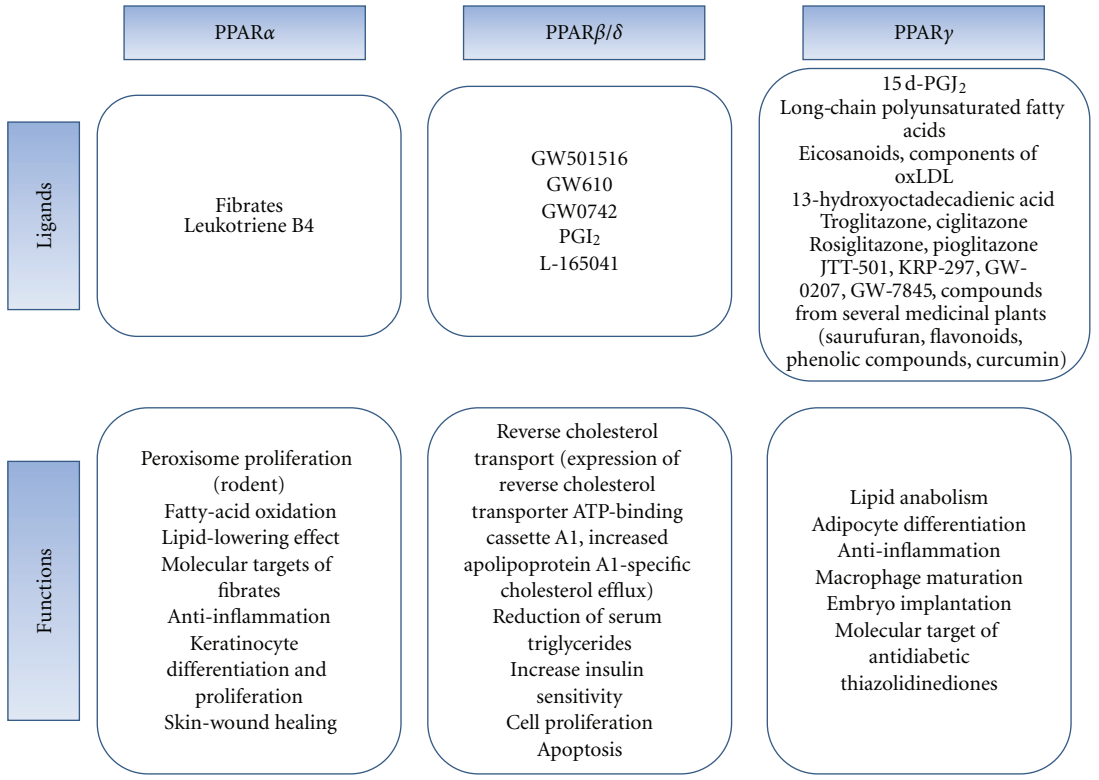

Figure 1: Summary of ligands and functions of each PPAR.

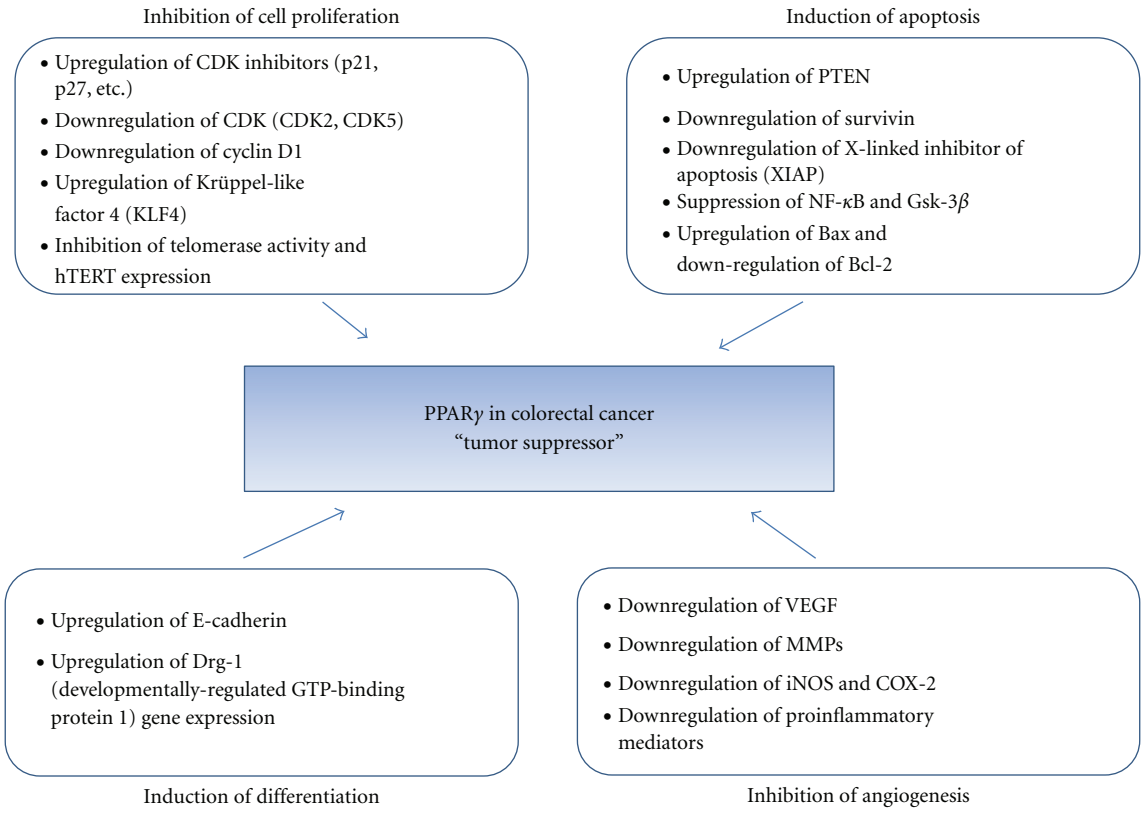

Figure 2: Potential molecular mechanisms for PPARγ as tumor suppressor in colorectal cancer.

Table 1: Potential molecular mechanisms for anticancer activity of PPARγ.

Actions and molecular mechanisms	References
(1) Inhibition of cell proliferation and induction of apoptosis	
(1) Upregulation of PTEN	[47, 48]
(2) Downregulation of survivin	[49]
(3) Downregulation of XIAP	[50, 51]
(4) Suppression of NF-κB	[52]
(5) Upregulation of cyclin-dependent kinase (CDK) inhibitors, downregulation of CDK, and downregulation of cyclin D1	[51, 53–57]
(6) Downregulation of COX-2	[51]
(7) Upregulation of Krüppel-like Factor4 (KLF4)	[58, 59]
(8) Upregulation of Bax and downregulation of Bcl-2	[51, 60, 61]
(9) Inhibition of telomerase activity and hTERT expression	[62]
(2) Induction of cellular differentiation	
Upregulation of E-cadherin and Drg-1 gene expression	[63]
(3) Inhibition of angiogenesis	
(1) Downregulation of vascular endothelial growth factor (VEGF)	[64]
(2) Downregulation of matrix metalloproteinases (MMP)	[65]
(3) Downregulation of iNOS and COX-2	[66–72]
(4) Downregulation of proinflammatory mediators	[73]

3.1.1. Inhibition of Cell Proliferation and Induction of Apoptosis

(1) Upregulation of PTEN. The Phosphatase and Tensin Homolog (PTEN) tumor suppressor gene modulates several cellular functions, including cell migration, survival, and proliferation by inhibiting phosphatidylinositol 3-kinase (PI-3K)-mediated signaling cascades [74]. Previous studies have demonstrated that rosiglitazone, a synthetic ligand for PPARγ, upregulates PTEN expression in Caco2 colorectal cancer cells [47]. Dai et al. also show that treatment of colon cancer cells with rosiglitazone stimulates expression of tumor suppressor gene PTEN. This effect is probably mediated through the binding of PPARγ on PPRE in the promoter of PTEN [48]. Inhibition of the PI-3K/Akt pathway by increased PTEN expression is believed to underlie this effect of the PPARγ ligand.

(2) Downregulation of Survivin. Survivin is one of the inhibitors of apoptosis protein (IAP) family since it is overexpressed in almost every human tumor that has been studied, but is barely detectable in most normal adult tissues [75]. Overexpression of survivin is associated with poor clinical outcome with reduced tumor cell apoptosis in patients with colorectal cancer [76, 77]. PPARγ agonist GW7845 induced cell death through downregulation of survivin in colorectal cancer cells [49].

(3) Downregulation of X-Linked Inhibitor of Apoptosis (XIAP). XIAP can inhibit apoptosis by binding and thereby inactivating caspases including caspase-9 and the effector caspases (-3 and -7) [78]. Qiao et al. showed that 15d-PGJ_2 and troglitazone mediate XIAP downregulation in colon cancer cells by facilitating ubiquitination and proteasomal degradation [50]. In addition, Lee et al. demonstrated that pioglitazone induces apoptosis through downregulation of XIAP via unknown mechanism in colorectal cancer cell lines [51].

(4) Suppression of NF-κB and GSK-3β. The transcription factor NF-κB is involved in the regulation of various genes, including metalloproteinases (MMPs), inflammatory response genes, and a number of antiapoptotic genes including cIAP1, cIAP2, and glycogen synthase kinase-3 (GSK-3) [79]. Its activation is also associated with cell proliferation, cell cycle progression, promotion of tumor growth, angiogenesis, and metastasis through the expression of genes participating in malignant conversion and tumor promotion [80–82]. Ban et al. showed that PPARγ agonist, troglitazone inhibits colon cancer cell growth via inactivation of NF-κB by suppressing GSK-3β activity [52].

(5) Upregulation of Cyclin-Dependent Kinase (CDK) Inhibitors, Downregulation of CDK and Downregulation of Cyclin D1. Interestingly, CDK5 protein expression and kinase activity were significantly inhibited by ciglitazone, which was associated with ciglitazone-induced antiproliferation in colon cancer HT-29 cells [53]. Cyclin D1 is involved in G1/S progression and increased proliferation. PPARγ activation in intestinal epithelial cells results in the inhibition of cell cycle and S-phase entry though a decrease in cyclin D1 expression [54, 55]. PPARγ ligand treatment not only decreases the protein level of cyclin D1, but also increases the CDK inhibitors $p21^{CIP}$ and $p27^{KIP1}$ through both increased transcriptional activity and inhibition of proteasome degradation in colorectal cancer cells [56, 57]. Ciglitazone also inhibited G1/S cell cycle progression through upregulation of p27 and inhibition of Cdk2 activity in HT-29 cells [56]. Fajas et al. [83] suggested that PPARγ activation in the presence of RB results in G1 arrest, whereas in the absence of RB, cells accumulate in G2/M, endoduplicate, and undergo apoptosis. Lee et al. [51] also showed that pioglitazone treatment leads to G2/M block through downregulation of cyclin B1 and cdc2 and upregulation of p21 in RB-deficient human colorectal cancer SNU-C4 and SNU-C2A cells. Thus, these studies suggest that the antiproliferative or proapoptotic effects of PPARγ agonist are associated with its ability to regulate the expression of various genes which are involved in controlling the cell cycle and cell survival/death.

(6) Downregulation of COX-2. Most of the current studies showed that COX-2 contributes to tumorigenesis through various mechanisms and overexpression of COX-2 can

stimulate tumor growth, invasion, and metastasis [84, 85]. A previous study showed that pioglitazone induces apoptosis through the downregulation of COX-2, activation of caspase-3, downregulation of Bcl-2 and upregulation of Bax in RB-deficient human colorectal cancer cells [51].

(7) Upregulation of Krüppel-Like Factor 4 (KLF4). KLF4 is a member of the Krüppel-like zinc finger transcription factor family. It is extensively expressed in the epithelial cells of the gastrointestinal tract [86–88]. Over-expression of KLF4 in colon cancer cells caused inhibition of DNA synthesis and cell growth [89, 90]. Zhi and Tseng demonstrated that 15d-PGJ_2 inhibits proliferation of HT-29 human colon cancer cells and induces upregulation of KLF4 mRNA and protein through the activation of MEK/ERK and STAT-dependent pathway [58]. They provided a novel mechanism for the antitumorigenic actions of 15d-PGJ_2. In addition, rosiglitazone treatment of colorectal cancer cells caused to G1 arrest because increased expression of KLF4 by rosiglitazone leads to increased expression of p21 and decreased expression of cyclin D1 [59]. These data suggest that KLF4 is a nodal player in a network of PPARγ-regulated genes.

(8) Upregulation of Bax and Downregulation of Bcl-2. In colon cancer cells, treatment of the PPARγ ligands (pioglitazone, troglitazone) induces apoptosis through upregulation of the proapoptotic protein Bax and downregulation of the antiapoptpotic protein Bcl-2 [51, 60, 61]. Alternative expression of Bax and Bcl-2 causes apoptosis by the release of cytochrome c and subsequent activation of several effector caspases.

(9) Inhibition of Telomerase Activity and hTERT Expression through Modulation of the Myc/Mad/Max Network. The telomerase stabilizes telomere length by adding TTAGGG repeats to telomeres [91, 92]. Telomerase activity has been detected in almost all human tumors [93, 94] but not in adjacent normal cells [95, 96]. Human telomerase is composed of human telomerase RNA, telomerase-associated protein 1 and human telomerase reverse transcriptase (hTERT) [91, 97]. The forced expression of hTERT in normal human cells has been reported to increase their lifespan [98], while the expression of dominant-negative hTERT in human cancer cells has been known to inhibit telomerase and cause telomere shortening [99, 100]. A recent study shows that 15d-PGJ_2 and rosiglitazone inhibit Caco-2 colon cancer cell proliferation through the inhibition of telomerase activity and hTERT expression. In addition, it was demonstrated that the inhibition of hTERT expression in Caco-2 cells depends on the downregulation of c-Myc and the upregulation of Mad 1 by PPARγ ligands [62].

3.1.2. Induction of Cellular Differentiation. PPARγ has been demonstrated to induce differentiation in solid tumors both *in vitro* and *in vivo* [101]. In colon cancer cells, activation of PPARγ by troglitazone treatment inhibits growth and metastasis through differentiation-promoting effects, such as the marked increase in p21 Waf-1, developmentally regulated GTP-binding protein 1 (DRG-1), and E-cadherin in human colon cancer cells [63]. These effects involve modulation of the E-cadherin/β-catenin system and upregulation of Drg-1 gene expression.

3.1.3. Inhibition of Angiogenesis. Angiogenesis, a formation of new capillaries from the preexisting vessels, is a complex process involved in the degradation of the basement membrane by cellular proteases, the penetration and migration of endothelial cells into the extracellular matrix, endothelial cell proliferation, tube formation, and vessel stabilization [102]. Inhibition of angiogenesis may contribute to the mechanism by which PPARγ agonists halt the cancer process. Several studies demonstrated that PPARγ agonist inhibits angiogenesis through the following mechanisms.

(1) Downregulation of Vascular Endothelial Growth Factor (VEGF). VEGF is involved in angiogenesis [103, 104]. VEGF expression is increased in several cancers including colorectal and other tumors [105, 106]. It was shown that rosiglitazone inhibited angiogenesis via the downregulation of VEGF and VEGF mRNA in pancreatic cancer xenografts [64].

(2) Downregulation of Matrix Metalloproteinases (MMPs). The process of cancer cell invasion is dependent on the degradation of the extracellular matrix (ECM) by MMPs. MMPs are a family of proteases cleaving several macromolecules of the ECM [107]. 15d-PGJ_2 has been reported to have inhibitory effects on the proliferation and invasiveness of colon cancer cell lines which are associated with G1 cell cycle arrest and downregulation of MMP-7 synthesis [65].

(3) Downregulation of iNOS and COX-2. It has been shown that both COX-2 and inducible nitric oxide synthase (iNOS) are overexpressed in various human cancers [108]. It was reported that iNOS is associated with altered expression of important modulators of angiogenesis [108]. 15d-PGJ_2 downregulates iNOS [66–68] and COX-2 [69–71]. The expression of COX-2 and iNOS is regulated by NF-κB. The recent several studies have demonstrated that 15d-PGJ_2 can act as a negative regulator of proinflammatory signaling through blocking the NF-κB activation pathway at multiple levels via covalent modification of NF-κB or its regulators [72]. Thus, antiangiogenic effects of 15d-PGJ_2 might be associated with disruption of NF-κB and subsequent blockade of iNOS and COX-2 expression.

(4) Downregulation of Proinflammatory Mediators. The potential mechanism of angiogenesis inhibition by 15d-PGJ_2 may involve downregulation of pro-inflammatory mediators. Both physiological and pathological angiogenesis can be stimulated by pro-inflammatory cytokines, such as IL-1 and TNF-α. Certain cytokines (e.g., IL-6 and CSF-1) can influence the phenotype and the function of tumor-associated macrophages and indirectly stimulate tumor invasiveness and angiogenesis [109]. Tumor-associated macrophages play an important role in tumor progression due to production

Table 2: Potential molecular mechanisms for procarcinogenic activity of PPARγ.

Actions and molecular mechanisms	References
(1) Stimulation of tumor cell growth	
(1) Upregulation of β-catenin and c-Myc expression	[110]
(2) Upregulation of COX-2	[111]
(2) Induction of angiogenesis	
(1) Upregulation of VEGF and VEGF receptor	[112–114]
(2) Upregulation of MMP-1	[115]

of several angiogenic factors, such as VEGF, IL-8, inflammatory cytokines (IL-1 and IL-10) and proteases (MMP-2 and MMP-9) [109]. Thus, 15d-PGJ_2 inhibits angiogenesis through suppression of such pro-inflammatory cytokines [73]. Induction of several pro-inflammatory cytokines, such as TNF-α, IL-1, and IL-8, is regulated at the transcription level by NF-κB. It is still unclear whether 15d-PGJ_2 exerts an anti-angiogenic effect through inhibition of NF-κB-dependent induction of pro-inflammatory mediators or through downregulation of cancer cell-derived pro-inflammatory cytokine release which is NF-κB-independent. Hence, further investigations are necessary to clarify the signaling pathways that delineate the anti-angiogenic effects of 15d-PGJ_2.

3.2. The Role of PPARγ as a Tumor Promoter in Colorectal Cancer. In contrast to above described, PPARγ has been known to have procarcinogenic activity such as stimulation of tumor cell growth and induction of angiogenesis. This review describes some mechanisms for it as summarized in Table 2 and Figure 3.

3.2.1. Stimulation of Tumor Cell Growth. Although the majority of publications indicate that PPARγ agonists have potent antiproliferative properties in several types of cancer cells, there are some reports demonstrating the cell growth promoting effects of 15d-PGJ_2 and other PPARγ ligands. It was shown that activation of PPARγ by troglitazone increased the frequency and the size of colon tumors in C57BL/6J-$APC^{Min/+}$ mice [116, 117]. In addition, a recent study shows that low concentration of 15d-PGJ_2 and pioglitazone can promote the growth of APC-mutated HT-29 colon cancer cells *in vitro* and *in vivo* [110].

(1) Upregulation of β-Catenin and c-Myc Expression. The Wnt/β-catenin pathway plays a critical role in the development of colon cancer [118]. Choi et al. showed that low concentrations of 15d-PGJ_2 and pioglitazone promote the HT-29 colon cancer cells *in vitro* and *in vivo* through increase in β-catenin and c-Myc expression [110].

(2) Upregulation of COX-2. As previously mentioned, 15d-PGJ_2 is one of the major final products of COX-2. Since abnormal overexpression of COX-2 was observed in several cancer cells, COX-2 has been shown to contribute to carcinogenesis by promoting cell proliferation and angiogenesis as well as by protecting cells from apoptosis [119]. The regulation of COX-2 synthesis occurs mainly at the transcriptional level, although mRNA stabilization is also involved. A recent study has shown that 15d-PGJ_2 enhances COX-2 expression through ROS-Akt-driven AP-1 activation in human breast cancer cells [111].

3.2.2. Induction of Angiogenesis. It has been reported that PPARγ agonist can induce angiogenesis in various cell lines. Several studies provided some molecular mechanisms for induction of angiogenesis by PPARγ agonist. Here, this paper summarizes the potential molecular mechanisms for enhanced metastasis and invasion by PPARγ agonist clarified until now.

(1) Upregulation of Expression of VEGF and VEGF Receptors. It was shown that the mRNA expression of VEGF was augmented by 15d-PGJ_2 and troglitazone in vascular smooth muscle cell, human monocytes/macrophages, human colorectal cancer cells and human coronary artery endothelial cells [112, 113]. More recently, 15d-PGJ_2 and troglitazone have been reported to increase the expression of VEGF and its receptors (Flt-1 and KDR) in myofibroblasts [114].

(2) Upregulation of MMP-1. Kim et al. reported that 15d-PGJ_2 enhances the angiogenesis by upregulation of MMP-1 [115]. MMP-1 is a major proteinase degrading native fibrillar collagens. MMP-1 is produced by a variety of cell types, including endothelium. It is implicated in several pathological processes such as tumor invasion and restenosis [120]. In addition, Kim et al. suggested that iron may contribute to increased metastasis and invasiveness by 15d-PGJ_2 in human breast cancer cells [115]. Thus, these studies suggest the regulation of MMP-1 expression by 15d-PGJ_2 may be more complex than expected.

4. The Role of PPARβ/δ in Colorectal Cancer

PPARβ/δ is also expressed in the colon and can be activated by fatty acids. In recent studies, it was shown that PPARβ/δ plays a central role in the differentiation of Paneth cells and innate immunity [121]. The role of PPARβ/δ in colorectal cancer is more controversial than that of PPARγ. Recent studies have shown that PPARβ/δ is involved in the pathogenesis of colorectal cancer [122]. Inactivation of APC upregulates PPARβ/δ expression in colorectal cancer cells [122]. It has also been reported that PPARβ/δ levels increase in colorectal tumor after treatment with the potent carcinogen azoxymethane (AOM) [123]. The increased expression of PPARβ/δ could potentially be activated by endogenous ligands such as COX-derived prostacyclin [123]. It was proposed that PPARβ/δ activation would initiate the expression of target genes, which still remain to be identified, and enhance cell growth. In support of this model, PPARβ/δ-null HCT116 cells have reduced tumorigenecity in a xenograft model [124]. PPARβ/δ expression levels in colorectal cancers are higher than in normal mucosa, supporting the hypothesis

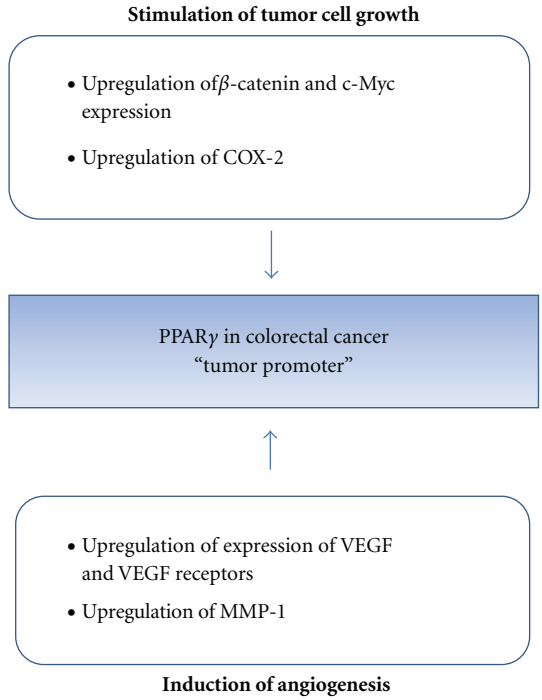

Figure 3: Potential molecular mechanisms for PPARγ as tumor promoter in colorectal cancer.

that APC suppresses activity of β-catenin/Tcf-4 transcription of target genes, including PPARβ/δ, c-myc, and cyclin D1 [122, 123]. PPARβ/δ expression and activity are also induced by oncogenic *K-ras* in rat intestinal epithelial cells [125]. These studies support a procarcinogenic role of PPARβ/δ in colorectal cancer.

A few mechanisms have been proposed to explain the procarcinogenic effect of PPARβ/δ. Di-Poï et al. suggested that PPARβ/δ activation increases the expression of 3-phosphoinositide-dependent-protein kinae 1 (PDPK1) and integrin-linked kinase (ILK), and decreases the expression of PTEN, causing increased phosphorylation of AKT, leading to antiapoptotic signaling and enhanced cell survival [126]. Another related mechanism is derived from the observation that ligand activation of PPARβ/δ increases the expression of VEGF through a PPARβ/δ-dependent mechanism, causing increased phosphorylation of AKT, which promotes cell survival by blocking apoptosis [127]. In addition, Kwak et al. [128] demonstrated that PPARβ/δ-binding aptamers suppressed transcription from natural promoters of VEGF-A and COX-2 and inhibited tumorigenic potential of colon-cancer cells. These data suggest that PPARβ/δ play an important role in transcription of tumor-promoting genes such as VEGF-A and COX-2.

However, other studies conflict with those reports. Targeted deletion of APC alleles reduces PPARβ/δ expression in mouse intestine [129]. PPARβ/δ expression in human colorectal cancers or intestinal polyps of $APC^{Min/+}$ mice are either unchanged or downregulated as compared with normal controls (reviewed in [130, 131]).

The conflicting results about the effect of PPARβ/δ on intestinal tumorigenesis in $APC^{Min/+}$- and AOM-treated mice may be related to differences in the specific targeting strategy employed to delete PPARβ/δ [127]. Deletion of PPARβ/δ exon 4 and/or 5, which encodes an essential portion of the DNA-binding domain, is thought to disrupt PPARβ/δ function as a nuclear transcriptional factor and to inhibit colonic carcinogenesis [127, 132]. Increased expression of VEGF in colon tumors was suppressed by loss of PPARβ/δ expression [133]. These findings indicate that PPARβ/δ has an important role in promoting colonic tumorigenesis. The deletion of exon 8 [134, 135], the last PPARβ/δ exon, is postulated to generate a hypomorphic PPARβ/δ protein that remains at least partly functional.

In a mouse mammary tumor model, treatment with the PPARβ/δ agonist GW501516 accelerated tumor formation, while a PPARγ agonist GW7845 delayed tumor growth [136]. This observation suggests that there are distinct mechanistic differences between PPARγ and PPARβ/δ in regulating tumor progression. A recent study showed that PPARβ/δ confers resistance to PPARγ-induced apoptosis by increasing the expression of survivin [49].

Recently, Yang et al. [137] showed that the specific knockdown of PPARβ/δ in colon-cancer cell lines results in more malignant morphologies, larger colonies and less CEA production, and enhances cell-fibronectin adhesion, without effects on cell invasion and migration. These findings indicate that PPARβ/δ may facilitate differentiation and inhibit the cell-fibronectin adhesion of colon cancer, having a protective role in the carcinogenesis and progression of colon cancer. Further immunohistochemistry data reveal that the expression of PPARβ/δ is closely associated with the differentiation and tumor-node-metastasis stage of rectal cancer. It was also shown that PGI_2 and L-165041, a synthetic PPARδ

ligand, activate PPARδ and upregulate PPARδ-mediated 14-3-3ε expression. 14-3-3ε binds and sequesters Bad in cytosol. PGI_2-induced 14-3-3ε upregulation is accompanied by augmented Bad sequestration and protects HT-29 cells from Bad-triggered mitochondrial leakage of proapoptotic factors and the consequent apoptosis [138].

5. Conclusion and Future Directions

Even though the extensive studies to clarify the role of PPARs in colorectal cancer using several PPAR agonists and gene knockout experiments were performed, there are still many controversies about them. PPAR ligands induce many physiological changes, including increased oxidation of fatty acids, which contributes to decreasing serum lipids and reducing body weight; and inhibition of inflammatory signaling. There are good reasons to suggest that PPAR agonists should be potential candidates for treating and preventing colorectal cancer, because obesity and chronic inflammation are major risk factors for colorectal cancer. It is interesting to note that there is an overlap in target genes regulated by each PPAR, but the physiological effects induced by selective PPAR agonists are unique owing to the complexity of the PPAR-dependent and the PPAR-independent effects that each agonist induces. To completely understand the role of PPARs in colorectal cancer, it is necessary to dissect the complex regulation of PPAR expression and to examine interactions of each PPAR with other nuclear receptors and signalling molecules involved in cell proliferation and cell death in the near future.

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References

[1] A. Jemal, F. Bray, M. M. Center, J. Ferlay, E. Ward, and D. Forman, "Global cancer statistics," *CA Cancer Journal for Clinicians*, vol. 61, no. 2, pp. 69–90, 2011.

[2] A. Jemal, R. Siegel, J. Xu, and E. Ward, "Cancer statistics, 2010," *CA Cancer Journal for Clinicians*, vol. 60, no. 5, pp. 277–300, 2010.

[3] D. M. Parkin, F. Bray, J. Ferlay, and P. Pisani, "Global cancer statistics, 2002," *CA Cancer Journal for Clinicians*, vol. 55, no. 2, pp. 74–108, 2005.

[4] J. Waters and D. Cunningham, "The changing face of chemotherapy in colorectal cancer," *British Journal of Cancer*, vol. 84, no. 1, pp. 1–7, 2001.

[5] D. J. Mangelsdorf, C. Thummel, M. Beato et al., "The nuclear receptor super-family: the second decade," *Cell*, vol. 83, no. 6, pp. 835–839, 1995.

[6] I. Issemann and S. Green, "Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators," *Nature*, vol. 347, no. 6294, pp. 645–650, 1990.

[7] K. Schoonjans, B. Staels, and J. Auwerx, "The peroxisome proliferator activated receptors (PPARs) and their effects on lipid metabolism and adipocyte differentiation," *Biochimica et Biophysica Acta*, vol. 1302, no. 2, pp. 93–109, 1996.

[8] T. M. Willson, P. J. Brown, D. D. Sternbach, and B. R. Henke, "The PPARs: from orphan receptors to drug discovery," *Journal of Medicinal Chemistry*, vol. 43, no. 4, pp. 527–550, 2000.

[9] J. I. Park, "The role of 15d-PGJ_2, a natural ligand for peroxisome proliferator-activated receptor γ (PPARγ), in cancer," in *Cellular and Genetic Practices for Translational Medicine*, pp. 169–195, Research Signpost, 2011.

[10] R. Grau, C. Punzón, M. Fresno, and M. A. Iñiguez, "Peroxisome-proliferator-activated receptor α agonists inhibit cyclo-oxygenase 2 and vascular endothelial growth factor transcriptional activation in human colorectal carcinoma cells via inhibition of activator protein-1," *Biochemical Journal*, vol. 395, no. 1, pp. 81–88, 2006.

[11] L. Rosenberg, J. R. Palmer, A. G. Zauber, M. E. Warshauer, P. D. Stolley, and S. Shapiro, "A hypothesis: nonsteroidal anti-inflammatory drugs reduce the incidence of large bowel cancer," *Journal of the National Cancer Institute*, vol. 83, no. 5, pp. 355–358, 1991.

[12] B. S. Reddy, C. V. Rao, and K. Seibert, "Evaluation of cyclooxygenase-2 inhibitor for potential chemopreventive properties in colon carcinogenesis," *Cancer Research*, vol. 56, no. 20, pp. 4566–4569, 1996.

[13] M. J. Thun, M. M. Namboodiri, and C. W. Heath Jr., "Aspirin use and reduced risk of fatal colon cancer," *The New England Journal of Medicine*, vol. 325, no. 23, pp. 1593–1596, 1991.

[14] R. F. A. Logan, J. Little, P. G. Hawtin, and J. D. Hardcastle, "Effect of aspirin and non-steroidal anti-inflammatory drugs on colorectal adenomas: case-control study of subjects participating in the Nottingham faecal occult blood screening programme," *British Medical Journal*, vol. 307, no. 6899, pp. 285–289, 1993.

[15] D. M. Schreinemachers and R. B. Everson, "Aspirin use and lung, colon, and breast cancer incidence in a prospective study," *Epidemiology*, vol. 5, no. 2, pp. 138–146, 1994.

[16] D. A. Kujubu, B. S. Fletcher, B. C. Varnum, R. W. Lim, and H. R. Herschman, "TIS10, a phorbol ester tumor promoter-inducible mRNA from Swiss 3T3 cells, encodes a novel prostaglandin synthase/cyclooxygenase homologue," *The Journal of Biological Chemistry*, vol. 266, no. 20, pp. 12866–12872, 1991.

[17] D. A. Jones, D. P. Carlton, T. M. McIntyre, G. A. Zimmerman, and S. M. Prescott, "Molecular cloning of human prostaglandin endoperoxide synthase type II and demonstration of expression in response to cytokines," *The Journal of Biological Chemistry*, vol. 268, no. 12, pp. 9049–9054, 1993.

[18] R. N. DuBois, J. Awad, J. Morrow, L. J. Roberts II, and P. R. Bishop, "Regulation of eicosanoid production and mitogenesis in rat intestinal epithelial cells by transforming growth factor-α and phorbol ester," *Journal of Clinical Investigation*, vol. 93, no. 2, pp. 493–498, 1994.

[19] H. Inoue, C. Yokoyama, S. Hara, Y. Tone, and T. Tanabe, "Transcriptional regulation of human prostaglandin-endoperoxide synthase-2 gene by lipopolysaccharide and phorbol ester in vascular endothelial cells. Involvement of both nuclear factor for interleukin-6 expression site and cAMP response element," *The Journal of Biological Chemistry*, vol. 270, no. 42, pp. 24965–24971, 1995.

[20] K. Subbaramaiah, N. Telang, J. T. Ramonetti et al., "Transcription of cyclooxygenase-2 is enhanced in transformed mammary epithelial cells," *Cancer Research*, vol. 56, no. 19, pp. 4424–4429, 1996.

[21] J. R. Mestre, K. Subbaramaiah, P. G. Sacks et al., "Retinoids suppress epidermal growth factor-induced transcription of cyclooxygenase-2 in human oral squamous carcinoma cells," *Cancer Research*, vol. 57, no. 14, pp. 2890–2895, 1997.

[22] M. Tsujii, S. Kawano, and R. N. Dubois, "Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 7, pp. 3336–3340, 1997.

[23] M. Tsujii and R. N. Dubois, "Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2," *Cell*, vol. 83, no. 3, pp. 493–501, 1995.

[24] M. Tsujii, S. Kawano, S. Tsuji, H. Sawaoka, M. Hori, and R. N. Dubois, "Cyclooxygenase regulates angiogenesis induced by colon cancer cells," *Cell*, vol. 93, no. 5, pp. 705–716, 1998.

[25] R. Masunaga, H. Kohno, D. K. Dhar et al., "Cyclooxygenase-2 expression correlates with tumor neovascularization and prognosis in human colorectal carcinoma patients," *Clinical Cancer Research*, vol. 6, no. 10, pp. 4064–4068, 2000.

[26] P. A. Craven, J. Pfanstiel, and F. R. DeRubertis, "Role of activation of protein kinase C in the stimulation of colonic epithelial proliferation and reactive oxygen formation by bile acids," *Journal of Clinical Investigation*, vol. 79, no. 2, pp. 532–541, 1987.

[27] T. Narisawa, N. E. Magadia, J. H. Weisburger, and E. L. Wynder, "Promoting effect of bile acids on colon carcinogenesis after intrarectal instillation of N-methyl-N′-nitro-N-nitrosoguanidine in rats," *Journal of the National Cancer Institute*, vol. 53, no. 4, pp. 1093–1097, 1974.

[28] U. G. Allinger, G. K. Johansson, J. A. Gustafsson, and J. J. Rafter, "Shift from a mixed to a lactovegetarian diet: influence on acidic lipids in fecal water—a potential risk factor for colon cancer," *American Journal of Clinical Nutrition*, vol. 50, no. 5, pp. 992–996, 1989.

[29] M. Makishima, A. Y. Okamoto, J. J. Repa et al., "Identification of a nuclear receptor for bite acids," *Science*, vol. 284, no. 5418, pp. 1362–1365, 1999.

[30] D. J. Parks, S. G. Blanchard, R. K. Bledsoe et al., "Bile acids: natural ligands for an orphan nuclear receptor," *Science*, vol. 284, no. 5418, pp. 1365–1368, 1999.

[31] J. L. Staudinger, B. Goodwin, S. A. Jones et al., "The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 6, pp. 3369–3374, 2001.

[32] M. Makishima, T. T. Lu, W. Xie et al., "Vitamin D receptor as an intestinal bile acid sensor," *Science*, vol. 296, no. 5571, pp. 1313–1316, 2002.

[33] H. Oshio, T. Abe, T. Onogawa et al., "Peroxisome proliferator-activated receptor α activates cyclooxygenase-2 gene transcription through bile acid transport in human colorectal cancer cell lines," *Journal of Gastroenterology*, vol. 43, no. 7, pp. 538–549, 2008.

[34] J. L. Tong, C. P. Zhang, F. Nie et al., "MicroRNA 506 regulates expression of PPARα in hydroxycamptothecin-resistant human colon cancer cells," *FEBS Letters*, vol. 585, no. 22, pp. 3560–3568, 2011.

[35] R. M. Evans, "The steroid and thyroid hormone receptor superfamily," *Science*, vol. 240, no. 4854, pp. 889–895, 1988.

[36] K. Schoonjans, G. Martin, B. Staels, and J. Auwerx, "Peroxisome proliferator-activated receptors, orphans with ligands and functions," *Current Opinion in Lipidology*, vol. 8, no. 3, pp. 159–166, 1997.

[37] J. J. Mansure, R. Nassim, and W. Kassouf, "Peroxisome proliferator-activated receptor γ in bladder cancer: a promising therapeutic target," *Cancer Biology and Therapy*, vol. 8, no. 7, pp. 1–9, 2009.

[38] R. N. Dubois, R. Gupta, J. Brockman, B. S. Reddy, S. L. Krakow, and M. A. Lazar, "The nuclear eicosanoid receptor, PPARγ, is aberrantly expressed in colonic cancers," *Carcinogenesis*, vol. 19, no. 1, pp. 49–53, 1998.

[39] P. Sarraf, E. Mueller, D. Jones et al., "Differentiation and reversal of malignant changes in colon cancer through PPARγ," *Nature Medicine*, vol. 4, no. 9, pp. 1046–1052, 1998.

[40] E. Mueller, P. Sarraf, P. Tontonoz et al., "Terminal differentiation of human breast cancer through PPARγ," *Molecular Cell*, vol. 1, no. 3, pp. 465–470, 1998.

[41] T. Kubota, K. Koshizuka, E. A. Williamson et al., "Ligand for peroxisome proliferator-activated receptor γ (troglitazone) has potent antitumor effect against human prostate cancer both in vitro and in vivo," *Cancer Research*, vol. 58, no. 15, pp. 3344–3352, 1998.

[42] A. Krishnan, S. A. Nair, and M. R. Pillai, "Biology of PPARγ in cancer: a critical review on existing lacunae," *Current Molecular Medicine*, vol. 7, no. 6, pp. 532–540, 2007.

[43] C. A. de la Lastra, S. Sánchez-Fidalgo, I. Villegas, and V. Motilva, "New pharmacological perspectives and therapeutic potential of PPAR-γ agonists," *Current Pharmaceutical Design*, vol. 10, no. 28, pp. 3505–3524, 2004.

[44] T. Wang, J. Xu, X. Yu, R. Yang, and Z. C. Han, "Peroxisome proliferator-activated receptor γ in malignant diseases," *Critical Reviews in Oncology/Hematology*, vol. 58, no. 1, pp. 1–14, 2006.

[45] P. Sarraf, E. Mueller, W. M. Smith et al., "Loss-of-function mutations in PPARγ associated with human colon cancer," *Molecular Cell*, vol. 3, no. 6, pp. 799–804, 1999.

[46] T. Ikezoe, C. W. Miller, S. Kawano et al., "Mutational analysis of the peroxisome proliferator-activated receptor γ in human malignancies," *Cancer Research*, vol. 61, no. 13, pp. 5307–5310, 2001.

[47] L. Patel, I. Pass, P. Coxon, C. P. Downes, S. A. Smith, and C. H. Macphee, "Tumor suppressor and anti-inflammatory actions of PPARγ agonists are mediated via upregulation of PTEN," *Current Biology*, vol. 11, no. 10, pp. 764–768, 2001.

[48] Y. Dai, L. Qiao, K. W. Chan et al., "Loss of XIAP sensitizes rosiglitazone-induced growth inhibition of colon cancer in vivo," *International Journal of Cancer*, vol. 122, no. 12, pp. 2858–2863, 2008.

[49] D. Wang, W. Ning, D. Xie, L. Guo, and R. N. Dubois, "Peroxisome proliferator-activated receptor δ confers resistance to peroxisome proliferator-activated receptor γ-induced apoptosis in colorectal cancer cells," *Oncogene*, vol. 31, no. 8, pp. 1013–1023, 2012.

[50] L. Qiao, Y. Dai, Q. Gu et al., "Downregulation of X-linked inhibitor of apoptosis synergistically enhanced peroxisome proliferator-activated receptor γ ligand-induced growth inhibition in colon cancer," *Molecular Cancer Therapeutics*, vol. 7, no. 7, pp. 2203–2211, 2008.

[51] C. J. Lee, J. S. Han, C. Y. Seo et al., "Pioglitazone, a synthetic ligand for PPARγ, induces apoptosis in RB-deficient human colorectal cancer cells," *Apoptosis*, vol. 11, no. 3, pp. 401–411, 2006.

[52] J. O. Ban, D. H. Kwak, J. H. Oh et al., "Suppression of NF-κB and GSK-3β is involved in colon cancer cell growth inhibition by the PPAR agonist troglitazone," *Chemico-Biological Interactions*, vol. 188, no. 1, pp. 75–85, 2010.

[53] E. Kim, F. Chen, C. C. Wang, and L. E. Harrison, "CDK5 is a novel regulatory protein in PPARγ ligand-induced antiproliferation," *International Journal of Oncology*, vol. 28, no. 1, pp. 191–194, 2006.

[54] S. Kitamura, Y. Miyazaki, S. Hiraoka et al., "PPARγ agonists inhibit cell growth and suppress the expression of cyclin D1 and EGF-like growth factors in ras-transformed rat intestinal epithelial cells," *International Journal of Cancer*, vol. 94, no. 3, pp. 335–342, 2001.

[55] A. Chen and J. Xu, "Activation of PPARγ by curcumin inhibits Moser cell growth and mediates suppression of gene expression of cyclin D1 and EGFR," *American Journal of Physiology—Gastrointestinal and Liver Physiology*, vol. 288, no. 3, pp. G447–G456, 2005.

[56] F. Chen and L. E. Harrison, "Ciglitazone-induced cellular anti-proliferation increases p27 kip1 protein levels through both increased transcriptional activity and inhibition of proteasome degradation," *Cellular Signalling*, vol. 17, no. 7, pp. 809–816, 2005.

[57] F. Chen, E. Kim, C. C. Wang, and L. E. Harrison, "Ciglitazone-induced p27 gene transcriptional activity is mediated through Sp1 and is negatively regulated by the MAPK signaling pathway," *Cellular Signalling*, vol. 17, no. 12, pp. 1572–1577, 2005.

[58] Y. C. Zhi and C. C. Tseng, "15-Deoxy-$\Delta^{12,14}$ prostaglandin J_2 upregulates krüppel-like factor 4 expression independently of peroxisome proliferator-activated receptor γ by activating the mitogen-activated protein kinase kinase/extracellular signal-regulated kinase signal transduction pathway in HT-29 colon cancer cells," *Molecular Pharmacology*, vol. 68, no. 5, pp. 1203–1213, 2005.

[59] J. Rageul, S. Mottier, A. Jarry et al., "KLF4-dependent, PPARγ-induced expression of GPA33 in colon cancer cell lines," *International Journal of Cancer*, vol. 125, no. 12, pp. 2802–2809, 2009.

[60] G. G. Chen, H. Xu, J. F. Y. Lee et al., "15-Hydroxy-eicosatetraenoic acid arrests growth of colorectal cancer cells via a peroxisome proliferator-activated receptor gamma-dependent pathway," *International Journal of Cancer*, vol. 107, no. 5, pp. 837–843, 2003.

[61] G. G. Chen, J. F. Y. Lee, S. H. Wang, U. P. F. Chan, P. C. Ip, and W. Y. Lau, "Apoptosis induced by activation of peroxisome-proliferator activated receptor-gamma is associated with Bcl-2 and NF-κB in human colon cancer," *Life Sciences*, vol. 70, no. 22, pp. 2631–2646, 2002.

[62] C. Toaldo, S. Pizzimenti, A. Cerbone et al., "PPARγ ligands inhibit telomerase activity and hTERT expression through modulation of the Myc/Mad/Max network in colon cancer cells," *Journal of Cellular and Molecular Medicine*, vol. 14, no. 6, pp. 1347–1357, 2010.

[63] T. Yoshizumi, T. Ohta, I. Ninomiya et al., "Thiazolidinedione, a peroxisome proliferator-activated receptor-gamma ligand, inhibits growth and metastasis of HT-29 human colon cancer cells through differentiation-promoting effects," *International Journal of Oncology*, vol. 25, no. 3, pp. 631–639, 2004.

[64] Y. D. Dong, X. P. Wang, and K. Wu, "Suppression of pancreatic carcinoma growth by activating peroxisome proliferator-activated receptor γ involves angiogenesis inhibition," *World Journal of Gastroenterology*, vol. 15, no. 4, pp. 441–448, 2009.

[65] D. Shen, C. Deng, and M. Zhang, "Peroxisome proliferator-activated receptor γ agonists inhibit the proliferation and invasion of human colon cancer cells," *Postgraduate Medical Journal*, vol. 83, no. 980, pp. 414–419, 2007.

[66] S. Giri, R. Rattan, A. K. Singh, and I. Singh, "The 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 inhibits the inflammatory response in primary rat astrocytes via downregulating multiple steps in phosphatidylinositol 3-kinase-Akt-NF-κB-p300 pathway independent of peroxisome proliferator-activated receptor γ," *Journal of Immunology*, vol. 173, no. 8, pp. 5196–5208, 2004.

[67] T. V. Petrova, K. T. Akama, and L. J. van Eldik, "Cyclopentenone prostaglandins suppress activation of microglia: downregulation of inducible nitric-oxide synthase by 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 8, pp. 4668–4673, 1999.

[68] P. K. Chatterjee, N. S. A. Patel, S. Cuzzocrea et al., "The cyclopentenone prostaglandin 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 ameliorates ischemic acute renal failure," *Cardiovascular Research*, vol. 61, no. 3, pp. 630–643, 2004.

[69] H. Inoue, T. Tanabe, and K. Umesono, "Feedback control of cyclooxygenase-2 expression through PPARγ," *The Journal of Biological Chemistry*, vol. 275, no. 36, pp. 28028–28032, 2000.

[70] H. Fahmi, J. P. Pelletier, F. Mineau, and J. Martel-Pelletier, "15d-PGJ_2 is acting as a "dual agent" on the regulation of COX-2 expression in human osteoarthritic chondrocytes," *Osteoarthritis and Cartilage*, vol. 10, no. 11, pp. 845–848, 2002.

[71] K. Subbaramaiah, D. T. Lin, J. C. Hart, and A. J. Dannenberg, "Peroxisome proliferator-activated receptor γ ligands suppress the transcriptional activation of cyclooxygenase-2. Evidence for involvement of activator protein-1 and CREB-binding protein/p300," *The Journal of Biological Chemistry*, vol. 276, no. 15, pp. 12440–12448, 2001.

[72] E. H. Kim and Y. J. Surh, "15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 as a potential endogenous regulator of redox-sensitive transcription factors," *Biochemical Pharmacology*, vol. 72, no. 11, pp. 1516–1528, 2006.

[73] Y. Azuma, M. Shinohara, P. L. Wang, and K. Ohura, "15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 inhibits IL-10 and IL-12 production by macrophages," *Biochemical and Biophysical Research Communications*, vol. 283, no. 2, pp. 344–346, 2001.

[74] K. M. Yamada and M. Araki, "Tumor suppressor PTEN: modulator of cell signaling, growth, migration and apoptosis," *Journal of Cell Science*, vol. 114, no. 13, pp. 2375–2382, 2001.

[75] D. C. Altieri, "Validating survivin as a cancer therapeutic target," *Nature Reviews Cancer*, vol. 3, no. 1, pp. 46–54, 2003.

[76] H. Kawasaki, D. C. Altieri, C. D. Lu, M. Toyoda, T. Tenjo, and N. Tanigawa, "Inhibition of apoptosis by survivin predicts shorter survival rates in colorectal cancer," *Cancer Research*, vol. 58, no. 22, pp. 5071–5074, 1998.

[77] A. I. Sarela, N. Scott, J. Ramsdale, A. F. Markham, and P. J. Guillou, "Immunohistochemical detection of the anti-apoptosis protein, survivin, predicts survival after curative resection of stage II colorectal carcinomas," *Annals of Surgical Oncology*, vol. 8, no. 4, pp. 305–310, 2001.

[78] T. Kaufmann, A. Strasser, and P. J. Jost, "Fas death receptor signalling: roles of Bid and XIAP," *Cell Death and Differentiation*, vol. 19, no. 1, pp. 42–50, 2012.

[79] S. Ghosh, M. J. May, and E. B. Kopp, "NF-κB and rel proteins: evolutionarily conserved mediators of immune responses," *Annual Review of Immunology*, vol. 16, pp. 225–260, 1998.

[80] F. R. Greten, L. Eckmann, T. F. Greten et al., "IKKβ links inflammation and tumorigenesis in a mouse model of colitis-associated cancer," *Cell*, vol. 118, no. 3, pp. 285–296, 2004.

[81] E. Pikarsky, R. M. Porat, I. Stein et al., "NF-κB functions as a tumour promoter in inflammation-associated cancer," *Nature*, vol. 431, no. 7007, pp. 461–466, 2004.

[82] A. Garg and B. B. Aggarwal, "Nuclear transcription factor-κB as a target for cancer drug development," *Leukemia*, vol. 16, no. 6, pp. 1053–1068, 2002.

[83] L. Fajas, V. Egler, R. Reiter, S. Miard, A. M. Lefebvre, and J. Auwerx, "PPARγ controls cell proliferation and apoptosis in an RB-dependent manner," *Oncogene*, vol. 22, no. 27, pp. 4186–4193, 2003.

[84] S. Lu, G. Yu, Y. Zhu, and M. C. Archer, "Cyclooxygenase-2 overexpression in MCF-10F human breast epithelial cells inhibits proliferation, apoptosis and differentiation, and causes partial transformation," *International Journal of Cancer*, vol. 116, no. 6, pp. 847–852, 2005.

[85] S. Gately and W. W. Li, "Multiple roles of COX-2 in tumor angiogenesis: a target for antiangiogenic therapy," *Seminars in Oncology*, vol. 31, no. 7, pp. 2–11, 2004.

[86] D. T. Dang, J. Pevsner, and V. W. Yang, "The biology of the mammalian Krüppel-like family of transcription factors," *International Journal of Biochemistry and Cell Biology*, vol. 32, no. 11-12, pp. 1103–1121, 2000.

[87] J. L. Shie, Z. Y. Chen, M. J. O'Brien, R. G. Pestell, M. E. Lee, and C. C. Tseng, "Role of gut-enriched Krüppel-like factor in colonic cell growth and differentiation," *American Journal of Physiology—Gastrointestinal and Liver Physiology*, vol. 279, no. 4, pp. G806–G814, 2000.

[88] J. J. Bieker, "Krüppel-like factors: three fingers in many pies," *The Journal of Biological Chemistry*, vol. 276, no. 37, pp. 34355–34358, 2001.

[89] Z. Y. Chen, J. L. Shie, and C. C. Tseng, "Upregulation of gut-enriched krüppel-like factor by interferon-γ in human colon carcinoma cells," *FEBS Letters*, vol. 477, no. 1-2, pp. 67–72, 2000.

[90] D. T. Dang, X. Chen, J. Feng, M. Torbenson, L. H. Dang, and V. W. Yang, "Overexpression of Krüppel-like factor 4 in the human colon cancer cell line RKO leads to reduced tumorigenecity," *Oncogene*, vol. 22, no. 22, pp. 3424–3430, 2003.

[91] K. Dhaene, E. van Marck, and R. Parwaresch, "Telomeres, telomerase and cancer: an up-date," *Virchows Archiv*, vol. 437, no. 1, pp. 1–16, 2000.

[92] J. W. Shay and W. E. Wright, "Telomerase therapeutics for cancer: challenges and new directions," *Nature Reviews Drug Discovery*, vol. 5, no. 7, pp. 577–584, 2006.

[93] E. Hiyama, T. Yokoyama, N. Tatsumoto et al., "Telomerase activity in gastric cancer," *Cancer Research*, vol. 55, no. 15, pp. 3258–3262, 1995.

[94] C. Chadeneau, K. Hay, H. W. Hirte, S. Gallinger, and S. Bacchetti, "Telomerase activity associated with acquisition of malignancy in human colorectal cancer," *Cancer Research*, vol. 55, no. 12, pp. 2533–2536, 1995.

[95] A. G. Bodnar, M. Ouellette, M. Frolkis et al., "Extension of life-span by introduction of telomerase into normal human cells," *Science*, vol. 279, no. 5349, pp. 349–352, 1998.

[96] H. Vaziri and S. Benchimol, "Reconstitution of telomerase activity in normal human cells leads to elongation of telomeres and extended replicative life span," *Current Biology*, vol. 8, no. 5, pp. 279–282, 1998.

[97] A. K. Meeker and A. M. de Marzo, "Recent advances in telomere biology: implications for human cancer," *Current Opinion in Oncology*, vol. 16, no. 1, pp. 32–38, 2004.

[98] C. P. Morales, S. E. Holt, M. Ouellette et al., "Absence of cancer-associated changes in human fibroblasts immortalized with telomerase," *Nature Genetics*, vol. 21, no. 1, pp. 115–118, 1999.

[99] W. C. Hahn, S. A. Stewart, M. W. Brooks et al., "Inhibition of telomerase limits the growth of human cancer cells," *Nature Medicine*, vol. 5, no. 10, pp. 1164–1170, 1999.

[100] F. Delhommeau, A. Thierry, D. Feneux et al., "Telomere dysfunction and telomerase reactivation in human leukemia cell lines after telomerase inhibition by the expression of a dominant-negative hTERT mutant," *Oncogene*, vol. 21, no. 54, pp. 8262–8271, 2002.

[101] H. Kawamata, M. Tachibana, T. Fujimori, and Y. Imai, "Differentiation-inducing therapy for solid tumors," *Current Pharmaceutical Design*, vol. 12, no. 3, pp. 379–385, 2006.

[102] W. Risau, "Mechanisms of angiogenesis," *Nature*, vol. 386, no. 6626, pp. 671–674, 1997.

[103] N. Ferrara, H. P. Gerber, and J. LeCouter, "The biology of VEGF and its receptors," *Nature Medicine*, vol. 9, no. 6, pp. 669–676, 2003.

[104] P. Carmeliet, "VEGF as a key mediator of angiogenesis in cancer," *Oncology*, vol. 69, no. 3, pp. 4–10, 2005.

[105] J. C. Lee, N. H. Chow, S. T. Wang, and S. M. Huang, "Prognostic value of vascular endothelial growth factor expression in colorectal cancer patients," *European Journal of Cancer*, vol. 36, no. 6, pp. 748–753, 2000.

[106] R. T. P. Poon, S. T. Fan, and J. Wong, "Clinical implications of circulating angiogenic factors in cancer patients," *Journal of Clinical Oncology*, vol. 19, no. 4, pp. 1207–1225, 2001.

[107] M. D. Sternlicht and Z. Werb, "How matrix metalloproteinases regulate cell behavior," *Annual Review of Cell and Developmental Biology*, vol. 17, pp. 463–516, 2001.

[108] B. B. Aggarwal, S. Shishodia, S. K. Sandur, M. K. Pandey, and G. Sethi, "Inflammation and cancer: how hot is the link?" *Biochemical Pharmacology*, vol. 72, no. 11, pp. 1605–1621, 2006.

[109] S. Minuzzo, L. Moserle, S. Indraccolo, and A. Amadori, "Angiogenesis meets immunology: cytokine gene therapy of cancer," *Molecular Aspects of Medicine*, vol. 28, no. 1, pp. 59–86, 2007.

[110] I. K. Choi, Y. H. Kim, J. S. Kim, and J. H. Seo, "PPAR-γ ligand promotes the growth of APC-mutated HT-29 human colon cancer cells in vitro and in vivo," *Investigational New Drugs*, vol. 26, no. 3, pp. 283–288, 2008.

[111] E. H. Kim, H. K. Na, D. H. Kim et al., "15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 induces COX-2 expression through Akt-driven AP-1 activation in human breast cancer cells: a potential role of ROS," *Carcinogenesis*, vol. 29, no. 4, pp. 688–695, 2008.

[112] K. Yamakawa, M. Hosoi, H. Koyama et al., "Peroxisome proliferator-activated receptor-γ agonists increase vascular endothelial growth factor expression in human vascular smooth muscle cells," *Biochemical and Biophysical Research Communications*, vol. 271, no. 3, pp. 571–574, 2000.

[113] M. Inoue, H. Itoh, T. Tanaka et al., "Oxidized LDL regulates vascular endothelial growth factor expression in human macrophages and endothelial cells through activation of peroxisome proliferator-activated receptor-γ," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 21, no. 4, pp. 560–566, 2001.

[114] V. Chintalgattu, G. S. Harris, S. M. Akula, and L. C. Katwa, "PPAR-γ agonists induce the expression of VEGF and its receptors in cultured cardiac myofibroblasts," *Cardiovascular Research*, vol. 74, no. 1, pp. 140–150, 2007.

[115] D. H. Kim, J. H. Kim, E. H. Kim et al., "15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 upregulates the expression of heme oxygenase-1 and subsequently matrix metalloproteinase-1 in human breast cancer cells: possible roles of iron and ROS," *Carcinogenesis*, vol. 30, no. 4, pp. 645–654, 2009.

[116] A. M. Lefebvre, I. Chen, P. Desreumaux et al., "Activation of the peroxisome proliferator-activated receptor γ promotes the development of colon tumors in C57BL/6J-APC$^{Min/+}$ mice," *Nature Medicine*, vol. 4, no. 9, pp. 1053–1057, 1998.

[117] E. Saez, P. Tontonoz, M. C. Nelson et al., "Activators of the nuclear receptor PPARγ enhance colon polyp formation," *Nature Medicine*, vol. 4, no. 9, pp. 1058–1061, 1998.

[118] R. Z. Karim, G. M. K. Tse, T. C. Putti, R. A. Scolyer, and C. S. Lee, "The significance of the Wnt pathway in the pathology of human cancers," *Pathology*, vol. 36, no. 2, pp. 120–128, 2004.

[119] D. Wang and R. N. Dubois, "Prostaglandins and cancer," *Gut*, vol. 55, no. 1, pp. 115–122, 2006.

[120] A. Józkowicz, I. Huk, A. Nigisch, J. Cisowski, G. Weigel, and J. Dulak, "Prostaglandin-J_2 upregulates expression of matrix metalloproteinase-1 independently of activation of peroxisome proliferator-activated receptor-γ," *Acta Biochimica Polonica*, vol. 50, no. 3, pp. 677–689, 2003.

[121] F. Varnat, B. B. Heggeler, P. Grisel et al., "PPARβ/δ regulates paneth cell differentiation via controlling the hedgehog signaling pathway," *Gastroenterology*, vol. 131, no. 2, pp. 538–553, 2006.

[122] T. C. He, T. A. Chan, B. Vogelstein, and K. W. Kinzler, "PPARδ is an APC-regulated target of nonsteroidal anti-inflammatory drugs," *Cell*, vol. 99, no. 3, pp. 335–345, 1999.

[123] R. A. Gupta, J. Tan, W. F. Krause et al., "Prostacyclin-mediated activation of peroxisome proliferator-activated receptor δ in colorectal cancer," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 24, pp. 13275–13280, 2000.

[124] B. H. Park, B. Vogelstein, and K. W. Kinzler, "Genetic disruption of PPARδ decreases the tumorigenicity of human colon cancer cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 5, pp. 2598–2603, 2001.

[125] J. Shao, H. Sheng, and R. N. Dubois, "Peroxisome proliferator-activated receptors modulate K-Ras-mediated transformation of intestinal epithelial cells," *Cancer Research*, vol. 62, no. 11, pp. 3282–3288, 2002.

[126] N. Di-Poï, N. S. Tan, L. Michalik, W. Wahli, and B. Desvergne, "Antiapoptotic role of PPARβ in keratinocytes via transcriptional control of the Akt1 signaling pathway," *Molecular Cell*, vol. 10, no. 4, pp. 721–733, 2002.

[127] D. Wang, H. Wang, Y. Guo et al., "Crosstalk between peroxisome proliferator-activated receptor δ and VEGF stimulates cancer progression," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 50, pp. 19069–19074, 2006.

[128] H. Kwak, I. Hwang, J. H. Kim, Y. K. Mee, J. S. Yang, and S. Jeong, "Modulation of transcription by the peroxisome proliferator-activated receptor δ-binding RNA aptamer in colon cancer cells," *Molecular Cancer Therapeutics*, vol. 8, no. 9, pp. 2664–2673, 2009.

[129] K. R. Reed, O. J. Sansom, A. J. Hayes et al., "PPARδ status and Apc-mediated tumourigenesis in the mouse intestine," *Oncogene*, vol. 23, no. 55, pp. 8992–8996, 2004.

[130] J. M. Peters and F. J. Gonzalez, "Sorting out the functional role(s) of peroxisome proliferator-activated receptor-β/δ (PPARβ/δ) in cell proliferation and cancer," *Biochimica et Biophysica Acta*, vol. 1796, no. 2, pp. 230–241, 2009.

[131] J. M. Peters, H. E. Hollingshead, and F. J. Gonzalez, "Role of peroxisome-proliferator-activated receptor β/δ (PPARβ/δ) in gastrointestinal tract function and disease," *Clinical Science*, vol. 115, no. 3-4, pp. 107–127, 2008.

[132] K. Nadra, S. I. Anghel, E. Joye et al., "Differentiation of trophoblast giant cells and their metabolic functions are dependent on peroxisome proliferator-activated receptor β/δ," *Molecular and Cellular Biology*, vol. 26, no. 8, pp. 3266–3281, 2006.

[133] X. Zuo, Z. Peng, M. J. Moussalli et al., "Targeted genetic disruption of peroxisome proliferator-activated receptor-δ and colonic tumorigenesis," *Journal of the National Cancer Institute*, vol. 101, no. 10, pp. 762–767, 2009.

[134] F. S. Harman, C. J. Nicol, H. E. Marin, J. M. Ward, F. J. Gonzalez, and J. M. Peters, "Peroxisome proliferator-activated receptor-δ attenuates colon carcinogenesis," *Nature Medicine*, vol. 10, no. 5, pp. 481–483, 2004.

[135] J. M. Peters, S. S. T. Lee, W. Li et al., "Growths, adipose, brain, and skin alterations resulting from targeted disruption of the mouse peroxisome proliferator-activated receptor $\beta(\delta)$," *Molecular and Cellular Biology*, vol. 20, no. 14, pp. 5119–5128, 2000.

[136] L. Kopelovich, Y. Yin, R. G. Russell et al., "Peroxisome proliferator-activator receptor δ and γ agonists differentially alter tumor differentiation and progression during mammary carcinogenesis," *Cancer Research*, vol. 65, no. 9, pp. 3950–3957, 2005.

[137] L. Yang, B. Olsson, D. Pfeifer et al., "Knockdown of peroxisome proliferator-activated receptor-β induces less differentiation and enhances cell-fibronectin adhesion of colon cancer cells," *Oncogene*, vol. 29, no. 4, pp. 516–526, 2010.

[138] K. K. Wu and J. Y. Liou, "Cyclooxygenase inhibitors induce colon cancer cell apoptosis via PPARδ $\rightarrow$ 14-3-3ε pathway," *Methods in Molecular Biology*, vol. 512, pp. 295–307, 2009.

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Review Article

PPARγ in Inflammatory Bowel Disease

Vito Annese, Francesca Rogai, Alessia Settesoldi, and Siro Bagnoli

Gastroenterology Unit, Careggi, University Hospital, 50134 Florence, Italy

Correspondence should be addressed to Vito Annese, annesev@aou-careggi.toscana.it

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Peroxisome proliferator-activated receptor gamma (PPARγ) is member of a family of nuclear receptors that interacts with nuclear proteins acting as coactivators and corepressors. The colon is a major tissue which expresses PPARγ in epithelial cells and, to a lesser degree, in macrophages and lymphocytes and plays a role in the regulation of intestinal inflammation. Indeed, both natural and synthetic PPARγ ligands have beneficial effects in different models of experimental colitis, with possible implication in the therapy of inflammatory bowel disease (IBD). This paper will specifically focus on potential role of PPARγ in the predisposition and physiopathology of IBD and will analyze its possible role in medical therapy.

1. Introduction

The peroxisome proliferator-activated receptor gamma (PPARγ) is a nuclear receptor highly expressed in adipose tissue but also intestine, playing a key role in regulation of insulin resistance and inflammation. Recently its role in intestinal diseases, especially colon cancer and intestinal inflammation, is emerging. The discovery that it is the major functional receptor mediating the aminosalicylate activities in inflammatory bowel diseases (IBD) has further enhanced the interest for the role of this receptor in the regulation of gut homeostasis, with possible implication for newer therapeutic targeting. After an extensive search of medical literature in English language from the PubMed database, we aim in this paper to focus on potential role of PPARγ in the predisposition and physiopathology of IBD and to analyze its role in experimental colitis and potential therapy for IBD.

2. IBD and PPARγ: Friend or Foe

The inflammatory bowel diseases (IBD), Crohn's disease (CD), and ulcerative colitis (UC) are common causes of gastrointestinal illness characterised by chronic, relapsing intestinal inflammation, often presenting in early childhood [1]. The incidence varies according to geographical location and in Northern Europe IBD may affect upto one in two hundred of the population [2]. The division into CD and UC is made on the basis of clinical, radiological, endoscopic, and histological features. Common clinical features of CD include abdominal pain, diarrhea, weight loss, and fever. Rectal blood loss is not always a feature and up to 10% of patients with CD may not have diarrhea. Inflammatory changes are patchy in distribution and may occur anywhere within the gastrointestinal tract from the mouth to the anus. Approximately 40% of patients with CD will have disease involving both small and large bowel, in 30% the disease is limited to the small bowel, and 27% percent will have colonic disease only. A small minority of patients will have involvement of the more proximal gastrointestinal tract. Inflammation is transmural and histological examination of bowel and lymph nodes will demonstrate epithelioid cell granulomas in 60–70% of cases. In contrast, patients with UC usually present with bloody diarrhea [3]. There may be associated abdominal pain, urgency, and tenesmus. The disease is limited to the mucosal layer of the colon; it will always involve the rectum and may extend proximally in a continuous fashion.

Current knowledge of aetiology is incomplete, but increasing evidence points towards a combination of environmental triggers in a genetically susceptible individual. More specifically, the intestinal inflammation is thought to result from an inappropriate immune response to microbial antigens of commensal microorganisms [4]. Both diseases manifest themselves primarily in the gastrointestinal tract yet

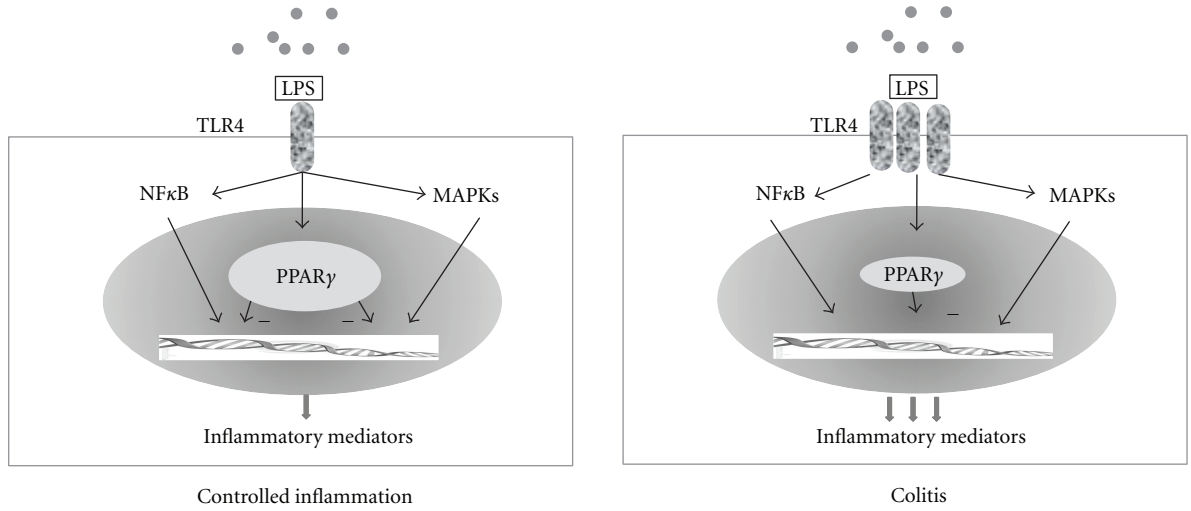

Figure 1: An hypothetical model of influence of PPARγ expression in ulcerative colitis. Induction of PPARγ expression in epithelial cells by bacterial lipopolysaccharide- (LPS-) activated TLR4, in turn leads to break NFκB and MAPK pathways to produce inflammatory mediators. The reduced expression of PPARγ together with TLR4 upregulation might enhance the inflammatory mediators production thus resulting in mucosal damage.

can, in principle, affect all of the organ systems of the body. IBD is also associated with an increased risk of colorectal cancer, which itself is already the third most common cancer in developed countries [2].

The progress in gene discovery in complex disease genetics has increased rapidly in recent years, boosted by the advent of genomewide association (GWA) studies. Few complex diseases have seen as much rapid progress as CD and UC thanks specially to the international inflammatory bowel disease genetics consortium (IIBDGC) who collected around the world some 20,000 cases for each of CD and UC (http://www.ibdgenetics.org/). The statistical power of such large sample sets has proven highly effective in identifying multiple susceptibility loci, even where these confer only modestly increased risk of disease. To date there are 99 IBD susceptibility loci: 71 associated with Crohn's disease, 47 with ulcerative colitis, and 28 with both CD and UC [5, 6]. Amongst these are multiple genes involved in IL23/Th17 signaling (IL23R, IL12B, JAK2, TYK2, and STAT3), genes involved in autophagy, intracellular bacteria processing and innate immunity (NOD2, IRGM, and ATG16L1), and genes involved in barrier (HNF4A, LAMB1, CDH1, and GNA1e). However, from these studies, included the recently reported data with the immunochip from the IIBDGC at DDW 2012 [7], no striking signal of PPARγ gene polymorphisms is emerged, with P values of tagging SNPs ranging from 0.005 to 0.01 (personal communication). Poliska et al. have investigated the association of four polymorphisms of PPARγ and IBD; they found haplotypes with both protective and increased risk [8]. Other studies, however, lead to conflicting results [9–13]. Accordingly, a meta-analysis of seven studies with over one thousand UC and CD found no significant association of the Pro12 Ala polymorphism of PPARγ with IBD [14].

In contrast, PPARγ is highly expressed in colonic epithelial cells and to a lesser degree into macrophages and lymphocytes [15]. In addition, its expression in the colon is closely linked to intestinal-microbial interaction. Using quantitative PCR, western blot, and immunohistochemical assay, a 60% decreased expression of PPARγ was observed at the mRNA and protein levels in the colon of UC patients, compared with CD and controls [16]. This impaired expression was found in both inflamed and noninflamed areas and limited to epithelial cells, suggesting that this modified expression is not secondary to the inflammatory process (Figure 1). A possible explanation is the occurrence of epigenetic changes [16]; this hypothesis is corroborated by the demonstration of similar levels of PPARγ in peripheral mononuclear cells of IBD patients and controls and lack of significant polymorphisms of PPARγ in UC patients. Another intriguing possibility is that the Toll-like receptor 4 (TLR4) signaling to PPARγ is impaired in UC. The resulting imbalance between elevated levels of TLR4 and reduced expression of PPARγ may lead to loss of mucosal tolerance to luminal LPS, resulting in mucosal inflammation [16]. In contrast, Yamamoto-Furusho et al. reported that the mRNA PPARγ expression was significantly reduced in the mucosa with active UC compared to the mucosa of patients in remission, with a significant correlation with disease activity [17].

More recently, another important role of PPARγ in the modulation of intestinal inflammation has been put forward. In healthy individual, immune cells and gut mucosa remain largely inactive towards 10^{14} bacteria of the intestinal microflora. This tolerance is attributed to the prominent presence of regulatory immune cells that may be triggered by the resident microflora and whose function is antagonistic to inflammatory pathways stimulated by pathogenic bacteria [18]. The effector cells are M1 macrophages and dendritic (De) cells secreting inflammatory mediators including factors stimulating additional resting macrophages, dendritic cells precursors (monocytes), and T cells. De and M1 present

antigen to resting T cells while secreting cytokines (IL12, IFN-γ, TNF-α, and IL-23) and induce the differentiation to proinflammatory T-helper, specifically Th1 and Th17. The immune response kills the invading bacteria, but may also cause indiscriminate tissue damage. In sterile organ systems, the inflammatory process usually ceases once the antigen population is eliminated. However, in the gut because of the resident microflora, the antigen population cannot be eliminated and the mounted inflammation could be more harmful for the host than the invading bacteria itself, for example, increasing gut permeability and infiltration of bacteria in the lamina propria. In healthy individuals, the gut mucosa contains various regulatory factors such as M2 macrophages, tolerogenic dendritic cells (Dt), and T regulatory cells. This regulatory pathway, by binding to ligands recognized as self to specific receptors, induces the differentiation and switches from M1 to M2 and from De to Dt. One such receptor is PPARγ expressed in T cells, dendritic cells, macrophages, and epithelial cells [19, 20].

3. PPARγ: Structure, Function, and Expression in the Gut

PPARγ belongs to the nuclear receptor family consisting of approximately 50 transcription factors implicated in many biological function. It is an essential nuclear receptor controlling the expression of a large number of regulatory genes in lipid metabolisms, insulin sensitization, inflammation, and cell proliferation [48].

Similarly to other nuclear hormone receptors, PPARγ displays a central DNA-binding domain, a C-terminal ligand-binding domain, and two transcription-activation function motifs (AF-1 and AF-2) [49]. Binding of ligands to PPARγ leads to a conformational change in the receptor which allows recruitment of co-activator proteins to then induce transcriptional activation. The transcriptional activity of PPARγ is regulated by post-translational changes such as phosphorylation or ubiquitination. The activation requires heterodimerization within the nucleus with another nuclear receptor named retinoid X receptor α (RXRα), leading to bind a specific DNA sequence elements known as peroxisome proliferator elements (PPREs) [50]. PPARγ interferes with inflammatory pathways by interactions with transcription factors such as nuclear factor kappa B (NF-κB), activating protein-1 (AP-1), signal transducer and activator of transcription (STAT), and nuclear factor-activated T cell (NFAT). For example, PPARγ is able to form a complex with the NF-κB subunit p65 at a nuclear level and this complex is exported from the nucleus leading to an altered expression of proinflammatory NF-κB-mediated gene expression. Inhibition of NF-κB in response to the activity of PPARγ ligands attenuates the expression of various cytokines in colonic epithelial cells such as IL-1β, COX-2, IL-6, IL-8, TNF-α, INF-γ, iNOS, and chemokines [51, 52]. Its expression has been initially investigated in adipose tissue where it plays a key role in adipocyte differentiation and insulin responses. More recently the colon has been found to highly express PPARγ in epithelial cells but also macrophages and lymphocytes [16, 52, 53]. Regulation of expression is incompletely understood; in vivo mRNA expression is negatively influenced by a long-term hypocaloric diet and fasting and positively by obesity and a rich in fatty acids diet. More recently a close link between intestinal microbial flora and PPARγ expression has been demonstrated. The stimulation of expression is probably multifactorial and involves the LPS recognition by the toll-like receptor (TLR), especially LPS of gram-negative bacteria and TLR4. Another alternative way of stimulation is the production through the bacteria of volatile fatty acid butyrate [15].

4. Experimental Model of Colitis

The initial evidence of the involvement of PPARγ in the regulation of intestinal inflammation derives from the observation of the use of PPARγ synthetic agonist thiazolidinedione (TZD) in mice dextran sodium sulfate- (DSS-) induced colitis. In the study by Su CG et al., both troglitazone and rosiglitazone dramatically reduced the colonic inflammation in mice and in addition significantly attenuated cytokine gene expression in colon cancer cell lines through NF-κB inhibition [21]. This first evidence was subsequently confirmed in another model of experimental colitis induced in mice by intrarectal administration of 2,4,6-trinitrobenzene sulfonic acid (TNBS) [51]. TZD given preventively significantly reduced mortality, severity of macroscopic and histological lesions, and markers of inflammation. So far several studies have reported similar prophylactic and therapeutic efficacy of PPARγ agonists in different animal models (mice, rats, and pigs) with different models of colitis induced by chemical compounds [22–34], ischaemia [35–38], bacteria [39], or genetically modified animals [43–47, 65, 66] (Table 1) [40–42]. Moreover a beneficial effect of PPARγ ligands has been demonstrated in colon carcinogenesis. Of interest, the use of probiotics (VSL#3), conjugated linoleic acid, n-3 polyunsaturated fatty acids, cannabidiol, punic acid, α-eleostearic acid, and a polyphenolic compound has prove beneficial effect on animal model of intestinal inflammation through the activation of PPARγ [67] (Table 1).

Taken together lessons from animal studies suggest that: (a) natural and synthetic PPARγ ligands are both effective in the treatment of acute and chronic animal models of inflammation; (b) the prophylactic effect is more pronounced than the therapeutic effect; (c) the therapeutic effect is apparently dependent by the abundance of PPARγ in the target tissue as demonstrated by the genetically modified animals. This information translated into clinical ground could suggest a major role of PPARγ agonists in maintenance rather than induction of remission in IBD patients. Moreover, with PPARγ being expressed not only in the epithelial cells but also in macrophages, T, and B cells, more investigations are needed to disclose which cell type expression of PPARγ is more crucial for the potential therapeutic effect.

5. Dietary Modulation of PPARγ

A large number of dietary nutrients are able to modulate PPARγ (see Table 2). Fatty acids and their metabolites can

Table 1: Anti-inflammatory properties of PPARγ agonists in experimental models.

Model	PPARγ modulator	Effect	Authors
Acute colitis			
DSS	Troglitazone	↓ Colonic inflammation	Su et al. 1999 [21]
		↓ Cytokine gene expression	
	Rosiglitazone	Reduced inflammation	Saubermann et al. 2002 [22]
		More severe colitis	Ramakers et al. 2007 [23]
	Pioglitazone	Prevention colitis	Takagi et al. 2002 [24]
		Recovery from colitis	Hontecillas et al. 2011 [25]
		Reduced CXCL10 level	Schaefer et al. 2005 [26]
	PUFA	Accelerated remission	
	CLA	Delayed onset of colitis	Bassaganya-Riera 2006 [27]
	CLA + VSL#3	Improvement of colitis	Bassaganya-Riera et al. 2012 [28]
	α-Eleostearic acid	Improvement of colitis	Lewis et al. 2011 [29]
TNBS	Troglitazone	Reduced inflammation	Desreumaux et al. 2001 [30]
	Rosiglitazone	Reduced inflammation	
		Reduced inflammation	Sànchez-Hidalgo et al. 2007 [31]
	Pioglitazone	Reduced CXCL10 level	Schaefer et al. 2005 [26]
	FMOC-L-leu	Reduced inflammation	Rocchi et al. 2001 [32]
	5-ASA	Reduced inflammation	Rousseaux et al. 2005 [33]
	5-ASA in PPARγ+/−	No efficacy of 5-ASA	
Acetic acid ischaemia	THSG	Attenuated colon lesions	Zeng et al. 2011 [34]
	Rosiglitazone	Protection	Nakaijma et al. 2001 [35]
	15-d-PGJ2	Reduced injury	Cuzzocrea et al. 2003 [36]
	NS-398	Protection	Sato et al. 2005 [37]
	Glutamine	Protection	Sato et al. 2006 [38]
Bacterial	CLA	Attenuated inflammation	Hontecillas et al. 2002 [39]
Chronic colitis			
DSS	Triglitazone	↓Cell proliferation	Tanaka et al. 2001 [40]
TNBS	Rosiglitazone	Protection	Sànchez-Hidalgoet al. 2005 [41]
CD4-CD45RBhi	CLA	Reduced inflammation	Bassaganya-Riera et al. 2004 [42]
IL-10 KO	Rosiglitazone	Slow onset colitis	Lytle et al. 2005 [43]
SAMP1/Yirfc	Rosiglitazone	Decreased severity	Sugawara et al. 2005 [44]
Genetic models			
PPARγ +/−	Ischaemia	More severe damage	Desreumaux et al. 2001 [30]
			Nakaijma et al. 2011 [35]
			Saubermann et al. 2002 [22]
	DSS + PUA	Loss protective effect PUA	Hontecillas et al. 2011 [25]
AdPPARγ			Katayama et al. 2003 [39]
SAMP1/yifc			Sugawara et al. 2005 [44]
PPARγCre+			Bassaganya-Riera et al. 2004 [42]
PPAR$\gamma\Delta$Mφ	DSS	Increased susceptibility	Shah et al. 2007 [45]
PPARγfifi	DSS	Accelerated colitis	Guri et al. 2010 [46]
		Worsen colonic lesions	Mohapatra et al. 2010 [47]

5-ASA: 5-aminosalycilic acid; 15dPGJ2: 15-deoxy-Δ12,14-prostaglandin J2; CLA: conjugated linoleic acid; PUFA: n-3 polyunsaturated fatty acids; DSS: dextransodiumsulphate; FMOC-L-leu: fluorenylmethyloxycarbonyl-L-leucine; IL-10 KO: interleukin 10 knockout mice; PPARγCre: PPARγ conditional knockout mice; TNBS: 2,4,6-trinitrobenzene sulfonic acid; PUA: punicic acid; THSG: 2,3,5,4′-tetrahydroxystilbene-2-O-beta-D-glucoside.

Table 2: Nutrients with demonstrated anti-inflammatory effects mediated through PPARγ.

Nutrient	Dietary source	Models	Authors
α-linoleic acid	Green vegetables, flax	Intestinal epithelial cells	Marion-Letellier 2008 [54]
Docosahexaenoic Eicosapentaenoic ac.	Fish	Intestinal epithelial cells	Marion-Letellier 2008 [54]
Conjugated linoleic acid	Beef, bovine milk	Intestinal epithelial cells DSS colitis	Allred et al. 2008 [55]
Glutamine	Beef, chicken, fish	Ischaemia reperfusion	Sato et al. 2006 [38]
Curcumin	Tumeric powder	TNBS colitis	Salh et al. 2003, Deguchi et al. 2007 [56]/[57]
Capsaicin	Cayenne pepper	Intestinal epithelial cells	Kim et al. 2004 [58]
Ginsenoids	Ginseng	Adypocites	Han et al. 2006, Hwang et al. 2007 [59]/[60]
Resveratrol	Grapes, wine, peanuts	Intestinal epithelial cells	Morikawa et al. 2007 [61]
Butyrate	Unabsorbed carbohydrate	Intestinal epithelial cells	Schwab et al. 2007 [62]
Vitamin E	Nuts, seeds, oils	Colon cancer cell lines	Campbell et al. 2003 [63]
Selenium	Plant foods	Macrophages	Vunta et al. 2007 [64]

Table 3: Efficacy of rosiglitazone therapy in ulcerative colitis (*P values < 0.05).

Authors	N° pts	Study design	Treatment	% Efficacy		
				Response	Remission	Mucosal healing
Lewis et al. 2001 [68]	15	Open 12 weeks	4 mg tid	—	27	20
Liang and Ouyang 2006 [69]	42	Random versus 5-ASA	4 mg	—	71 versus 57*	—
Lewis et al. 2008 [70]	105	12 wks versus Plac	4 mg tid	44 versus 23*	17 versus 2*	8 versus 2
Pederson and Brynskov 2010 [71]	14	Open versus 5-ASA	4 mg versus 1 enema	= 5-ASA	= 5-ASA	—

affect gene expression by binding to PPARγ. The effect of n-3 PUFAs is well documented; linoleic acid is the major PUFA in human diet and several derivatives like conjugated linoleic acid (CLA), nitrolinoleic acid, and gamma linoleic acid have shown activation property on PPARγ [54, 55]. Another fatty acid-derived metabolite known to be a strong PPARγ inducer is the prostaglandin 15d-PGJ2 as demonstrated in several animal models [36]. Glutamine is the preferential substrate of enterocytes and is considered essential in stress situations. In a rodent model of ischemia reperfusion, glutamine also acted as PPARγ agonist, as protective effect was abrogated by a PPARγ inhibitor [38]. Various spicy foods such as curcumin and capsaicin have been shown to activate PPARγ. The anti-inflammatory property of curcumin is also expressed by the inhibition of NF-κB, but is clearly blocked by PPARγ inhibitor [56–58]. Also ginsenosides, compounds derived by ginseng, may have opposite effects being ginsenoside 20S a strong inducer and Rh2 an inhibitor of PPARγ [59, 60]. Finally, other inducers are flavonoids, epigallocatechingallate derived from green tea, resveratrol derived from grapes and wine, butyrate, and micronutrients such as vitamin E and selenium [61–64, 72] (Table 2).

6. PPARγ and Therapy of Ulcerative Colitis

5-ASA is one of the oldest anti-inflammatory agents used for treatment of IBD, although the mechanism underlying its effects is still unknown. It is the mainstay of therapy for the majority of patients with UC for the induction of remission, maintenance, and possibly chemoprevention of colorectal cancer [73]. Recently, functional, biological, pharmacological, and chemical evidence has shown that aminosalicylates are a new functional synthetic ligand for PPARγ in colonic epithelial cells [33]. PPARγ is indeed the key receptor mediating the 5-ASA activity, by trans-repressing several key target genes such as nuclear factor κB, signal transducers, and activators of transcription.

Since in animal models treatment with PPARγ ligands has been demonstrated to attenuate inflammatory cytokines production such as IL-1β and TNF-α, it has been hypothesized the use of PPARγ ligands, like thiazolidinedione (TZD), in the therapy of UC [15]. One potential candidate is rosiglitazone, an antidiabetic drug. A first open-label pilot study in 15 patients with mild to moderate UC refractory to 5-ASA has evaluated the efficacy of the PPARγ ligand rosiglitazone (4 mg orally twice daily) (Table 3). These patients were refractory to conventional treatment, including corticosteroids and immune modulators. After 12 weeks of treatment, a striking reduction in disease activity index score was reported, with clinical and endoscopic remission in 27% and 20% of patients, respectively [68]. Liang and Quayang performed a clinical trial in China in 42 patients with mild to moderate UC [69]. Patients were allocated alternatively to the treatment of rosiglitazone 4 mg/day plus 5-ASA 2 gr or sulfasalazine 3 gr, while the control group received 5-ASA or sulfasalazine alone for 4 weeks. The remission rate was greater in the rosiglitazone group (71.4% versus 57.1%), with a significant improvement of the histologic score ($P < 0.05$). Moreover in the treatment group the PPARγ expression was increased compared to baseline [69].

Recently, a randomized multicenter double-blind, placebo-controlled trial has been published by using rosiglitazone 4 mg orally twice daily versus placebo for 12 weeks in 105 patients with mild to moderate ulcerative colitis [70]. Disease activity was measured by Mayo score with a primary endpoint of a clinical response (≥2 points reduction) at week 12, while clinical remission, endoscopic remission, and quality of life changes were considered secondary outcomes. After 12 weeks of therapy, 23 patients (44%) treated with rosiglitazone and 12 patients (23%) treated with placebo achieved clinical response ($P = 0.04$). Remission was achieved in 9 patients (17%) treated with rosiglitazone and 1 patient (2%) of the placebo arm ($P = 0.01$). However, endoscopic remission was uncommon in either arms (8% versus 2%; $P = 0.34$). Clinical improvement was clearly evident already at 4 week ($P = 0.049$), while quality of life was significantly improved at week 8 ($P = 0.01$), but not at week 4 and 12. The safety profile was remarkably safe, with adverse events occurring at similar rates in both groups; in particular edema and weight gain, as expected, were more common in the rosiglitazone group. Of interest, no cases of symptomatic hypoglycemia were reported.

Pederson and Brynskov reported the use of rosiglitazone enema compared to mesalazine in fourteen patients with distal UC [71]. Rosiglitazone had a similar effect compared to mesalazine enema, with a significant reduction of Mayo score ($P < 0.01$). In addition rosiglitazone restored the PPARγ activity in the inflamed area which was fourfold reduced before treatment compared with noninflamed areas and controls.

Although substantial research has focused on potential anti-inflammatory effects of TZD PPARγ ligands, their mechanism of action, particularly in the colon, is not well defined. The 5-ASA compounds largely used in UC are able to bind to PPARγ [33]. In the study of Lewis et al. [70] the majority of patients were on concomitant therapy with 5-ASA. Since rosiglitazone has a higher affinity to PPARγ compared to 5-ASA, one possible explanation of the efficacy is a more powerful stimulation and anti-inflammatory property of PPARγ. Alternatively, the effect could be mediated at the mucosal level, where the PPARγ is largely expressed [74]. Of note, large clinical trials with rosiglitazone in the treatment of psoriasis, another inflammatory disease, did not demonstrate efficacy, thus suggesting a "topical" and not a systemic effect in patients with UC [75].

Being also involved in cell proliferation, apoptosis, and modulation of cytokine production with antitumorigenic effect, PPARγ is also extremely important for the basis of chemoprevention strategies against colorectal cancer. For these reasons, there is an active ongoing research to disclose and investigate safer PPARγ agonists, with topical effect and direct targeting of the colon, possibly void of metabolic and systemic effect.

7. PPARγ and Therapy of Crohn's Disease

Recent data have suggested that the role of PPARγ in IBD physiopathology is not limited to UC but might involve also CD. Based on SAMP1/YitFc animal example, developing a spontaneous ileitis due to a defect of expression of PPARγ in ileal crypts, the polymorphisms of PPARγ has been tested in CD. Sugawara et al. [44] demonstrated that two intronic SNPs exhibited a significant lower frequency in CD compared to controls. However, these findings have not been independently replicated yet. Moreover, no data are available by using PPARγ ligands in medical therapy of CD, in which 5-ASA compounds are generally believed to be of little or no efficacy [73].

8. Conclusion and Take-Home Messages

PPARγ receptors are widely and highly expressed in the colon, being a key regulator factor of bacteria-induced mucosal inflammation. Moreover, they are directly involved in the mechanism of action of mesalazine, which is largely used and effective in UC. In addition, they are involved in the process of tumor suppression, especially in the colon. Therefore, beside the potential interest in the IBD physiopathology and genetic predisposition which is still under evaluation, it is highly expected that new molecules specifically targeting the intestinal receptors and void of action in the adipose tissue and insulin action could be developed and tested. Several tens of compounds have been already synthetized, some with 30–50-fold higher affinity against PPARγ and potentially higher efficacy than 5-ASA. These compounds are not that far from clinical application with potential implication in controlling the inflammation, better handling of host-bacterial interactions, and possible chemoprevention. In addition, a better understanding of the role of microbiota on PPARγ receptors should be elucidated, since some commensal bacterial or natural ligands of foods may directly activate and increase the expression of PPARγ, thus determining a "biologic" anti-inflammatory action.

References

[1] D. K. Podolsky, "Inflammatory bowel disease," *New England Journal of Medicine*, vol. 347, no. 6, pp. 417–429, 2002.

[2] J. Cosnes, C. Gowerrousseau, P. Seksik, and A. Cortot, "Epidemiology and natural history of inflammatory bowel diseases," *Gastroenterology*, vol. 140, no. 6, pp. 1785–1794, 2011.

[3] S. Danese and C. Fiocchi, "Ulcerative colitis," *New England Journal of Medicine*, vol. 365, pp. 1713–1725, 2011.

[4] B. Khor, A. Gardet, and R. J. Xavier, "Genetics and pathogenesis of inflammatory bowel disease," *Nature*, vol. 474, no. 7351, pp. 307–317, 2011.

[5] C. A. Anderson, G. Boucher, C. W. Lees et al., "Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47," *Nature Genetics*, vol. 43, no. 3, pp. 246–252, 2011.

[6] A. Franke, D. P. B. McGovern, J. C. Barrett et al., "Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci," *Nature Genetics*, vol. 42, no. 12, pp. 1118–1125, 2010.

[7] J. H. Cho, "Immunochip-based analysis of a large IBD case-control cohort identifies 50 novel loci, refining definitions of disease pathways," *Gastroenterology*, vol. 142, supplement 1, pp. S149–S150, 2012.

[8] S. Poliska, A. Penyige, P. L. Lakatos et al., "Association of peroxisome proliferator-activated receptor gamma polymorphisms with inflammatory bowel disease in a hungarian cohort," *Inflammatory Bowel Diseases*, vol. 18, no. 3, pp. 472–479, 2012.

[9] Y. Aoyagi, S. Nagata, T. Kudo et al., "Peroxisome proliferator-activated receptor γ 2 mutation may cause a subset of ulcerative colitis," *Pediatrics International*, vol. 52, no. 5, pp. 729–734, 2010.

[10] U. K. Shrestha, O. Karimi, J. B. A. Crusius et al., "Distribution of peroxisome proliferator-activated receptor-gamma polymorphisms in Chinese and Dutch patients with inflammatory bowel disease," *Inflammatory Bowel Diseases*, vol. 16, no. 2, pp. 312–319, 2010.

[11] O. Atug, V. Tahan, F. Eren et al., "Pro12Ala polymorphism in the peroxisome proliferator-activated receptor-gamma (PPARγ) gene in inflammatory bowel disease," *Journal of Gastrointestinal and Liver Diseases*, vol. 17, no. 4, pp. 433–437, 2008.

[12] J. Mwinyi, C. Grete-Wenger, J. J. Eloranta, and G. A. Kullak-Ublick, "The impact of PPARγ genetic variants on IBD susceptibility and IBD disease course," *PPAR Research*, vol. 2012, Article ID 349469, 13 pages, 2012.

[13] V. Andersen, J. Christensen, A. Ernst et al., "Polymorphisms in NF-κB, PXR, LXR, PPARγ and risk of infammatory bowel disease," *World Journal of Gastroenterology*, vol. 17, no. 2, pp. 197–206, 2011.

[14] Z.-F. Zhang, N. Yang, G. Zhao, L. Zhu, and L.-X. Wang, "Association between the Pro12Ala polymorphism of peroxisome proliferator-activated receptor gamma 2 and inflammatory bowel disease: a meta-analysis," *PLoS One*, vol. 7, no. 1, Article ID e30551, 2012.

[15] L. Dubuquoy, C. Rousseaux, X. Thuru et al., "PPARγ as a new therapeutic target in inflammatory bowel diseases," *Gut*, vol. 55, no. 9, pp. 1341–1349, 2006.

[16] L. Dubuquoy, E. Å Jansson, S. Deeb et al., "Impaired expression of peroxisome proliferator-activated receptor γin ulcerative colitis," *Gastroenterology*, vol. 124, no. 5, pp. 1265–1276, 2003.

[17] J. K. Yamamoto-Furusho, A. Peñaloza-Coronel, F. Sánchez-Muñoz, R. Barreto-Zuñiga, and A. Dominguez-Lopez, "Peroxisome proliferator-activated receptor-gamma (PPAR-γ) expression is downregulated in patients with active ulcerative colitis," *Inflammatory Bowel Diseases*, vol. 17, no. 2, pp. 680–681, 2011.

[18] K. Wendelsdorf, J. Bassaganya-Riera, R. Hontecillas, and S. Eubank, "Model of colonic inflammation: immune modulatory mechanisms in inflammatory Bowel disease," *Journal of Theoretical Biology*, vol. 264, no. 4, pp. 1225–1239, 2010.

[19] B. M. Spiegelman, "PPAR-γ: adipogenic regulator and thiazolidinedione receptor," *Diabetes*, vol. 47, no. 4, pp. 507–514, 1998.

[20] A. Mansén, H. Guardiola-Diaz, J. Rafter, C. Branting, and J. Å. Gustafsson, "Expression of the peroxisome proliferator-activated receptor (PPAR) in the mouse colonic mucosa," *Biochemical and Biophysical Research Communications*, vol. 222, no. 3, pp. 844–851, 1996.

[21] C. G. Su, X. Wen, S. T. Bailey et al., "A novel therapy for colitis utilizing PPAR-γ ligands to inhibit the epithelial inflammatory response," *Journal of Clinical Investigation*, vol. 104, no. 4, pp. 383–389, 1999.

[22] L. J. Saubermann, A. Nakajima, K. Wada et al., "Peroxisome proliferator-activated receptor gamma agonist ligands stimulate a Th2 cytokine response and prevent acute colitis," *Inflammatory Bowel Diseases*, vol. 8, no. 5, pp. 330–339, 2002.

[23] J. D. Ramakers, M. I. Verstege, G. Thuijls, A. A. Te Velde, R. P. Mensink, and J. Plat, "The PPARγ agonist rosiglitazone impairs colonic inflammation in mice with experimental colitis," *Journal of Clinical Immunology*, vol. 27, no. 3, pp. 275–283, 2007.

[24] T. Takagi, Y. Naito, N. Tomatsuri et al., "Pioglitazone, a PPAR-γ ligand, provides protection from dextran sulfate sodium-induced colitis in mice in association with inhibition of the NF-κB-cytokine cascade," *Redox Report*, vol. 7, no. 5, pp. 283–289, 2002.

[25] R. Hontecillas, W. T. Horne, M. Climent et al., "Immunoregulatory mechanisms of macrophage PPAR-γ in mice with experimental inflammatory bowel disease," *Mucosal Immunology*, vol. 4, no. 3, pp. 304–313, 2011.

[26] K. L. Schaefer, S. Denevich, C. Ma et al., "Intestinal antiinflammatory effects of thiazolidenedione peroxisome proliferator-activated receptor-γ ligands on T helper type 1 chemokine regulation include nontranscriptional control mechanisms," *Inflammatory Bowel Diseases*, vol. 11, no. 3, pp. 244–252, 2005.

[27] J. Bassaganya-Riera and R. Hontecillas, "CLA and n-3 PUFA differentially modulate clinical activity and colonic PPAR-responsive gene expression in a pig model of experimental IBD," *Clinical Nutrition*, vol. 25, no. 3, pp. 454–465, 2006.

[28] J. Bassaganya-Riera, M. Viladomiu, M. Pedragosa, C. De Simone, and R. Hontecillas, "Immunoregulatory mechanisms underlying prevention of colitis-associated colorectal cancer by probiotic bacteria," *PLoS One*, vol. 7, no. 4, Article ID e34676, 2012.

[29] S. N. Lewis, L. Brannan, A. J. Guri et al., "Dietary α-eleostearic acid ameliorates experimental inflammatory Bowel disease in mice by activating peroxisome proliferator-activated receptor-γ," *Plos One*, vol. 6, no. 8, Article ID e24031, 2011.

[30] P. Desreumaux, L. Dubuquoy, S. Nutten et al., "Attenuation of colon inflammation through activators of the retinoid X receptor (RXR)/peroxisome proliferator-activated receptor γ (PPARγ) heterodimer: a basis for new therapeutic strategies," *Journal of Experimental Medicine*, vol. 193, no. 7, pp. 827–838, 2001.

[31] M. Sánchez-Hidalgo, A. R. Martín, I. Villegas, and C. Alarcón de la Lastra, "Rosiglitazone, a PPARγ ligand, modulates signal transduction pathways during the development of acute TNBS-induced colitis in rats," *European Journal of Pharmacology*, vol. 562, no. 3, pp. 247–258, 2007.

[32] S. Rocchi, F. Picard, J. Vamecq et al., "A unique PPARγ ligand with potent insulin-sensitizing yet weak adipogenic activity," *Molecular Cell*, vol. 8, no. 4, pp. 737–747, 2001.

[33] C. Rousseaux, B. Lefebvre, L. Dubuquoy et al., "Intestinal antiinflammatory effect of 5-aminosalicylic acid is dependent on peroxisome proliferator-activated receptor-γ," *Journal of Experimental Medicine*, vol. 201, no. 8, pp. 1205–1215, 2005.

[34] C. Zeng, J. H. Xiao, M. J. Chang, and J. L. Wang, "Beneficial effects of THSG on acetic acid-induced experimental colitis: involvement of upregulation of PPAR-γ and inhibition of the NF-κB inflammatory pathway," *Molecules*, vol. 16, pp. 8552–8568, 2011.

[35] A. Nakajima, K. Wada, H. Miki et al., "Endogenous PPARγ mediates anti-inflammatory activity in murine ischemia-reperfusion injury," *Gastroenterology*, vol. 120, no. 2, pp. 460–469, 2001.

[36] S. Cuzzocrea, B. Pisano, L. Dugo et al., "Rosiglitazone and 15-deoxy-Δ12,14-prostaglandin J 2, ligands of the peroxisome proliferator-activated receptor-γ (PPAR-γ), reduce ischaemia/

reperfusion injury of the gut," *British Journal of Pharmacology*, vol. 140, no. 2, pp. 366–376, 2003.

[37] N. Sato, R. A. Kozar, L. Zou et al., "Peroxisome proliferator-activated receptor γ mediates protection against cyclooxygenase-2-induced gut dysfunction in a rodent model of mesenteric ischemia/reperfusion," *Shock*, vol. 24, no. 5, pp. 462–469, 2005.

[38] N. Sato, F. A. Moore, B. C. Kone et al., "Differential induction of PPAR-γ by luminal glutamine and iNOS by luminal arginine in the rodent postischemic small bowel," *American Journal of Physiology. Gastrointestinal and Liver Physiology*, vol. 290, no. 4, pp. G616–G623, 2006.

[39] R. Hontecillas, M. J. Wannemeulher, D. R. Zimmerman et al., "Nutritional regulation of porcine bacterial-induced colitis by conjugated linoleic acid," *Journal of Nutrition*, vol. 132, no. 7, pp. 2019–2027, 2002.

[40] T. Tanaka, H. Kohno, S. I. Yoshitani et al., "Ligands for peroxisome proliferator-activated receptors α and γ inhibit chemically induced colitis and formation of aberrant crypt foci in rats," *Cancer Research*, vol. 61, no. 6, pp. 2424–2428, 2001.

[41] M. Sánchez-Hidalgo, A. R. Martín, I. Villegas, and C. Alarcón De La Lastra, "Rosiglitazone, an agonist of peroxisome proliferator-activated receptor gamma, reduces chronic colonic inflammation in rats," *Biochemical Pharmacology*, vol. 69, no. 12, pp. 1733–1744, 2005.

[42] J. Bassaganya-Riera, K. Reynolds, S. Martino-Catt et al., "Activation of PPAR γ and δ by conjugated linoleic acid mediates protection from experimental inflammatory bowel disease," *Gastroenterology*, vol. 127, no. 3, pp. 777–791, 2004.

[43] C. Lytle, T. J. Tod, K. T. Vo, J. W. Lee, R. D. Atkinson, and D. S. Straus, "The peroxisome proliferator-activated receptor γ ligand rosiglitazone delays the onset of inflammatory bowel disease in mice with interleukin 10 deficiency," *Inflammatory Bowel Diseases*, vol. 11, no. 3, pp. 231–243, 2005.

[44] K. Sugawara, T. S. Olson, C. A. Moskaluk et al., "Linkage to peroxisome proliferator-activated receptor-γ in SAMP1/YitFc mice and in human Crohn's disease," *Gastroenterology*, vol. 128, no. 2, pp. 351–360, 2005.

[45] Y. M. Shah, K. Morimura, and F. J. Gonzalez, "Expression of peroxisome proliferator-activated receptor-γ in macrophage suppresses experimentally induced colitis," *American Journal of Physiology. Gastrointestinal and Liver Physiology*, vol. 292, no. 2, pp. G657–G666, 2007.

[46] A. J. Guri, S. K. Mohapatra, W. T. Horne, R. Hontecillas, and J. Bassaganya-Riera, "The Role of T cell PPAR γ in mice with experimental inflammatory bowel disease," *BMC Gastroenterology*, vol. 10, article no. 60, 2010.

[47] S. K. Mohapatra, A. J. Guri, M. Climent et al., "Immunoregulatory actions of epithelial cell PPAR γ at the colonic mucosa of mice with experimental inflammatory bowel disease," *PLoS One*, vol. 5, no. 4, Article ID e10215, 2010.

[48] J. Auwerx, E. Baulieu, M. Beato et al., "A unified nomenclature system for the nuclear receptor superfamily," *Cell*, vol. 97, no. 2, pp. 161–163, 1999.

[49] T. M. Willson, P. J. Brown, D. D. Sternbach, and B. R. Henke, "The PPARs: from orphan receptors to drug discovery," *Journal of Medicinal Chemistry*, vol. 43, no. 4, pp. 527–550, 2000.

[50] S. A. Kliewer, K. Umesono, D. J. Noonan, R. A. Heyman, and R. M. Evans, "Convergence of 9-cis retinoic acid and peroxisome proliferator signalling pathways through heterodimer formation of their receptors," *Nature*, vol. 358, no. 6389, pp. 771–774, 1992.

[51] K. Yamazaki, M. Shimizu, M. Okuno et al., "Synergistic effects of RXRα and PPARγ ligands to inhibit growth in human colon cancer cells—phosphorylated RXRα is a critical target for colon cancer management," *Gut*, vol. 56, no. 11, pp. 1557–1563, 2007.

[52] L. Dubuquoy, S. Dharancy, S. Nutten, S. Pettersson, J. Auwerx, and P. Desreumaux, "Role of peroxisome proliferator-activated receptor γ and retinoid X receptor heterodimer in hepatogastroenterological diseases," *Lancet*, vol. 360, no. 9343, pp. 1410–1418, 2002.

[53] B. M. Spiegelman, "PPARγ in monocytes: less pain, any gain?" *Cell*, vol. 93, no. 2, pp. 153–155, 1998.

[54] R. Marion-Letellier, M. Butler, P. Déchelotte, R. J. Playford, and S. Ghosh, "Comparison of cytokine modulation by natural peroxisome proliferator-activated receptor γ ligands with synthetic ligands in intestinal-like Caco-2 cells and human dendritic cells—potential for dietary modulation of peroxisome proliferator-activated receptor γ in intestinal inflammation," *American Journal of Clinical Nutrition*, vol. 87, no. 4, pp. 939–948, 2008.

[55] C. D. Allred, D. R. Talbert, R. C. Southard, X. Wang, and M. W. Kilgore, "PPARγ1 as a molecular target of eicosapentaenoic acid in human colon cancer (HT-29) cells," *Journal of Nutrition*, vol. 138, no. 2, pp. 250–256, 2008.

[56] B. Salh, K. Assi, V. Templeman et al., "Curcumin attenuates DNB-induced murine colitis," *American Journal of Physiology. Gastrointestinal and Liver Physiology*, vol. 285, no. 1, pp. G235–G243, 2003.

[57] Y. Deguchi, A. Andoh, O. Inatomi et al., "Curcumin prevents the development of dextran sulfate sodium (DSS)-induced experimental colitis," *Digestive Diseases and Sciences*, vol. 52, no. 11, pp. 2993–2998, 2007.

[58] C. S. Kim, W. H. Park, J. Y. Park et al., "Capsaicin, a spicy component of hot pepper, induces apoptosis by activation of the peroxisome proliferator-activated receptor γ in HT-29 human colon cancer cells," *Journal of Medicinal Food*, vol. 7, no. 3, pp. 267–273, 2004.

[59] K. L. Han, M. H. Jung, J. H. Sohn, and J. K. Hwang, "Ginsenoside 20(S)-protopanaxatriol (PPT) activates peroxisome proliferator-activated receptor γ (PPARγ) in 3T3-L1 adipocytes," *Biological and Pharmaceutical Bulletin*, vol. 29, no. 1, pp. 110–113, 2006.

[60] J. T. Hwang, S. H. Kim, M. S. Lee et al., "Anti-obesity effects of ginsenoside Rh2 are associated with the activation of AMPK signaling pathway in 3T3-L1 adipocyte," *Biochemical and Biophysical Research Communications*, vol. 364, no. 4, pp. 1002–1008, 2007.

[61] K. Morikawa, C. Ikeda, M. Nonaka et al., "Epigallocatechin gallate-induced apoptosis does not affect adipocyte conversion of preadipocytes," *Cell Biology International*, vol. 31, no. 11, pp. 1379–1387, 2007.

[62] M. Schwab, V. Reynders, S. Loitsch, D. Steinhilber, J. Stein, and O. Schröder, "Involvement of different nuclear hormone receptors in butyrate-mediated inhibition of inducible NFκB signalling," *Molecular Immunology*, vol. 44, no. 15, pp. 3625–3632, 2007.

[63] S. E. Campbell, W. L. Stone, S. G. Whaley, M. Qui, and K. Krishnan, "Gamma tocopherol upregulates peroxisome proliferator activated receptor (PPAR) gamma expression in SW 480 human colon cancer cell lines," *BMC Cancer*, vol. 3, article no. 25, 2003.

[64] H. Vunta, F. Davis, U. D. Palempalli et al., "The anti-inflammatory effects of selenium are mediated through 15-deoxy-Δ12,14-prostaglandin J2 in macrophages," *Journal of Biological Chemistry*, vol. 282, no. 25, pp. 17964–17973, 2007.

[65] J. Bassaganya-Riera and R. Hontecillas, "Dietary conjugated linoleic acid and n-3 polyunsaturated fatty acids in inflammatory Bowel disease," *Current Opinion in Clinical Nutrition and Metabolic Care*, vol. 13, no. 5, pp. 569–573, 2010.

[66] K. Katayama, K. Wada, A. Nakajima et al., "A novel PPARγ gene therapy to control inflammation associated with inflammatory bowel disease in a murine model," *Gastroenterology*, vol. 124, no. 5, pp. 1315–1324, 2003.

[67] R. Marion-Letellier, P. Déchelotte, M. Lacucci, and S. Ghosh, "Dietary modulation of peroxisome proliferator-activated receptor gamma," *Gut*, vol. 58, no. 4, pp. 586–593, 2009.

[68] J. D. Lewis, G. R. Lichtenstein, R. B. Stein et al., "An open-label trial of the PPARγ ligand rosiglitazone for active ulcerative colitis," *American Journal of Gastroenterology*, vol. 96, no. 12, pp. 3323–3328, 2001.

[69] H. L. Liang and Q. Ouyang, "A clinical trial of rosiglitazone and 5-aminosalicylate combination for ulcerative colitis," *Zhonghua Nei ke Za Zhi*, vol. 45, no. 7, pp. 548–551, 2006.

[70] J. D. Lewis, G. R. Lichtenstein, J. J. Deren et al., "Rosiglitazone for active ulcerative colitis: a randomized placebo-controlled trial," *Gastroenterology*, vol. 134, no. 3, pp. 688–695, 2008.

[71] G. Pedersen and J. Brynskov, "Topical rosiglitazone treatment improves ulcerative colitis by restoring peroxisome proliferator-activated receptor-γ activity," *American Journal of Gastroenterology*, vol. 105, no. 7, pp. 1595–1603, 2010.

[72] S. Ulrich, S. M. Loitsch, O. Rau et al., "Peroxisome proliferator-activated receptor γ as a molecular target of resveratrol-induced modulation of polyamine metabolism," *Cancer Research*, vol. 66, no. 14, pp. 7348–7354, 2006.

[73] N. J. Talley, M. T. Abreu, J. P. Achkar et al., "An evidence-based systematic review on medical therapies for inflammatory bowel disease," *American Journal of Gastroenterology*, vol. 106, supplement 1, pp. S2–S25, 2011.

[74] L. Fajas, D. Auboeuf, E. Raspé et al., "The organization, promoter analysis, and expression of the human PPARγ gene," *Journal of Biological Chemistry*, vol. 272, no. 30, pp. 18779–18789, 1997.

[75] C. N. Ellis, J. N. Barker, A. E. Haig, C. A. Parker, S. Daly, and D. A. Jayawardene, "Placebo response in two long-term randomized psoriasis studies that were negative for rosiglitazone," *American Journal of Clinical Dermatology*, vol. 8, no. 2, pp. 93–102, 2007.

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Review Article

The Role of Peroxisome Proliferator-Activated Receptors in the Esophageal, Gastric, and Colorectal Cancer

Alessandra Fucci, Tommaso Colangelo, Carolina Votino, Massimo Pancione, Lina Sabatino, and Vittorio Colantuoni

Department of Biological, Geological and Environmental Sciences, University of Sannio, 82100 Benevento, Italy

Correspondence should be addressed to Vittorio Colantuoni, colantuoni@unisannio.it

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Tumors of the gastrointestinal tract are among the most frequent human malignancies and account for approximately 30% of cancer-related deaths worldwide. Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that control diverse cellular functions such as proliferation, differentiation, and cell death. Owing to their involvement in so many processes, they play crucial roles also in the development and physiology of the gastrointestinal tract. Consistently, PPARs deregulation has been implicated in several pathophysiological conditions, including chronic inflammation and cancer development. This paper summarizes the current knowledge on the role that the various PPAR isoforms play in the pathogenesis of the esophageal, gastric, and intestinal cancer. Elucidation of the molecular mechanisms underlying PPARs' signaling pathways will provide insights into their possible use as predictive biomarkers in the initial stages of the process. In addition, this understanding will provide the basis for new molecular targets in cancer therapy and chemoprevention.

1. Introduction

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear hormone receptor superfamily. Three subtypes, PPARα, PPARβ/δ, and PPARγ, have been identified so far. PPARα is expressed in the liver, kidney, small intestine, heart, and muscle, where it activates fatty acid catabolism and controls lipoprotein assembly in response to long-chain unsaturated fatty acids, eicosanoids, and hypolipidemic drugs (e.g., fenofibrate) [1, 2]. PPARβ/δ is more broadly expressed and is implicated in fatty acid oxidation, keratinocyte differentiation, wound healing, and macrophage response to VLDL metabolism. This isoform has been implicated in transcriptional-repression functions and has been shown to repress the activity of PPARα or PPARγ target genes [2–7]. PPARγ1 and γ2 are generated from a single-gene *PPARG* by differential promoter usage and alternative splicing [8–12]. PPARγ1 is expressed in colon, immune system (e.g., monocytes and macrophages), and other tissues where it participates in the modulation of inflammation, cell proliferation, and differentiation. PPARγ2 contains 28 additional amino acids at the N-terminus, as compared to PPARγ1, and is expressed in adipose tissue where it plays a pivotal role in adipocyte differentiation, lipid storage, and energy dissipation [12]. *PPARG3* and *PPARG4* are splicing variants of *PPARG1* mRNA and give rise to the same PPARγ1 protein [8, 9, 12]. Since PPARγ is also involved in glucose metabolism improving insulin sensitivity, selective ligands such as the thiazolidinediones (TZD) are used as insulin-sensitizing drugs in type 2 diabetes [2, 4, 5].

As all nuclear receptors, PPARs share a modular structure with four distinct domains [13, 14]. The A/B domain at the N-terminus is the key determinant of isotype-selective gene function and harbors a ligand-independent transcriptional activating function (AF-1) motif. The C domain is the DNA binding domain, with the typical two-zinc-finger structure with which the receptor binds the major groove of the double helix DNA of the peroxisome proliferator response elements (PPREs). They are formed by direct repeats (DRs) of the core

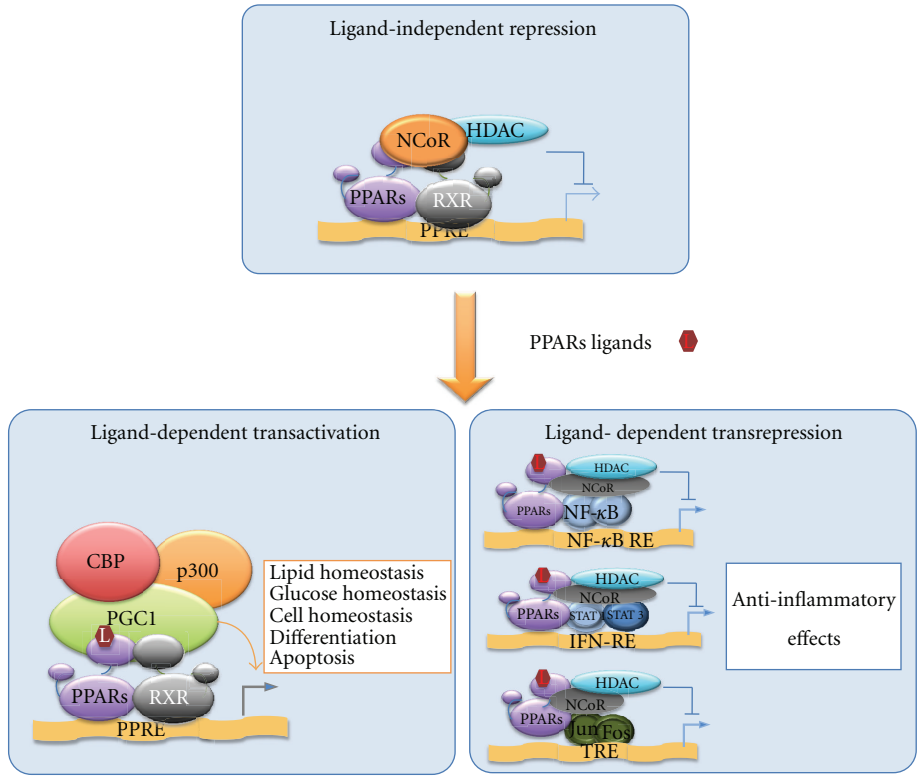


Figure 1: PPARs-mediated mechanisms of transcriptional regulation. In the absence of ligands, PPARs bind the promoters of their target genes and repress transcription by recruiting the corepressor complex. In the presence of ligands, PPARs can induce either ligand-dependent transactivation or transrepression. Transactivation involves PPARs heterodimerization with the retinoid X receptors (RXRs) followed by recognition of specific PPAR response elements (PPREs) and interaction with coactivators. Transrepression involves interference with other signal transduction pathways, including NFκB, STAT, and AP1. NFκB-RE: NFκB response element; IFN-RE = "interferon-stimulated gene factor" responsive element; TRE = O-tetradecanoylphorbol 13-acetate-responsive element.

sequence AGG(A/T)CA, separated by one or two nucleotides (DR1 and DR2, resp.). The D domain or hinge region allows receptor dimerization and DNA binding. The E/F domain contains the ligand-binding domain (LBD), a large binding pocket in which a variety of natural and synthetic ligands, such as fatty acids, eicosanoids, linoleic acid derivatives, as well as oxidized and nitrated fatty acids, accommodate. In addition, this domain exhibits the ligand-dependent transcriptional-activating function (AF2) motif on the C-terminus helix 12 [13]. Both the D and E/F domains are required to the dimerization with the 9-cis retinoic acid receptor (RXR) with which PPARs form permissive heterodimers bound to their cognate PPREs. Several genes involved in lipid metabolism and energy homeostasis, as well as genes modulating cell proliferation, differentiation, and survival, have functional PPREs in their regulatory regions [1, 2, 13, 15].

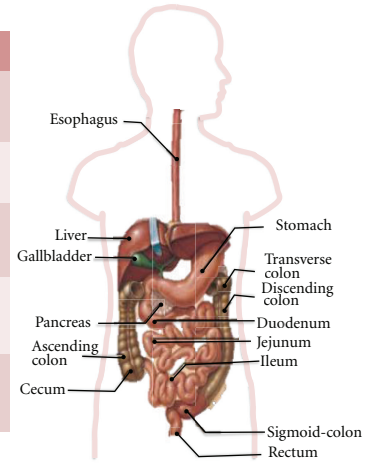
PPARs regulate gene expression through distinct mechanisms: ligand-dependent transactivation, ligand-independent repression, and ligand-dependent transrepression (Figure 1) [16, 17]. Ligand-dependent transactivation is considered the "classical mode of action" of PPARs: upon ligand binding, the helix 12 of the LBD folds back exposing the AF2 motif that governs the recruitment of transcriptional coactivators. These, in turn, facilitate the assembly of the general transcriptional machinery at PPRE-containing promoters [16, 17]. In the absence of ligand, PPARs repress transcription of target genes by recruiting corepressor complexes (e.g., NCoR and SMRT). Finally, recent studies have disclosed an additional "nongenomic" mode of action defined "transrepression" that involves gene repression in a ligand-dependent manner through protein-protein interactions with NFκB, AP1, Smads, STATs, and NFATs [17–19]. In contrast to transcriptional activation and repression, transrepression does not involve binding to PPREs but is attained through the recruitment and stabilization of the corepressor complexes on the promoters of pro-inflammatory genes. This mechanism might explain the anti-inflammatory properties of PPARs [17–20].

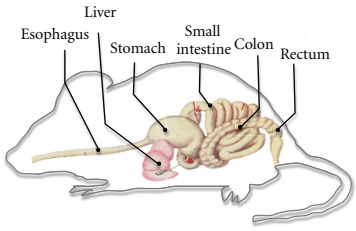
2. Gastrointestinal Cancers

The inner lining of the digestive tract is composed by high-proliferating cells located at the basis of the epithelium and by differentiated cells undergoing continuous replacement. The rapid cell turnover required for the maintenance of the mucosa homeostasis and the response to an adverse environment, such as toxins and carcinogens present in digested foods, makes the digestive tract a common site of

PPARs in human GI Tract

GI tract	Isoform	Physiological function	Pathological function
Esophagus	PPARα PPARβ/δ PPARγ	NA NA NA	NA NA Promotes cancer development [55–57]
Stomach	PPARα PPARβ/δ PPARγ	NA Unknown/controversial [45] Differentiation; H+ seretion [62, 75]	NA Unknown/controversial [45] Protective against cancer [58–60, 63]
Small intestine	PPARα PPARβ/δ PPARγ	Differentiation, anti-inflammatory [27]; Proliferation homeostasis [27, 28, 46]; NA	Differentiation, lipid metabolism [27]; Differentiation/proliferation [28, 45]; NA
Large intestine	PPARα PPARβ/δ PPARγ	Differentiation and lipid sensing [27, 28, 37] Differentiation of paneth cells [47] Differentiation; host defense [74]	Differentiation [37] Unknown/contrastant Protective against cancer [74, 75]
Rectum	PPARα PPARβ/δ PPARγ	NA Differentiation of paneth cells [47] Differentiation; host defense [74]	Protective against cancer[44] Unknown/contrastant Protective against cancer [74, 75]





PPARs in murine GI Tract

GI tract	Isoform	Physiological function	Pathological function
Esophagus	PPARα PPARβ/δ PPARγ	NA NA NA	NA NA Promotes cancer development [55–57]
Stomach	PPARα PPARβ/δ PPARγ	NA NA Differentiation; H+ seretion [62, 63]	NA NA Protective againstcancer [58–60, 63]
Small intestine	PPARα PPARβ/δ PPARγ	Differentiation; lipid metabolism anti-inflammatory effects; host defence [44] Proliferation/differentiation [45, 50] NA	Protective against cancer; anti-angiogenic; anti-inflammatory effects [44] Unknown/controversial [45, 46, 50] NA
Large intestine	PPARα PPARβ/δ PPARγ	Differentiation; lipid sensing; anti-inflammatory effects; host defence [44] Differentiation of panethcells; anti-inflammatory [47] Differentiation; host defense; anti-inflammatory effects [60–68]	Protective against cancer [44] Unknown/contrastant [46] Protective against cancer [60–68, 75]
Rectum	PPARα PPARβ/δ PPARγ	NA Differentiation of paneth cells [47] Differentiation; anti-inflammatory [60–68, 75]	Protective against cancer [44] Unknown/contrastant [46] Protective against cancer[60–68, 75]

Figure 2: Physiological and pathological functions of the three PPAR isoforms in the human and murine GI tract.

cancer development in humans. In particular, esophagus, stomach, and colon are at high risk of developing cancer: indeed esophageal (EC), gastric (GC), and colorectal cancers (CRC) represent very common malignancies and account for approximately 30% of cancer-related deaths worldwide [21].

Esophageal cancer (EC) is the sixth most common cause of cancer-related death worldwide. Barrett's esophagus is the premalignant condition that appears to predispose to the adenocarcinoma trough a metaplasia-dysplasia-carcinoma sequence. The molecular mechanisms underlying the events leading to the conversion of the normal squamous epithelium to a metaplastic columnar epithelium are poorly understood. However, chronic activation of NFκB, together with the increase of COX-2 and gastrin expression due to gastroesophageal reflux could be responsible for chronic inflammation-related cancer promotion [22, 23].

Gastric cancer (GC) remains the second leading cause of cancer-related death worldwide. More than 90% of these tumors are adenocarcinomas originating from the glandular epithelium of the gastric mucosa [21]. Also in this case, inflammation plays a pivotal role in tumor development. In particular, *H. pylori* infection is the major causative agent of chronic gastritis and gastrointestinal metaplasia characterized by infiltration of inflammatory cells, enhanced expression of chemokines, NFκB activation, COX-2 overexpression, and upregulation of Wnt signaling pathway leading to aberrant cell proliferation, excessive angiogenesis, and inhibition of apoptosis [24].

Colorectal cancer (CRC) is the third most common cancer in men and the second in women [21, 25]. In the vast majority of cases, CRC occurs sporadically and only in 5–10% is due to inherited mutations [25]. The risk of CRC development increases significantly in people with inflammatory bowel diseases (IBDs), such as ulcerative colitis (UC) and Crohn's disease (CD). Chronic inflammation processes induce development of colitis-associated cancers (CAC) generally initiated by mutations in *TP53* or by COX2 overexpression and followed by *APC* inactivation at later stages [26].

Although the genetic and epigenetic alterations responsible for the different gastrointestinal (GI) cancers are still unknown, a pivotal role of inflammation in their pathogenesis is emerged. In particular, COX2 overexpression contributes to this process inhibiting apoptosis and promoting angiogenesis and invasiveness of tumor cells. Concordantly, epidemiologic studies have demonstrated that the long-term and regular use of nonsteroidal anti-inflammatory drugs (NSAIDs, COX2 inhibitors) reduces the mortality from digestive tract malignancies [24, 25].

In search of new strategies for the treatment of GI cancers, PPARs have attracted increasing attention mainly because of their anti-inflammatory effects, accompanied by prodifferentiation and proapoptotic functions [23–26]. PPARs are heterogeneously expressed along in GI tract and their role in the pathophysiology of these neoplasms is beginning to emerge (Figure 2) [27–29].

3. PPARα

PPARα is mainly expressed in the mucosa of the small and large intestine where "senses" the total flux of dietary fatty acids delivered [27, 28]. In these contexts, PPARα regulates genes involved in lipid metabolism, inflammation, cell cycle progression, and angiogenesis [30–34]. Given its role in these latter processes, PPARα has been suggested to contribute to tumor formation and/or progression. To date, no data are available on its involvement in GC and EC, whereas its role in CRC has been investigated both *in vitro* and *in vivo*. PPARα is correlated with a reduced expression of MYC-related genes, such as cyclin D1, caspase3, NFκB, STAT1, and EGFR. PPARα activation inhibits capillary tube formation *in vitro* and angiogenesis *in vivo* through a mechanism that involves deconstruction of the cytoskeleton**,** reduction of bFGF-induced Akt activity and COX-2 expression. PPARα also reduces the neovascularization, modulating the expression of VEGF, FGFs, members of the arachidonic acid P450 mono-oxygenases, thrombospondin, and endostatin [31–35]. *In vitro* PPARα induces apoptosis through modulation of Bcl-2 and Bad proteins [35, 36]. PPARα ligands, in addition, downregulate oncogenes and upregulate antiproliferative genes, supporting a tumor suppressor role [37]. In CRC cell lines, PPARα is modulated by the activation of the MAPK pathways; specifically, phosphorylation of specific amino acid residues located at the PPARα N-terminus region by JNK and p38 MAPK enhances its ligand-dependent transcriptional activity. This, in turn, promotes apoptosis, differentiation, and anti-inflammatory effects mainly through inhibition of iNOS, COX-2, and TNF-α. On the contrary, activation of ERK-MAPK signaling pathway reduces PPARα activity [38, 39]. Growing evidence obtained in animal models suggests that PPARα has anti-inflammatory effects *in vivo* but the precise and direct role it plays in intestinal inflammation is not fully elucidated. The data indicate that PPARα has anti-inflammatory effects in a mouse model of chemically induced colitis; PPARα-deficient mice exhibit enhanced inflammation; exposure to PPARα ligands controls colonic inflammation through inhibition of proinflammatory cytokines. Collectively, the evidence supports that PPARα activation leads to mitigation of IBD progression [40–42]. Unfortunately, the precise and correct assessment of PPARα function in CRC is made even more complicated by species-specific differences. The data obtained from mice models indicate that PPARα ligands play a potential role in suppressing polyp formation in *Apc*-deficient mice, an animal model corresponding to human familial adenomatous polyposis [43]. A significant reduction in PPARα expression is detected in human CRC specimens and UC patients' mucosa, suggesting PPARα as a therapeutic target to prevent adenoma formation also in IBD-induced cancer formation [36, 41, 44]. Thus, in CRC PPARα seems to act as a tumor suppressor with antiangiogenic, anti-inflammatory, and, ultimately, antitumor activities.

4. PPARβ/δ

To date, no studies have demonstrated a role for PPARβ/δ in the esophageal epithelium. In gastric epithelium, it is highly expressed but whether it has any role in tumorigenesis is still poorly understood [27, 28]. *In vitro* studies report an inverse relationship between PPARβ/δ and NFκB, IL-1β, COX2, and the Wnt-β-catenin/TCF-4 pathways, suggesting a possible protective role in cancer development by virtue of its anti-inflammatory effects [45].

PPARβ/δ is also involved in the homeostatic regulation of proliferation/differentiation and modulation of the inflammatory response in cells of the small and large intestine [27, 28, 46]. Its physiologic role, however, is still unknown as well as it is controversial its function in inflammation and CRC development. In mouse models, PPARβ/δ activation by selective ligands in small and large intestine induces terminal differentiation of epithelial and Paneth cells that play an important role in immunity and host defense [27, 28, 46–48]. Emerging evidence suggests also that PPARβ/δ can suppress inflammatory bowel diseases through a PPARβ/δ-dependent and ligand-independent downregulation of inflammatory signaling [47, 48]. These effects may be due, in part, to interference with NFκB signaling or, alternatively, to inhibition of Paneth cell differentiation that, in turn, could contribute to exacerbate experimentally induced colitis in PPARβ/δ-null mice [47, 48]. In contrast, administration of a highly specific PPARβ/δ ligand does not ameliorate inflammation [49]. The role that PPARβ/δ serves in the interplay between inflammation and cancer and in colon carcinogenesis remains debatable. In fact, *in vivo* and *in vitro* experiments have provided conflicting results suggesting that PPARβ/δ ligand activation can either potentiate or attenuate the process [50]. Its expression and/or activity is increased after loss of *APC* or activation of *K-RAS* expression [51, 52]. PPARβ/δ has also been shown to be a target of APC/β-catenin/T-cell factor- (TCF-) 4-pathway and, in turn, to modulate further downstream targets, such as c-myc and cyclin D1 [53].

5. PPARγ

PPARγ is the best-studied isoform in the GI cancer context. Its role in esophageal cancer development is debated: its activation *in vitro* reduces cell growth and induces apoptosis, implying that PPARγ ligands could have a potential use as chemotherapeutic agents in the treatment of patients affected by dysplastic Barrett's esophagus [54]. In contrast, xenografted mice treated with PPARγ agonists show an increased tumor growth. This discrepancy has been ascribed to *in vivo* effects of "tumor interactions," differences in PPARγ activation magnitude and PPARγ-independent

effects of thiazolidinediones. Recently, it has been reported that PPARγ expression increases in less differentiated human Barrett's adenocarcinoma, supporting a role for PPAR in inhibiting the development of these tumors [54–57].

As far as GC, PPARγ agonists reduce the proliferation of human cells lines *in vitro* although the effects appear to be dependent upon cell differentiation [58–60]. In contrast, PPARγ silencing in GC cell lines reduces cell viability, suggesting that PPARγ overexpression may induce tumorigenesis [61]. PPARγ agonists induce gastric acid secretion via serum and glucocorticoid inducible kinase (SGK1) upregulation [62]. Although this stimulation should favor the formation of gastric ulcers, PPARγ agonists have been reported to foster ulcer healing, suggesting that the potentially "dangerous" effect on gastric acid secretion is overridden by the simultaneous protective effects [62]. The critical importance of PPARγ in gastric carcinogenesis *in vivo* has recently been provided: PPARγ heterozygous-deficient mice show an increased susceptibility to carcinogen-induced GC and shorter survival rate than PPARγ wild-type bearing mice, implying a tumor suppressor function. In this animal model, thiazolidinediones act as chemopreventive agents in a PPARγ-dependent manner [63].

Several studies have addressed the role of *PPARG* in CRC development. *PPARG* mRNA is detected in the normal human mucosa of the caecum and colon, as well as in adenocarcinomas and CRC-derived cell lines. Although PPARγ function in colon carcinogenesis has been controversial for long time, more recent evidence supports a role as tumor suppressor [64–68]. PPARγ agonists induce cell cycle arrest, differentiation, and apoptosis. In particular, p16, p21 and p27, as well as the tumor suppressor gene, PTEN are upregulated while β-catenin, COX-2, VEGF, Bcl-2, and NFκB target genes are downregulated. Finally, PPARγ reduces the epithelial mesenchymal transition (EMT), a well-known process that allows cancer cells to acquire invasive ability, a prerequisite for metastasis formation. Consistent with the evidence *in vitro*, mouse models have shown that PPARγ ligands reduce the growth of tumors originated from subcutaneously injected human CRC cells and the number of aberrant crypt foci (ACF) in a chemically induced model of IBD [60, 69].

Loss-of-function mutations in *PPARG* have been reported in 8% of human CRCs, implying a protective effect [70]. Although these mutations have been classified as "very rare events" [70, 71], increasing evidence suggests that PPARγ activity is attenuated during the transition from adenoma to adenocarcinoma, likely explaining why PPARγ agonists are effective in blocking the early stages of tumorigenesis (i.e., ACF formation is inhibited while little or no effect is detected in advanced tumor stages) [71–74]. PPARγ-reduced activity may, at least in part, involve its phosphorylation by the mitogen-activated kinase ERK1/2, and its ligand-independent SUMOylation, two posttranslational modifications that negatively modulate PPARγ activity [72, 73]. In addition to loss-of-function mutations and inactivating posttranslational modifications, low *PPARG* expression has been found in 35% of sporadic CRCs due to epigenetic events such as DNA methylation and repressive histone modifications [75–77]. Interestingly, the epigenetic repression appears to be associated with a more aggressive course, EMT activation, and patients' worse prognosis, further supporting the notion that PPARγ is an independent prognostic factor in CRC [75, 78]. Reduced PPARγ levels have been detected in patients affected by IBDs, such as UC and CD, suggesting that impaired *PPARG* expression precedes and is not secondary to the inflammatory process and likely contributes to the pathogenesis of IBDs [79–81]. Concordantly, *PPARG* genetic variations have recently been correlated with a different risk of IBD incidence [81, 82]. Low PPARγ levels have also been found in peripheral mononuclear cells of IBD patients in the absence of specific *PPARG* mutations. Epigenetic events or abnormal signaling pathways carried out by natural ligands or microorganisms of the colon microenvironment might account for the impaired *PPARG* expression in UC and CD patients [82].

6. PPARs and ncRNA

PPARs deregulation during tumorigenesis of the GI tract has been attributed to gene mutations, altered levels of expression and, more recently, epigenetic modifications. These latter events, however, have been identified as "critical" only for *PPARG* expression while no evidence has been provided for their involvement in PPARα and PPARβ/δ regulation [69, 76, 77]. A novel mechanism of gene regulation is emerging that involves noncoding RNAs (ncRNAs). They are recognized as important regulators of physiological and pathological processes playing critical roles in DNA structure, RNA production, protein translation, and protein functions [83]. The term ncRNA includes both micro-RNAs (miRNA) and long noncoding RNAs (lncRNA). MicroRNAs are small noncoding RNAs that inhibit protein translation or induce degradation of their target mRNAs upon binding to cognate recognition sites usually located in the 3′ untranslated region [84]. Over one third of protein-coding genes is potentially regulated by miRNAs thus affecting important biological functions among which tumorigenesis [84, 85]. A handful of miRNAs have been identified to promote or inhibit tumor initiation, progression, and metastasis, influencing oncogenes or tumour suppressor genes or acting as oncogenes or tumor suppressors themselves [86, 87]. Although the role of ncRNAs in the regulation of PPARs expression or activity is beyond the scope of this review, we would like to mention the miRNAs directly involved in PPARs regulation. miR-21 and miR-10b downregulate PPARα in liver, while miR-506 targets this receptor in human CRC cell lines [88–91]. PPARγ is negatively regulated by miR-27 and miR-130 family members in preadipocytes, hampering adipocyte differentiation [92–97]. In addition, miR-27 reduces PPARγ expression in LPS-stimulated macrophages, inhibiting its anti-inflammatory activity [92]. More recently, miR-27 has been implicated in downregulation of PPARγ in cardiomyocytes and also in neuroblastoma and breast cancer [95, 97]. miR-122 targets PPARβ/δ in liver [98]. Lastly, PPAR activity may also be repressed via miR-dependent targeting of PPARs-coregulators [99]. All together these observations indicate that miRNAs may

exert coordinating and redundancy-limiting actions on the gene-expression networks controlled by PPARs [99].

In addition to miRNAs, also long noncoding RNAs (lcnRNAs) regulate nuclear receptors and thus, potentially, PPARs expression and activity. LncRNAs are, in general, defined as non-protein coding transcripts longer than 200 nucleotides that might directly affect gene expression through the interaction with transcriptional activators/repressors inducing or repressing gene transcription [83]. Several studies suggest a critical role of lncRNAs also in the epigenetic-dependent gene regulation by orienting chromatin-modifying factors/complexes to specific locations in the genome and in the nucleus [83, 100, 101]. The direct involvement of lncRNA in PPARs expression has not been described so far; the ncRNA SRA (steroid receptor RNA activator) has recently been reported to associate with PPARγ and modulate transcription of PPAR-target genes [102]. A more recent work provides evidence of a new mechanism of nuclear receptor activity regulation: the ncRNA Gas5 acts as a decoy RNA inhibiting the activity of the glucocorticoid receptor on its target genes [103].

Although the relationship between PPARs and ncRNAs in cancer is only at the beginning to emerge, it is conceivable that miRNAs may regulate PPARs expression influencing the development of GI cancers at different levels.

7. Conclusions and Future Directions

Dietary, environmental, and genetic factors contribute to the etiology, pathogenesis and risk with of gastrointestinal cancers. The link between PPARs and environmental factors in the development of GI tumors is strong, reciprocal but still poorly understood at molecular level. Inflammation plays a crucial role in the development of premalignant lesions of the esophagus, stomach, and colon rectum that, up to now, has only indirectly been proved through expression/correlation studies. PPARs impact diverse aspects of cancer development such as signaling pathways, metabolic interactions, cell cycle, and inflammation.

Here, we have overviewed the most recent evidence of the literature supporting the hypothesis that the events underlying chronic inflammatory conditions and their evolution towards GI tumors could be at least in part orchestrated by the pro- and antitumor effects mediated by PPARs. Their expression and activity in tumor cells are modulated by genetic and epigenetic alterations; miRNAs are emerging as a new pathogenetic player. Intriguingly, dietary and life styles as well as environmental factors may influence PPARs function and impact cancer predisposition through epigenetic mechanisms. Hence, understanding how the individual genetic background and environmental factors contribute to PPARs deregulation and hence to the establishment of an inflammatory status or a tumor condition is mandatory. The studies reported here suggest, in addition, a rationale for novel strategies in cancer treatment whereby PPARs ligands might directly interfere with tumor growth and promote anticancer activity. More direct data and deeper evidence are still awaited to appraise the benefits that these agonists may provide in the prevention and treatment of GI tract inflammations and tumors. Clinical trials suggest that PPARs ligands may not be so effective as a single agent in advanced tumors but they could be effective in combination with a classical chemotherapy and additional anticancer agents such as epigenetic drugs, recently introduced into the therapeutic armamentarium. Another promising translational outcome of these studies is the possibility to identify PPAR alterations in premalignant lesions so that they can be used as prognostic biomarkers. In conclusion, elucidation of these pathways could provide biomarkers or new therapeutic targets with broad implications for cancer prevention, risk prediction, and prognosis.

Authors' Contribution

A. Fucci and T. Colangelo contributed equally to the work.

References

[1] B. Desvergne and W. Wahli, "Peroxisome proliferator-activated receptors: nuclear control of metabolism," *Endocrine Reviews*, vol. 20, no. 5, pp. 649–688, 1999.

[2] S. Kersten and W. Wahli, "Peroxisome proliferator activated receptor agonists," *EXS*, vol. 89, pp. 141–151, 2000.

[3] L. Michalik, B. Desvergne, and W. Wahli, "Peroxisome-proliferator-activated receptors and cancers: complex stories," *Nature Reviews Cancer*, vol. 4, no. 1, pp. 61–70, 2004.

[4] R. M. Evans, G. D. Barish, and Y. X. Wang, "PPARs and the complex journey to obesity," *Nature Medicine*, vol. 10, no. 4, pp. 355–361, 2004.

[5] Z. Nahlé, "PPAR trilogy from metabolism to cancer," *Current Opinion in Clinical Nutrition and Metabolic Care*, vol. 7, no. 4, pp. 397–402, 2004.

[6] L. Michalik, J. Auwerx, J. P. Berger et al., "International union of pharmacology. LXI. Peroxisome proliferator-activated receptors," *Pharmacological Reviews*, vol. 58, no. 4, pp. 726–741, 2006.

[7] L. Michalik and W. Wahli, "Involvement of PPAR nuclear receptors in tissue injury and wound repair," *The Journal of Clinical Investigation*, vol. 116, no. 3, pp. 598–606, 2006.

[8] L. Fajas, J. C. Fruchart, and J. Auwerx, "PPARγ3 mRNA: a distinct PPARγ mRNA subtype transcribed from an independent promoter," *FEBS Letters*, vol. 438, no. 1-2, pp. 55–60, 1998.

[9] K. Al-Shali, H. Cao, N. Knoers, A. R. Hermus, C. J. Tack, and R. A. Hegele, "A single-base mutation in the peroxisome proliferator-activated receptor γ4 promoter associated with altered in vitro expression and partial lipodystrophy," *The Journal of Clinical Endocrinology & Metabolism*, vol. 89, no. 11, pp. 5655–5660, 2004.

[10] Y. Zhu, K. Alvares, Q. Huang, M. S. Rao, and J. K. Reddy, "Cloning of a new member of the peroxisome proliferator-activated receptor gene family from mouse liver," *Journal of Biological Chemistry*, vol. 268, no. 36, pp. 26817–26820, 1993.

[11] A. Meirhaeghe and P. Amouyel, "Impact of genetic variation of PPARγ in humans," *Molecular Genetics and Metabolism*, vol. 83, no. 1-2, pp. 93–102, 2004.

[12] P. Tontonoz, E. Hu, R. A. Graves, A. I. Budavari, and B. M. Spiegelman, "mPPARγ2: tissue-specific regulator of an adipocyte enhancer," *Genes and Development*, vol. 8, no. 10, pp. 1224–1234, 1994.

[13] C. Diradourian, J. Girard, and J. P. Pégorier, "Phosphorylation of PPARs: from molecular characterization to physiological relevance," *Biochimie*, vol. 87, no. 1, pp. 33–38, 2005.

[14] J. P. Renaud and D. Moras, "Structural studies on nuclear receptors," *Cellular and Molecular Life Sciences*, vol. 57, no. 12, pp. 1748–1769, 2000.

[15] D. J. Mangelsdorf and R. M. Evans, "The RXR heterodimers and orphan receptors," *Cell*, vol. 83, no. 6, pp. 841–850, 1995.

[16] J. Berger and D. E. Moller, "The mechanisms of action of PPARs," *Annual Review of Medicine*, vol. 53, pp. 409–435, 2002.

[17] M. Ricote and C. K. Glass, "PPARs and molecular mechanisms of transrepression," *Biochimica et Biophysica Acta*, vol. 1771, no. 8, pp. 926–935, 2007.

[18] A. Yessoufou and W. Wahli, "Multifaceted roles of peroxisome proliferator-activated receptors (PPARs) at the cellular and whole organism levels," *Swiss Medical Weekly*, vol. 140, p. w13071, 2010.

[19] J. Torchia, C. Glass, and M. G. Rosenfeld, "Co-activators and co-repressors in the integration of transcriptional responses," *Current Opinion in Cell Biology*, vol. 10, no. 3, pp. 373–383, 1998.

[20] M. Li, G. Pascual, and C. K. Glass, "Peroxisome proliferator-activated receptor γ-dependent repression of the inducible nitric oxide synthase gene," *Molecular and Cellular Biology*, vol. 20, no. 13, pp. 4699–4707, 2000.

[21] R. M. Thomas and L. H. Sobin, "Gastrointestinal cancer," *Cancer*, vol. 75, no. 1, pp. 154–170, 1995.

[22] O. H. Al-Taie, T. Graf, B. Illert et al., "Differential effects of PPARγ activation by the oral antidiabetic agent pioglitazone in Barrett's carcinoma in vitro and in vivo," *Journal of Gastroenterology*, vol. 44, no. 9, pp. 919–929, 2009.

[23] P. C. Konturek, A. Nikiforuk, J. Kania, M. Raithel, E. G. Hahn, and S. M. Mühldorfer, "Activation of NFκB represents the central event in the neoplastic progression associated with Barrett's esophagus: a possible link to the inflammation and overexpression of COX-2, PPARγ and growth factors," *Digestive Diseases and Sciences*, vol. 49, no. 7-8, pp. 1075–1083, 2004.

[24] C. Hafner, A. Reichle, and T. Vogt, "New indications for established drugs: combined tumor-stroma-targeted cancer therapy with PPARγ agonists, COX-2 inhibitors, mTOR antagonists and metronomic chemotherapy," *Current Cancer Drug Targets*, vol. 5, no. 6, pp. 393–419, 2005.

[25] R. E. Schoen, P. F. Pinsky, J. L. Weissfeld et al., "Colorectal-cancer incidence and mortality with screening flexible sigmoidoscopy," *The New England Journal of Medicine*, vol. 366, no. 25, pp. 2345–2357, 2012.

[26] L. Hartnett and L. J. Egan, "Inflammation, DNA methylation and colitis-associated cancer," *Carcinogenesis*, vol. 33, no. 4, pp. 723–731, 2012.

[27] C. Huin, L. Corriveau, A. Bianchi et al., "Differential expression of peroxisome proliferator-activated receptors (PPARs) in the developing human fetal digestive tract," *The Journal of Histochemistry & Cytochemistry*, vol. 48, no. 5, pp. 603–611, 2000.

[28] O. Braissant and W. Wahli, "Differential expression of peroxisome proliferator-activated receptor- α, -β_1, and -γ during rat embryonic development," *Endocrinology*, vol. 139, no. 6, pp. 2748–2754, 1998.

[29] S. A. Kliewer, B. M. Forman, B. Blumberg et al., "Differential expression and activation of a family of murine peroxisome proliferator-activated receptors," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 91, no. 15, pp. 7355–7359, 1994.

[30] S. Cuzzocrea, R. di Paola, E. Mazzon et al., "Role of endogenous and exogenous ligands for the peroxisome proliferators activated receptors alpha (PPAR-α) in the development of inflammatory bowel disease in mice," *Laboratory Investigation*, vol. 84, no. 12, pp. 1643–1654, 2004.

[31] M. Griffioen, W. T. Steegenga, I. J. M. Ouwerkerk, L. T. C. Peltenburg, A. G. Jochemsen, and P. I. Schrier, "Repression of the minimal HLA-B promoter by c-myc and p53 occurs through independent mechanisms," *Molecular Immunology*, vol. 35, no. 13, pp. 829–835, 1998.

[32] J. D. Watson, S. K. Oster, M. Shago, F. Khosravi, and L. Z. Penn, "Identifying genes regulated in a Myc-dependent manner," *Journal of Biological Chemistry*, vol. 277, no. 40, pp. 36921–36930, 2002.

[33] J. Varet, L. Vincent, P. Mirshahi et al., "Fenofibrate inhibits angiogenesis in vitro and in vivo," *Cellular and Molecular Life Sciences*, vol. 60, no. 4, pp. 810–819, 2003.

[34] T. Kasai, K. Miyauchi, T. Yokoyama, K. Aihara, and H. Daida, "Efficacy of peroxisome proliferative activated receptor (PPAR)-α ligands, fenofibrate, on intimal hyperplasia and constrictive remodeling after coronary angioplasty in porcine models," *Atherosclerosis*, vol. 188, no. 2, pp. 274–280, 2006.

[35] A. Pozzi and J. H. Capdevila, "PPARα ligands as antitumorigenic and antiangiogenic agents," *PPAR Research*, vol. 2008, Article ID 906542, 8 pages, 2008.

[36] G. Martinasso, M. Oraldi, A. Trombetta et al., "Involvement of PPARs in cell proliferation and apoptosis in human colon cancer specimens and in normal and cancer cell lines," *PPAR Research*, vol. 2007, Article ID 93416, 9 pages, 2007.

[37] F. Gizard, C. Amant, O. Barbier et al., "PPARα inhibits vascular smooth muscle cell proliferation underlying intimal hyperplasia by inducing the tumor suppressor p16INK4a," *The Journal of Clinical Investigation*, vol. 115, no. 11, pp. 3228–3238, 2005.

[38] P. M. Barger, A. C. Browning, A. N. Garner, and D. P. Kelly, "p38 mitogen-activated protein kinase activates peroxisome proliferator-activated receptor α: a potential role in the cardiac metabolic stress response," *Journal of Biological Chemistry*, vol. 276, no. 48, pp. 44495–44501, 2001.

[39] K. A. Burns and J. P. Vanden Heuvel, "Modulation of PPAR activity via phosphorylation," *Biochimica et Biophysica Acta*, vol. 1771, no. 8, pp. 952–960, 2007.

[40] L. Riccardi, E. Mazzon, S. Bruscoli et al., "Peroxisome proliferator-activated receptor-α modulates the anti-inflammatory effect of glucocorticoids in a model of inflammatory bowel disease in mice," *Shock*, vol. 31, no. 3, pp. 308–316, 2009.

[41] L. A. Moraes, L. Piqueras, and D. Bishop-Bailey, "Peroxisome proliferator-activated receptors and inflammation," *Pharmacology and Therapeutics*, vol. 110, no. 3, pp. 371–385, 2006.

[42] S. R. Pyper, N. Viswakarma, S. Yu, and J. K. Reddy, "PPARα: energy combustion, hypolipidemia, inflammation and cancer," *Nuclear receptor signaling*, vol. 8, article e002, 2010.

[43] M. Mutoh, N. Niho, and K. Wakabayashi, "Concomitant suppression of hyperlipidemia and intestinal polyp formation by increasing lipoprotein lipase activity in Apc-deficient mice," *Biological chemistry*, vol. 387, no. 4, pp. 381–385, 2006.

[44] M. W. Matthiessen, G. Pedersen, T. Albrektsen, S. Adamsen, J. Fleckner, and J. Brynskov, "Peroxisome proliferator-activated receptor expression and activation in normal human colonic epithelial cells and tubular adenomas," *Scandinavian Journal of Gastroenterology*, vol. 40, no. 2, pp. 198–205, 2005.

[45] C. W. Wu, J. Yu, and J. J. Y. Sung, "Peroxisome proliferator-activated receptor δ and gastric cancer," *Oncology Reports*, vol. 22, no. 3, pp. 451–457, 2009.

[46] J. M. Peters, H. E. Hollingshead, and F. J. Gonzalez, "Role of peroxisome-proliferator-activated receptor β/δ (PPARβ/δ) in gastrointestinal tract function and disease," *Clinical Science*, vol. 115, no. 3-4, pp. 107–127, 2008.

[47] F. Varnat, B. B. Heggeler, P. Grisel et al., "PPARβ/δ regulates paneth cell differentiation via controlling the hedgehog signaling pathway," *Gastroenterology*, vol. 131, no. 2, pp. 538–553, 2006.

[48] P. Escher, O. Braissant, S. Basu-Modak, L. Michalik, W. Wahli, and B. Desvergne, "Rat PPARs: quantitative analysis in adult rat tissues and regulation in fasting and refeeding," *Endocrinology*, vol. 142, no. 10, pp. 4195–4202, 2001.

[49] H. E. Hollingshead, K. Morimura, M. Adachi et al., "PPARβ/δ protects against experimental colitis through a ligand-independent mechanism," *Digestive Diseases and Sciences*, vol. 52, no. 11, pp. 2912–2919, 2007.

[50] O. Takayama, H. Yamamoto, B. Damdinsuren et al., "Expression of PPARδ in multistage carcinogenesis of the colorectum: implications of malignant cancer morphology," *British Journal of Cancer*, vol. 95, no. 7, pp. 889–895, 2006.

[51] T. C. He, T. A. Chan, B. Vogelstein, and K. W. Kinzler, "PPARδ is an APC-regulated target of nonsteroidal anti-inflammatory drugs," *Cell*, vol. 99, no. 3, pp. 335–345, 1999.

[52] J. Shao, H. Sheng, and R. N. DuBois, "Peroxisome proliferator-activated receptors modulate K-Ras-mediated transformation of intestinal epithelial cells," *Cancer Research*, vol. 62, no. 11, pp. 3282–3288, 2002.

[53] T. C. He, A. B. Sparks, C. Rago et al., "Identification of c-MYC as a target of the APC pathway," *Science*, vol. 281, no. 5382, pp. 1509–1512, 1998.

[54] W. Wang, R. Wang, Z. Zhang, D. Li, and Y. Yu, "Enhanced PPAR-γ expression may correlate with the development of Barrett's esophagus and esophageal adenocarcinoma," *Oncology Research*, vol. 19, no. 3-4, pp. 141–147, 2011.

[55] Y. Naito, T. Takagi, and T. Yoshikawa, "Gastrointestinal cytoprotection by PPARγ ligands," *PPAR Research*, vol. 2010, Article ID 108632, 8 pages, 2010.

[56] C. Tselepis, I. Perry, C. Dawson et al., "Tumour necrosis factor-α in Barrett's oesophagus: a potential novel mechanism of action," *Oncogene*, vol. 21, no. 39, pp. 6071–6081, 2002.

[57] M. M. M. Abdel-Latif, J. O'Riordan, H. J. Windle et al., "NF-κB activation in esophageal adenocarcinoma: relationship to Barrett's metaplasia, survival, and response to neoadjuvant chemoradiotherapy," *Annals of Surgery*, vol. 239, no. 4, pp. 491–500, 2004.

[58] H. Sato, S. Ishihara, K. Kawashima et al., "Expression of peroxisome proliferator-activated receptor (PPAR)γ in gastric cancer and inhibitory effects of PPARγ agonists," *British Journal of Cancer*, vol. 83, no. 10, pp. 1394–1400, 2000.

[59] S. Kitamura, Y. Miyazaki, S. Hiraoka et al., "PPARγ inhibits the expression of c-MET in human gastric cancer cells through the suppression of Ets," *Biochemical and Biophysical Research Communications*, vol. 265, no. 2, pp. 453–456, 1999.

[60] C. Grommes, G. E. Landreth, and M. T. Heneka, "Antineoplastic effects of peroxisome proliferator-activated receptor γ agonists," *Lancet Oncology*, vol. 5, no. 7, pp. 419–429, 2004.

[61] S. H. Liu, C. C. Shen, Y. C. Yi et al., "Honokiol inhibits gastric tumourigenesis by activation of 15-lipoxygenase-1 and consequent inhibition of peroxisome proliferator-activated receptor-γ and COX-2-dependent signals," *British Journal of Pharmacology*, vol. 160, no. 8, pp. 1963–1972, 2010.

[62] A. Rotte, A. F. MacK, M. Bhandaru et al., "Pioglitazone induced gastric acid secretion," *Cellular Physiology and Biochemistry*, vol. 24, no. 3-4, pp. 193–200, 2009.

[63] J. Lu, K. Imamura, S. Nomura et al., "Chemopreventive effect of peroxisome proliferator-activated receptor γ on gastric carcinogenesis in mice," *Cancer Research*, vol. 65, no. 11, pp. 4769–4774, 2005.

[64] E. Saez, P. Tontonoz, M. C. Nelson et al., "Activators of the nuclear receptor PPARγ enhance colon polyp formation," *Nature Medicine*, vol. 4, no. 9, pp. 1058–1061, 1998.

[65] P. Sarraf, E. Mueller, D. Jones et al., "Differentiation and reversal of malignant changes in colon cancer through PPARγ," *Nature Medicine*, vol. 4, no. 9, pp. 1046–1052, 1998.

[66] S. Drori, G. D. Girnun, L. Tou et al., "Hic-5 regulates an epithelial program mediated by PPARγ," *Genes and Development*, vol. 19, no. 3, pp. 362–375, 2005.

[67] W. Su, C. R. Bush, B. M. Necela et al., "Differential expression, distribution, and function of PPAR-γ in the proximal and distal colon," *Physiological Genomics*, vol. 30, no. 3, pp. 342–353, 2007.

[68] N. Niho, M. Takahashi, Y. Shoji et al., "Dose-dependent suppression of hyperlipidemia and intestinal polyp formation in Min mice by pioglitazone, a PPARγ ligand," *Cancer Science*, vol. 94, no. 11, pp. 960–964, 2003.

[69] L. Sabatino, A. Fucci, M. Pancione, and V. Colantuoni, "PPARG epigenetic deregulation and Its role in colorectal tumorigenesis," *PPAR Research*, vol. 2012, Article ID 687492, 12 pages, 2012.

[70] P. Sarraf, E. Mueller, W. M. Smith et al., "Loss-of-function mutations in PPARγ associated with human colon cancer," *Molecular Cell*, vol. 3, no. 6, pp. 799–804, 1999.

[71] T. Ikezoe, C. W. Miller, S. Kawano et al., "Mutational analysis of the peroxisome proliferator-activated receptor γ in human malignancies," *Cancer Research*, vol. 61, no. 13, pp. 5307–5310, 2001.

[72] E. Burgermeister and R. Seger, "MAPK kinases as nucleocytoplasmic shuttles for PPARγ," *Cell Cycle*, vol. 6, no. 13, pp. 1539–1548, 2007.

[73] T. M. Garcia-Bates, G. M. Lehmann, P. J. Simpson-Haidaris, S. H. Bernstein, P. J. Sime, and R. P. Phipps, "Role of peroxisome proliferator-activated receptor gamma and its ligands in the treatment of hematological malignancies," *PPAR Research*, vol. 2008, Article ID 834612, 18 pages, 2008.

[74] E. A. Thompson, "PPARγ physiology and pathology in gastrointestinal epithelial cells," *Molecules and Cells*, vol. 24, no. 2, pp. 167–176, 2007.

[75] M. Pancione, N. Forte, L. Sabatino et al., "Reduced β-catenin and peroxisome proliferator-activated receptor-γ expression levels are associated with colorectal cancer metastatic progression: correlation with tumor-associated macrophages, cyclooxygenase 2, and patient outcome," *Human Pathology*, vol. 40, no. 5, pp. 714–725, 2009.

[76] M. Pancione, L. Sabatino, A. Fucci et al., "Epigenetic silencing of peroxisome proliferator- activated receptor γ is a biomarker for colorectal cancer progression and adverse patients' outcome," *PLoS One*, vol. 5, no. 12, Article ID e14229, 2010.

[77] L. Sabatino, A. Fucci, M. Pancione et al., "UHRF1 coordinates peroxisome proliferator activated receptor gamma (PPARG) epigenetic silencing and mediates colorectal cancer progression," *Oncogene*. In press.

[78] S. Ogino, K. Shima, Y. Baba et al., "Colorectal cancer expression of peroxisome proliferator-activated receptor γ

(PPARG, PPARgamma) is associated with good prognosis," *Gastroenterology*, vol. 136, no. 4, pp. 1242–1250, 2009.

[79] J. K. Yamamoto-Furusho, A. Peñaloza-Coronel, F. Sánchez-Muñoz, R. Barreto-Zuñiga, and A. Dominguez-Lopez, "Peroxisome proliferator-activated receptor-gamma (PPAR-γ) expression is downregulated in patients with active ulcerative colitis," *Inflammatory Bowel Diseases*, vol. 17, no. 2, pp. 680–681, 2011.

[80] Z.-F. Zhang, N. Yang, G. Zhao, L. Zhu, and L.-X. Wang, "Association between the Pro12Ala polymorphism of peroxisome proliferator-activated receptor gamma 2 and inflammatory bowel disease: a meta-analysis," *PLoS One*, vol. 7, no. 1, Article ID e30551, 2012.

[81] V. Andersen, J. Christensen, A. Ernst et al., "Polymorphisms in NF-κB, PXR, LXR, PPARγ and risk of infammatory bowel disease," *World Journal of Gastroenterology*, vol. 17, no. 2, pp. 197–206, 2011.

[82] L. Dubuquoy, C. Rousseaux, X. Thuru et al., "PPARγ as a new therapeutic target in inflammatory bowel diseases," *Gut*, vol. 55, no. 9, pp. 1341–1349, 2006.

[83] A. M. W. Turner and K. V. Morris, "Controlling transcription with noncoding RNAs in mammalian cells," *BioTechniques*, vol. 48, no. 6, pp. 9–16, 2010.

[84] G. Stefani and F. J. Slack, "Small non-coding RNAs in animal development," *Nature Reviews Molecular Cell Biology*, vol. 9, no. 3, pp. 219–230, 2008.

[85] W. Filipowicz, S. N. Bhattacharyya, and N. Sonenberg, "Mechanisms of post-transcriptional regulation by microRNAs: Are the answers in sight?" *Nature Reviews Genetics*, vol. 9, no. 2, pp. 102–114, 2008.

[86] R. Garzon, G. A. Calin, and C. M. Croce, "MicroRNAs in cancer," *Annual Review of Medicine*, vol. 60, pp. 167–179, 2009.

[87] V. A. Krutovskikh and Z. Herceg, "Oncogenic microRNAs (OncomiRs) as a new class of cancer biomarkers," *BioEssays*, vol. 32, no. 10, pp. 894–904, 2010.

[88] K. Kida, M. Nakajima, T. Mohri et al., "PPARα is regulated by miR-21 and miR-27b in human liver," *Pharmaceutical Research*, vol. 28, no. 10, pp. 2467–2476, 2011.

[89] J. Zhou, K.-C. Wang, W. Wu et al., "MicroRNA-21 targets peroxisome proliferators-activated receptor-α in an autoregulatory loop to modulate flow-induced endothelial inflammation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 25, pp. 10355–10360, 2011.

[90] L. Zheng, G. C. Lv, J. Sheng, and Y. D. Yang, "Effect of miRNA-10b in regulating cellular steatosis level by targeting PPAR-α expression, a novel mechanism for the pathogenesis of NAFLD," *Journal of Gastroenterology and Hepatology*, vol. 25, no. 1, pp. 156–163, 2010.

[91] J. L. Tong, C. P. Zhang, F. Nie et al., "MicroRNA 506 regulates expression of PPAR alpha in hydroxycamptothecin-resistant human colon cancer cells," *FEBS Letters*, vol. 585, no. 22, pp. 3560–3568, 2011.

[92] C. Jennewein, A. von Knethen, T. Schmid, and B. Brüne, "MicroRNA-27b contributes to lipopolysaccharide-mediated peroxisome proliferator-activated receptor γ (PPARγ) mRNA destabilization," *Journal of Biological Chemistry*, vol. 285, no. 16, pp. 11846–11853, 2010.

[93] M. Karbiener, C. Fischer, S. Nowitsch et al., "microRNA miR-27b impairs human adipocyte differentiation and targets PPARγ," *Biochemical and Biophysical Research Communications*, vol. 390, no. 2, pp. 247–251, 2009.

[94] S. Y. Kim, A. Y. Kim, H. W. Lee et al., "miR-27a is a negative regulator of adipocyte differentiation via suppressing PPARγ expression," *Biochemical and Biophysical Research Communications*, vol. 392, no. 3, pp. 323–328, 2010.

[95] J.-J. Lee, A. Drakaki, D. Iliopoulos, and K. Struhl, "MiR-27b targets PPARγ toinhibit growth, tumor progression and the inflammatory response in neuroblastoma cells," *Oncogene*. In press.

[96] E. K. Lee, M. J. Lee, K. Abdelmohsen et al., "miR-130 suppresses adipogenesis by inhibiting peroxisome proliferator-activated receptor γ expression," *Molecular and Cellular Biology*, vol. 31, no. 4, pp. 626–638, 2011.

[97] J. Wang, Y. Song, Y. Zhang et al., "Cardiomyocyte overexpression of miR-27b induces cardiac hypertrophy and dysfunction in mice," *Cell Research*, vol. 22, no. 3, pp. 516–527, 2012.

[98] D. Gatfield, G. Le Martelot, C. E. Vejnar et al., "Integration of microRNA miR-122 in hepatic circadian gene expression," *Genes and Development*, vol. 23, no. 11, pp. 1313–1326, 2009.

[99] R. J. A. H. Eendebak, P. J. Lucassen, and C. P. Fitzsimons, "Nuclear receptors and microRNAs: Who regulates the regulators in neural stem cells?" *FEBS Letters*, vol. 585, no. 5, pp. 717–722, 2011.

[100] X. Wang, X. Song, C. K. Glass, and M. G. Rosenfeld, "The long arm of long noncoding RNAs: roles as sensors regulating gene transcriptional programs," *Cold Spring Harbor Perspectives in Biology*, vol. 3, no. 1, article a003756, 2011.

[101] L. Nie, H.-J. Wu, J.-M. Hsu et al., "Long non-coding RNAs: versatile master regulators of gene expression and crucial players in cancer," *American Journal of Translational Research*, vol. 4, no. 2, pp. 127–150, 2012.

[102] B. Xu, I. Gerin, H. Miao et al., "Multiple roles for the non-coding RNA SRA in regulation of adipogenesis and insulin sensitivity," *PLoS One*, vol. 5, no. 12, Article ID e14199, 2010.

[103] T. Kino, D. E. Hurt, T. Ichijo, N. Nader, and G. P. Chrousos, "Noncoding RNA Gas5 is a growth arrest- and starvation-associated repressor of the glucocorticoid receptor," *Science Signaling*, vol. 3, no. 107, p. ra8, 2010.

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Research Article

Time-Qualified Patterns of Variation of PPARγ, DNMT1, and DNMT3B Expression in Pancreatic Cancer Cell Lines

Valerio Pazienza,[1] Francesca Tavano,[2] Massimo Francavilla,[3] Andrea Fontana,[4] Fabio Pellegrini,[4] Giorgia Benegiamo,[1] Vincenzo Corbo,[5] Fabio Francesco di Mola,[2] Pierluigi Di Sebastiano,[2] Angelo Andriulli,[1] and Gianluigi Mazzoccoli[6]

[1] *Research Laboratory and Gastroenterology Unit, Scientific Institute and Regional General Hospital "Casa Sollievo della Sofferenza", 71013 San Giovanni Rotondo, Italy*
[2] *Research Laboratory and Surgery Unit, Scientific Institute and Regional General Hospital "Casa Sollievo della Sofferenza", 71013 San Giovanni Rotondo, Italy*
[3] *Computing Unit, Scientific Institute and Regional General Hospital "Casa Sollievo della Sofferenza", 71013 San Giovanni Rotondo, Italy*
[4] *Unit of Biostatistics, Scientific Institute and Regional General Hospital "Casa Sollievo della Sofferenza", 71013 San Giovanni Rotondo, Italy*
[5] *ARC-NET Miriam Cherubini Centre for Applied Research on Cancer, University and Hospital Trust of Verona, 37100 Verona, Italy*
[6] *Division of Internal Medicine and Chronobiology Unit, Department of Medical Sciences, Scientific Institute and Regional General Hospital "Casa Sollievo della Sofferenza", 71013 San Giovanni Rotondo, Italy*

Correspondence should be addressed to Gianluigi Mazzoccoli, g.mazzoccoli@tin.it

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Carcinogenesis is related to the loss of homeostatic control of cellular processes regulated by transcriptional circuits and epigenetic mechanisms. Among these, the activities of peroxisome proliferator-activated receptors (PPARs) and DNA methyltransferases (DNMTs) are crucial and intertwined. PPARγ is a key regulator of cell fate, linking nutrient sensing to transcription processes, and its expression oscillates with circadian rhythmicity. Aim of our study was to assess the periodicity of PPARγ and DNMTs in pancreatic cancer (PC). We investigated the time-related patterns of *PPARG, DNMT1*, and *DNMT3B* expression monitoring their mRNA levels by qRT-PCR at different time points over a 28-hour span in BxPC-3, CFPAC-1, PANC-1, and MIAPaCa-2 PC cells after synchronization with serum shock. *PPARG* and *DNMT1* expression in PANC-1 cells and *PPARG* expression in MIAPaCa-2 cells were characterized by a 24 h period oscillation, and a borderline significant rhythm was observed for the *PPARG, DNMT1*, and *DNMT3B* expression profiles in the other cell lines. The time-qualified profiles of gene expression showed different shapes and phase relationships in the PC cell lines examined. In conclusion, *PPARG* and *DNMTs* expression is characterized by different time-qualified patterns in cell lines derived from human PC, and this heterogeneity could influence cell phenotype and human disease behaviour.

1. Introduction

Cancer statistics rank pancreatic cancer as the fourth leading cause of malignancy-related death worldwide [1], and incidence and mortality rates are very similar, due to difficult early diagnosis, elevated aggressiveness, and chemotherapy resistance. Bad prognosis and lack of effective treatment are responsible for high lethality, so that there is pressing need to identify molecular biomarkers for prognostic assessment and target therapy. The preservation of tissue integrity is critical for organism survival and relies on tissue renewal, driven by stem cells that are capable of responding to injury and repairing tissue damage, caused by physical, chemicals, microbial, and mutagenic agents. Transcriptional mechanisms regulate cell processes underlying cell renewal and comprising proliferation, differentiation, cell death, and

apoptosis. Carcinogenesis relies on the loss of homeostatic mechanisms regulating cell proliferative, differentiative, and survival processes. Among the transcriptional regulators an important role is played by the peroxisome proliferator-activated receptors (PPARs), ligand-activated transcription factors belonging to the superfamily of nuclear hormone receptors, which are considered to be involved in the regulation of nutrient metabolism and energy homeostasis, and in various pathophysiological processes, such as metabolic derangement, inflammation, and cancerogenesis [2]. PPARs are crucial for the transduction of metabolic and nutritional signals into transcriptional responses and comprise three isoforms, PPARα, PPARβ/δ, and PPARγ, with a high degree of homology but with distinct biological activities [3]. PPARα is mainly involved in lipid metabolism, the function of PPARβ/δ is not entirely clear, and PPARγ regulates cell fate and differentiation decisions, as well as adipogenesis and fat storage [4–7]. PPARγ expression oscillates over a 24-hour span, and its circadian rhythmicity is crucial in the crosstalk between feeding/fasting cycles, nutrient sensing, metabolic pathways and transcriptional processes. The derangement of this crosstalk is involved in cancer development [8, 9]. High-affinity synthetic ligands, the thiazolidinedione, prompted the study of PPARγ signalling pathways in the regulation of metabolic processes and are currently evaluated as possible therapeutic tools to take advantage of PPARγ prodifferentiative effects in cancer treatment [10].

Transcriptional processes are regulated also by epigenetic mechanisms, such as acetylation/deacetylation and methylation/demethylation. DNA methyltransferases (DNMTs) play a critical role in epigenetic mechanisms attaching methyl groups to DNA, and in particular DNMT1 keeps up the methylation pattern during DNA replication, whereas DNMT3a and DNMT3b primarily catalyze *de novo* methylation [11–13]. An intriguing interaction between PPARγ and DNMTs has been recently suggested by the downregulation of DNA methyltransferases evidenced in immune cells following ligand-dependent PPARγ activation [14].

The aim of our study was to assess the time-related patterns of variation of PPARγ and DNMTs in pancreatic cancer using *in vitro* models represented by pancreatic cancer cell lines evaluated after synchronization.

2. Material and Methods

2.1. Cell Culture and Serum Shock Procedures. BxPC-3, CFPAC-1, PANC-1, and MIA PaCa-2 cells were cultured at 37°C in 5% CO_2 atmosphere in DMEM medium supplemented with 10% fetal bovine serum (FCS), 100 U/mL penicillin, and 100 ng/mL streptomycin (Invitrogen Life Technologies, Milan, Italy) while CFPAC-1 and MIA PaCa-2 were maintained in RPMI medium (Invitrogen Life Technologies, Milan, Italy). Cell synchronization was obtained by means of serum shock performed as follows: approximately 5×10^5 cells/6 wells were plated the day before the experiments. At the day of the experiments, culture medium was exchanged with serum-rich medium with 50% FBS, and after 2 hours this medium was replaced as described [15]. The cells were harvested over 28 hours at the indicated time points after serum shock.

2.2. Quantitative Real-Time Polymerase Chain Reaction. Total RNA was extracted from BxPC-3, CFPAC-1, PANC-1, and MIA PaCa-2 cells at the indicated time points after serum shock using the RNeasy Mini Kit (Qiagen S.P.A. Milano Italy) and subsequently digested by DNase I. cDNA was synthesized from 50 ng total RNA, and quantitative Real-Time PCR (qRT-PCR) was performed using QuantiFast Sybr Green PCR kit following the one-step protocol. For real-time RT-PCR, we used the following SYBR Green QuantiTect Primer purchased from Qiagen: *PPARG* (QT00029841), human *DNMT1* (QT00034335) and *DNMT3B* (QT00032067). Reactions were set up in 96-well plates using a 7700 Real-Time PCR System (Applied Biosystems, Foster City, CA), and all samples were assayed in triplicate. Optical data obtained were analyzed using the default and variable parameters available in the SDS software package (version 1.9.1; Applied Biosystems, Foster City, CA). Expression levels of target gene were normalized using the housekeeping control gene TATA-binding protein (TBP, QT00000721).

2.3. Statistical Analysis. Gene expression values were normalized, for each variable for each cell line, to the expression value of the first time point (T0) of sample collection after serum shock to reduce interassay level variability. Analysis of periodicity patterns was performed, for each time series of the normalized gene expression values, by fitting a least-squares linear regression of a single component (24 h) cosine waveform [16], using the MATLAB statistical package (MathWorks, Natick, Massachusetts, USA). The following parameters were estimated: "mesor" (the overall mean level of the wave); the "amplitude" (A, the range from the maximum and the minimum peaks of the best-fitted curve), and the "acrophase" ($a\emptyset$, the time in angular degrees, from the local midnight $\emptyset$, of the wave peak: acro = peak). P-values from F-statistics were reported for each fitted single cosinor model, to test the null hypothesis of zero amplitude (where the wave has no periodicity). Furthermore, a novel statistical approach was employed to compare the evolution of different time qualified profiles of gene expression in the cell lines, by means of suitable statistical contrasts from a multivariate periodic linear mixed model. In particular, for each comparison, two statistical contrasts were assessed, testing whether the rhythms have an identical or opposing waveform, respectively [17]. The periodic linear mixed model can be thought as the join assessment of many different cosinor models (each one including a specific number of harmonic terms). With respect to cosinor analysis, this novel statistical approach enables the comparison of the evolution of multiple biological rhythms by jointly representing all of them in terms of sine and cosine series into a multivariate linear mixed model, taking into account all their interdependencies (intra- and interoutcome correlation structures), as well as the collection of unequally spaced measures over time and heterogeneity between gene expressions. Moreover, any specific pairwise comparison between the biological rhythms

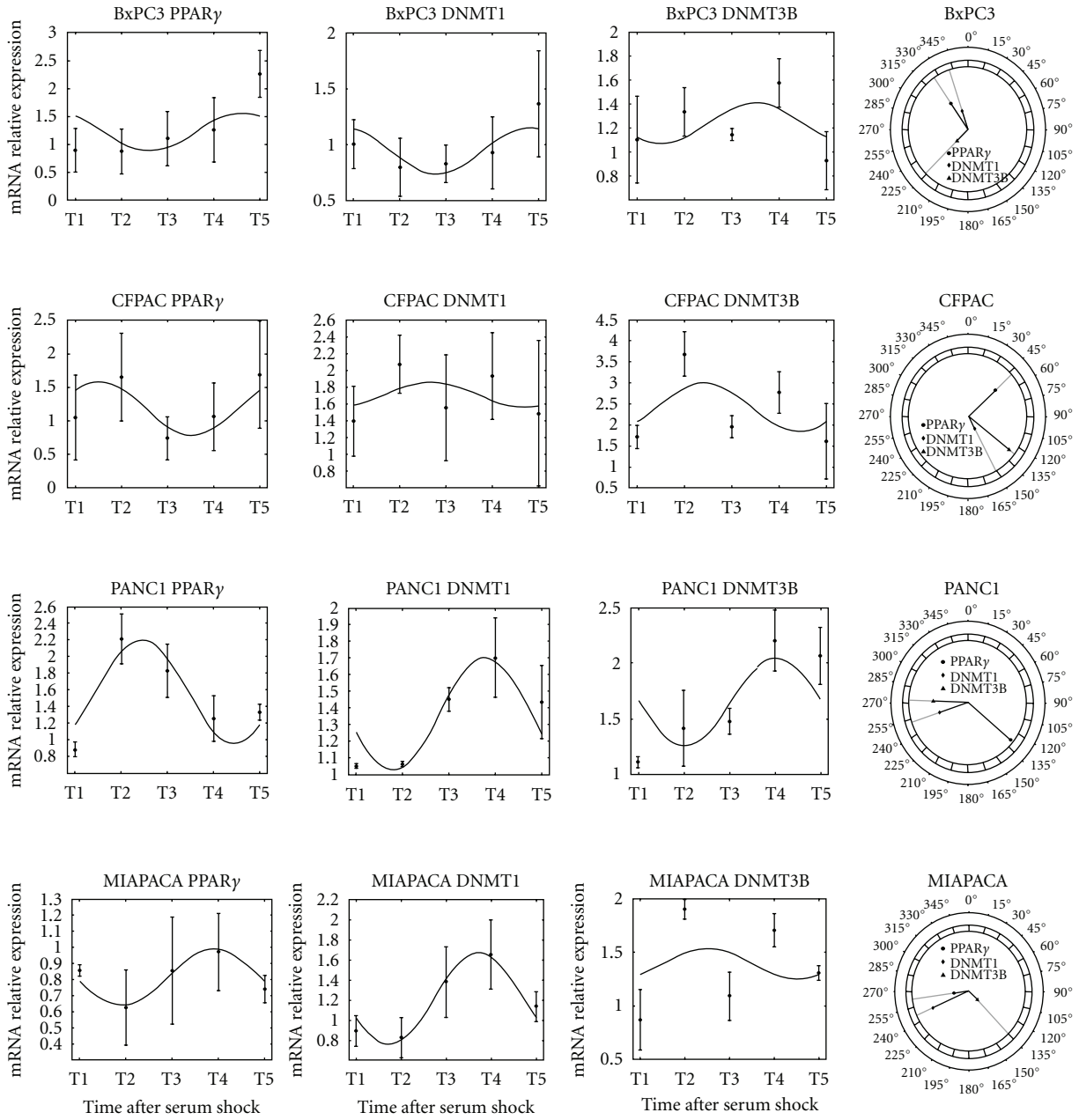

Figure 1: Chronograms displaying time qualified variations of *PPARG*, *DNMT1*, and *DNMT3B* expression level in pancreatic cancer cell lines. Original units standardized to T0 and combined for analyses. Polarograms of cosinor analysis showing the acrophases for the expression values of *PPARG*, *DNMT1*, and *DNMT3B*. Radial axis represents the time point (in degrees) after serum shock corresponding to the acme and vector length represents the amplitude of the oscillation.

can be performed by means of proper statistical contrasts. P-values <0.05 were considered for statistical significance. Statistical analyses were performed using MATLAB and SAS Release 9.1.3 (SAS Institute, Cary, NC).

3. Results

Results from cosinor analysis were reported in Table 1 and evidence a clear 24 h periodicity for the time-qualified variations of expression of *PPARG* ($P = 0.016$) and *DNMT1* ($P = 0.024$) in PANC-1 cells and *PPARG* ($P = 0.010$) in MIA PaCa-2 cells, whereas a borderline significant rhythm was observed for the other *PPARG*, *DNMT1*, and *DNMT3B* expression profiles in the examined cell lines (Figure 1).

Results from multivariate periodic regression analysis were reported in Table 2. Pairwise comparisons suggested that in BxPC-3 cells the time profiles of both *PPARG* and *DNMT1* showed flat shapes, whereas the time profiles of *PPARG* and *DNMT3B*, as well as those of *DNMT1* and *DNMT3B*, were opposing. In CFPAC-1 cells the time profiles

Table 1: Rhythm parameters from fitted single cosinor models for mRNA expression calculated on original values normalized to the first time point of sample collection after serum shock.

	BxPC3		
	PPARG	*DNMT1*	*DNMT3B*
Mesor	1.22	0.94	1.24
Amplitude	0.33	0.20	0.16
Acrophase	325.32	342.87	225.6
P-value	0.780	0.430	0.722
	CFPAC		
	PPARG	*DNMT1*	*DNMT3B*
Mesor	1.18	1.71	2.42
Amplitude	0.40	0.14	0.57
Acrophase	47.58	151.69	127.57
P-value	0.446	0.839	0.753
	PANC1		
	PPARG	*DNMT1*	*DNMT3B*
Mesor	1.58	1.36	1.65
Amplitude	0.62	0.33	0.39
Acrophase	129.72	251.25	272.44
P-value	0.016	0.024	0.630
	MIAPACA		
	PPARG	*DNMT1*	*DNMT3B*
Mesor	0.81	1.21	1.39
Amplitude	0.17	0.45	0.14
Acrophase	261.86	245.4	136.43
P-value	0.010	0.067	0.933

Overall gene expression levels were analyzed for time effect across the timepoints by single cosinor: fit of 24 h cosine to all data by least squares linear regression. Acrophase, the crest time of rhythm, is expressed in degrees. *P*-values refer to test for time effect (zero amplitude).

of all the expressions of *PPARG*, *DNMT1*, and *DNMT3B* were different (neither identical nor opposing). In PANC-1 cells the time profiles of *PPARG* and *DNMT1* were different, the time profiles of *PPARG* and *DNMT3B* were opposing, and the time profiles of *DNMT1* and *DNMT3B* had flat shapes. In MIA PaCa-2 cells the time profiles of all the expressions of *PPARG*, *DNMT1*, and *DNMT3B* were different (neither identical nor opposing) (Figure 2).

4. Discussion

Nycthemeral variations with a 24 h periodicity (circadian, from the Latin *circa* and *dies*) characterize behavior and physiology in the greater part of living organisms and contribute to homeostasis maintenance ensuring optimal timing of cellular phenomena in body systems, orchestrated by a complex network of transcriptional circuits [18–20]. Circadian rhythmicity is driven at the body level by a central pacemaker and master oscillator located in the hypothalamic suprachiasmatic nuclei (SCN) entrained by the light/dark cycle *via* the retinohypothalamic tract [21]. At the tissue-specific and single-cell levels the circadian rhythmicity is driven by molecular clocks ticked by transcription/translation feedback loops operated by a set of genes (so-called clock genes: *BMAL1, CLOCK, PER 1–3, CRY 1-2*) and their coded proteins, entrained by the SCN *via* humoral and neural outputs, and in a tissue-specific manner by other factors, such as feeding and temperature fluctuations [22–28]. The biological clocks control cell processes and tissue/organ functions driving the expression of genes coding for transcriptional factors, such as DBP (albumin D-site binding protein) and E4BP4 (adenoviral E4 protein-binding protein), which steer the expression of so-called clock controlled genes and tissue-specific output genes. The transcription factors DBP and E4BP4 among other processes control the circadian rhythmicity of PPARγ by binding to *PPARG* first exon D-sites with functional promoter activity [9].

Disruption of the circadian clock circuitry and alteration of the physiological circadian rhythmicity are considered to be involved in the processes underlying tumorigenesis [29–36].

Considering the important role played in the transcriptional processes by epigenetic mechanisms such as reversible or irreversible attachment of methyl groups to DNA catalyzed by DNMTs [37] and the recently evidenced interaction between PPARγ and DNMTs [14], we sought to evaluate if PPARγ and DNMTs show correspondent oscillation in pancreatic cancer, analyzing their time-related patterns of variation in synchronized pancreatic cancer cell lines.

Our data put in evidence important differences in the periodicity and in the phase relationships of *PPARG*, *DNMT1*, and *DNMT3B* expression levels among the diverse

Table 2: (a) Statistical contrasts from multivariate periodic regression analysis, along with a summarization of the decision to be drawn for each comparison, (b) interpretation rules: conclusions to be drawn from the joint hypotheses testing "identical biorhythms" (H_{01}) and "opposing biorhythms" (H_{02}).

(a)

BxPC3 statistical contrasts		
Hypotheses	*F*-value	*P*-value
H_{01}: Biorhythms of *PPARG* and *DNMT1* are identical	0.57	0.695
H_{02}: Biorhythms of *PPARG* and *DNMT1* are opposing	2.11	0.189
H_{01}: Biorhythms of *PPARG* and *DNMT3B* are identical	4.16	0.039
H_{02}: Biorhythms of *PPARG* and *DNMT3B* are opposing	0.46	0.764
H_{01}: Biorhythms of *DNMT1* and *DNMT3B* are identical	11.67	0.001
H_{02}: Biorhythms of *DNMT1* and *DNMT3B* are opposing	1.61	0.246
Decisions derived from statistical contrasts		
(1) Biorhythms of *PPARG* and *DNMT1* have flat shapes, although statistical tests slightly suggest that they could be identical (i.e., no sufficient statistical power). (2) Biorhythms of *PPARG* and *DNMT3B* are opposing. (3) Biorhythms of *DNMT1* and *DNMT3B* are opposing		
CFPAC statistical contrasts		
Hypotheses	*F*-value	*P*-value
H_{01}: Biorhythms of *PPARG* and *DNMT1* are identical	7.82	0.003
H_{02}: Biorhythms of *PPARG* and *DNMT1* are opposing	6.70	0.021
H_{01}: Biorhythms of *PPARG* and *DNMT3B* are identical	43.78	<0.001
H_{02}: Biorhythms of *PPARG* and *DNMT3B* are opposing	22.68	<0.001
H_{01}: Biorhythms of *DNMT1* and *DNMT3B* are identical	28.98	<0.001
H_{02}: Biorhythms of *DNMT1* and *DNMT3B* are opposing	38.02	<0.001
Decisions derived from statistical contrasts		
(1) Biorhythms of *PPARG* and *DNMT1* are different (neither identical nor opposing). (2) Biorhythms of *PPARG* and *DNMT3B* are different (neither identical nor opposing). (3) Biorhythms of *DNMT1* and *DNMT3B* are different (neither identical nor opposing)		
PANC1 statistical contrasts		
Hypotheses	*F*-value	*P*-value
H_{01}: Biorhythms of *PPARG* and *DNMT1* are identical	52.22	<0.001
H_{02}: Biorhythms of *PPARG* and *DNMT1* are opposing	5.78	0.010
H_{01}: Biorhythms of *PPARG* and *DNMT3B* are identical	16.14	0.002
H_{02}: Biorhythms of *PPARG* and *DNMT3B* are opposing	3.16	0.070
H_{01}: Biorhythms of *DNMT1* and *DNMT3B* are identical	1.96	0.196
H_{02}: Biorhythms of *DNMT1* and *DNMT3B* are opposing	3.57	0.063
Decisions derived from statistical contrasts		
(1) Biorhythms of *PPARG* and *DNMT1* are different (neither identical nor opposing). (2) Biorhythms of *PPARG* and *DNMT3B* are opposing, although statistical tests slightly suggest that they could be different at all. (3) Biorhythms of *DNMT1* and *DNMT3B* have flat shapes, although statistical tests slightly suggest that they could be identical		
MIAPACA statistical contrasts		
Hypotheses	*F*-value	*P*-value
H_{01}: Biorhythms of *PPARG* and *DNMT1* are identical	11.63	<0.001
H_{02}: Biorhythms of *PPARG* and *DNMT1* are opposing	7.23	0.018
H_{01}: Biorhythms of *PPARG* and *DNMT3B* are identical	9.89	0.001
H_{02}: Biorhythms of *PPARG* and *DNMT3B* are opposing	8.52	0.003
H_{01}: Biorhythms of *DNMT1* and *DNMT3B* are identical	17.71	<0.001
H_{02}: Biorhythms of *DNMT1* and *DNMT3B* are opposing	9.06	0.002

(a) Continued.

Decisions derived from statistical contrasts		
Hypotheses	*F*-value	*P*-value
(1) Biorhythms of *PPARG* and *DNMT1* are different (neither identical nor opposing). (2) Biorhythms of *PPARG* and *DNMT3B* are different (neither identical nor opposing). (3) Biorhythms of *DNMT1* and *DNMT3B* are different (neither identical nor opposing)		

(b)

		H_{01} "*identical biorhythms*"	
		Rejected	Not-rejected
H_{02} "*opposing biorhythms*"	Rejected	The biorhythms are different (neither identical nor opposing)	The biorhythms are identical
	Not-rejected	The biorhythms are opposing	The biorhythms have flat shape

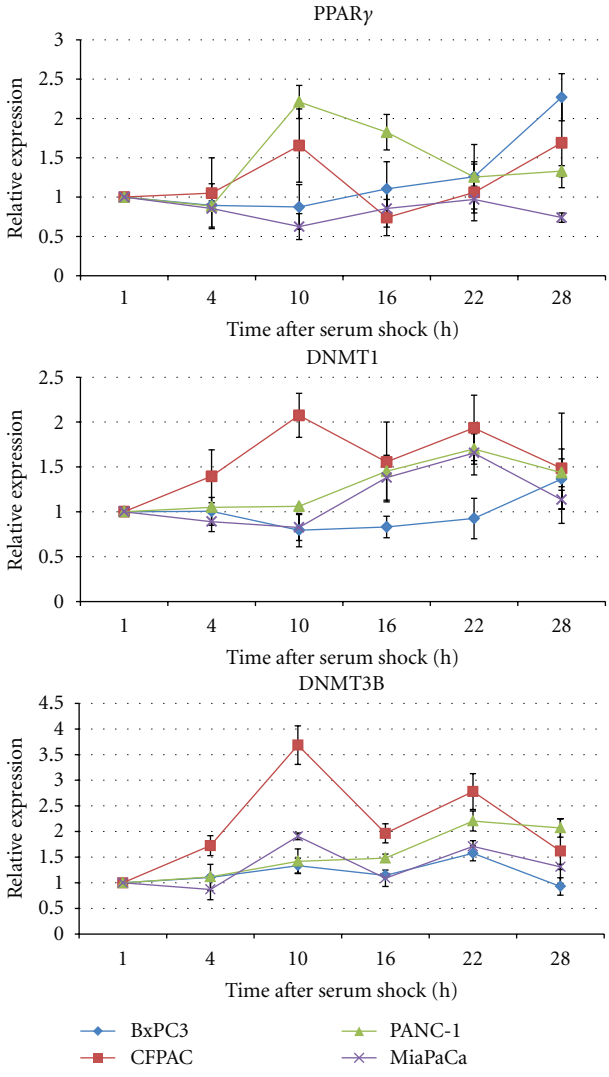

Figure 2: *x*-*y* plots showing the time-related profiles of expression level changes of *PPARG*, *DNMT1*, and *DNMT3B* in pancreatic cancer cell lines. Original units standardized to T0 and combined for analyses. Gene expression data assigned to actual collection time after serum shock.

cell lines examined, maybe related to a different genetic background in the diverse pancreatic cancer cells [38].

In the BxPC-3 cell line, mucin-producing cells derived from a human primary pancreatic adenocarcinoma, a borderline significant 24 h periodicity was evidenced for the *PPARG*, *DNMT1*, and *DNMT3B* expression patterns, and the time-qualified profiles of *PPARG* and *DNMT3B*, as well as the time qualified profiles of *DNMT1* and *DNMT3B*, were opposing.

In CFPAC-1 cells, derived from a pancreatic ductal adenocarcinoma liver metastasis of a patient with cystic fibrosis, a borderline significant rhythmicity with a 24 h period was found for the *PPARG*, *DNMT1*, and *DNMT3B* expression patterns, and the time-qualified profiles showed different shapes.

In PANC-1 cells, an epithelial-like cell line derived from a human pancreatic carcinoma, a clear 24 h periodicity was observed for the time qualified variations of *PPARG* and *DNMT1* expression, a borderline significant rhythmicity with a 24 h period was observed for the *DNMT3B* expression pattern, and the time qualified profiles of *PPARG* and *DNMT3B* were opposing, whereas those of *PPARG* and *DNMT1* were different, and the time qualified profiles of *DNMT1* and *DNMT3B* showed flat shapes.

In the MIA PaCa-2 cell line, established from a human pancreatic adenocarcinoma, a clear 24-h periodicity was observed for the time qualified variations of expression of *PPARG*, and a borderline significant rhythmicity with a 24 h period was observed for the *DNMT1* and *DNMT3B* expression patterns, and the time qualified profiles of *PPARG* and *DNMT1* as well as those of *PPARG* and *DNMT3B* and the time qualified profiles of *DNMT1* and *DNMT3B* were different (neither identical nor opposing).

The different time qualified profiles and phase relationships evidenced in the pancreatic cancer cell lines examined suggest that they rely on a dissimilar temporal architecture of transcriptional circuits and epigenetic mechanisms, which may influence cancer cell behavioral phenotype and possibly response to therapy.

Normal pancreatic duct epithelial cells do not seem to express PPARγ, whereas human pancreatic cancer cell lines

express the nuclear receptor, and drugs of the thiazolidinedione class transactivate the transcription of a peroxisome proliferator response element-driven promoter in a dose-dependent fashion [39]. Besides, immunohistochemical staining of resected specimens by means of a polyclonal PPARγ antibody has evidenced PPARγ protein expression in the nuclei of carcinoma cells in 90% of human pancreatic adenocarcinomas [40]. Selective PPARγ ligands inhibit pancreatic cancer cell growth in a dose-dependent manner and reduce the invasiveness of the tumor cells, suggesting a potential role for these agents in the adjuvant treatment of pancreatic cancer [41]. Furthermore, the first-line drug for the treatment of unresectable pancreatic cancer is represented by the nucleoside analog gemcitabine, and PPARγ ligands potentiate its cytotoxic action on human pancreatic cancer cells in a dosage-dependent manner and are tested to improve the prognosis of pancreatic cancer patients [42].

Inactivation of tumor suppressor genes is central to the development of all common forms of human cancer, and this inactivation often results from epigenetic silencing rather than intragenic mutations. A prevalent mechanism of tumor-suppressor gene inactivation in neoplastic disease is represented by transcriptional silencing by CpG island methylation, and the prototypic DNA methyltransferase, DNMT1, accounts for most methylation in mouse cells, but human cancer cells lacking DNMT1 retain significant genomic methylation and associated gene silencing [11]. In human cells, the mechanisms underlying locus-specific or global methylation patterns remain unclear, but genetic disruption of both DNMT1 and DNMT3b nearly eliminates methyltransferase activity and reduces genomic DNA methylation by greater than 95%. The importance of the DNA methyltransferase DNMT1 for the maintenance of cell methylation and its role in tumorigenesis have been highlighted by genetic experiments. DNMT1 is necessary and sufficient to maintain global methylation and aberrant CpG island methylation in human cancer cells, and selective depletion of DNMT1 with antisense inhibitors has been shown to induce demethylation and reactivation of silenced tumor-suppressor genes such as *CDKN2A*. Inactivation of both *DNMT1* and *DNMT3B* induces low levels of DNA methylation, whereas selective deletion of *DNMT1* alleles in cancer cells produces clones that retain CpG island methylation and associated tumor-suppressor gene silencing, suggesting that the two DNMTs cooperatively maintain DNA methylation and gene silencing in human cancer cells, providing convincing support that such methylation is indispensable for best possible neoplastic proliferation [11, 35].

In conclusion, the cell lines derived from human pancreatic cancers are characterized by different arrays of time qualified profiles of gene expression and epigenetic modifications, which could be related to particular genetic backgrounds and could impinge on cancer cell phenotype, suggesting variable temporal organization of cell processes that could conditionate disease behaviour and response to timed delivery of conventional chemotherapy.

Conflict of Interests

The authors declare that there is no conflict of interests with respect to the authorship and/or publication of this paper.

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References

[1] A. Jemal, R. Siegel, E. Ward, Y. Hao, J. Xu, and M. J. Thun, "Cancer statistics, 2009," *CA Cancer Journal for Clinicians*, vol. 59, no. 4, pp. 225–249, 2009.

[2] P. Charoensuksai and W. Xu, "PPARs in rhythmic metabolic regulation and implications in health and disease," *PPAR Research*, vol. 2010, Article ID 243643, 9 pages, 2010.

[3] P. Lefebvre, G. Chinetti, J. C. Fruchart, and B. Staels, "Sorting out the roles of PPARα in energy metabolism and vascular homeostasis," *Journal of Clinical Investigation*, vol. 116, no. 3, pp. 571–580, 2006.

[4] M. H. Hsu, U. Savas, K. J. Griffin, and E. F. Johnson, "Identification of peroxisome proliferator-responsive human genes by elevated expression of the peroxisome proliferator-activated receptor α in HepG2 cells," *Journal of Biological Chemistry*, vol. 276, no. 30, pp. 27950–27958, 2001.

[5] J. K. Reddy, "Nonalcoholic steatosis and steatohepatitis III. Peroxisomal β-oxidation, PPARα, and steatohepatitis," *American Journal of Physiology*, vol. 281, no. 6, pp. G1333–G1339, 2001.

[6] E. Ip, G. C. Farrell, G. Robertson, P. Hall, R. Kirsch, and I. Leclercq, "Central role of PPARα-dependent hepatic lipid turnover in dietary steatohepatitis in mice," *Hepatology*, vol. 38, no. 1, pp. 123–132, 2003.

[7] S. Yu, K. Matsusue, P. Kashireddy et al., "Adipocyte-specific gene expression and adipogenic steatosis in the mouse liver due to peroxisome proliferator-activated receptor γ1 (PPARγ1) overexpression," *Journal of Biological Chemistry*, vol. 278, no. 1, pp. 498–505, 2003.

[8] P. Ferré, "The biology of peroxisome proliferator-activated receptors: relationship with lipid metabolism and insulin sensitivity," *Diabetes*, vol. 53, no. 1, supplement, pp. S43–S50, 2004.

[9] S. Takahashi, I. Inoue, Y. Nakajima et al., "A promoter in the novel exon of hPPARγ directs the circadian expression of PPARγ," *Journal of Atherosclerosis and Thrombosis*, vol. 17, no. 1, pp. 73–83, 2010.

[10] B. Farrow, K. L. O'Connor, K. Hashimoto, T. Iwamura, and B. M. Evers, "Selective activation of PPARγ inhibits pancreatic cancer invasion and decreases expression of tissue plasminogen activator," *Surgery*, vol. 134, no. 2, pp. 206–212, 2003.

[11] I. Rhee, K. E. Bachman, B. H. Park et al., "DNMT1 and DNMT3b cooperate to silence genes in human cancer cells," *Nature*, vol. 416, no. 6880, pp. 552–556, 2002.

[12] M. Ripoli, R. Barbano, T. Balsamo et al., "Hypermethylated levels of E-cadherin promoter in Huh-7 cells expressing the HCV core protein," *Virus Research*, vol. 160, no. 1-2, pp. 74–81, 2011.

[13] G. Benegiamo, M. Vinciguerra, G. Mazzoccoli, A. Piepoli, A. Andriulli, and V. Pazienza, "DNA methyltransferases 1 and 3b expression in Huh-7 cells expressing HCV core protein of different genotypes," *Digestive Disease Science*, vol. 57, no. 6, pp. 1598–1603, 2012.

[14] J. Lei, H. Hasegawa, T. Matsumoto, and M. Yasukawa, "Peroxisome proliferator-activated receptor α and γ agonists together with TGF-β convert human CD4+CD25- T cells into functional Foxp3+ regulatory T cells," *Journal of Immunology*, vol. 185, no. 12, pp. 7186–7198, 2010.

[15] A. Balsalobre, F. Damiola, and U. Schibler, "A serum shock induces circadian gene expression in mammalian tissue culture cells," *Cell*, vol. 93, no. 6, pp. 929–937, 1998.

[16] W. Nelson, Y. L. Tong, J. K. Lee, and F. Halberg, "Methods for cosinor-rhythmometry," *Chronobiologia*, vol. 6, no. 4, pp. 305–323, 1979.

[17] G. Mazzoccoli, A. Fontana, M. Copetti et al., "Stage dependent destructuration of neuro-endocrine-immune system components in lung cancer patients," *Biomedicine and Pharmacotherapy*, vol. 65, no. 1, pp. 69–76, 2011.

[18] I. Edery, "Circadian rhythms in a nutshell," *Physiol Genomics*, vol. 3, no. 2, pp. 59–74, 2000.

[19] T. Matsuo, S. Yamaguchi, S. Mitsui, A. Emi, F. Shimoda, and H. Okamura, "Control mechanism of the circadian clock for timing of cell division in vivo," *Science*, vol. 302, no. 5643, pp. 255–259, 2003.

[20] G. Mazzoccoli, V. Pazienza, and M. Vinciguerra, "Clock genes and clock controlled genes in the regulation of metabolism rhythms," *Chronobiology International*, vol. 29, no. 3, pp. 227–251, 2012.

[21] M. H. Hastings, A. B. Reddy, and E. S. Maywood, "A clockwork web: circadian timing in brain and periphery, in health and disease," *Nature Reviews Neuroscience*, vol. 4, no. 8, pp. 649–661, 2003.

[22] F. Damiola, N. Le Minli, N. Preitner, B. Kornmann, F. Fleury-Olela, and U. Schibler, "Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus," *Genes and Development*, vol. 14, no. 23, pp. 2950–2961, 2000.

[23] R. Hara, K. Wan, H. Wakamatsu et al., "Restricted feeding entrains liver clock without participation of the suprachiasmatic nucleus," *Genes to Cells*, vol. 6, no. 3, pp. 269–278, 2001.

[24] K. A. Stokkan, S. Yamazaki, H. Tei, Y. Sakaki, and M. Menaker, "Entrainment of the circadian clock in the liver by feeding," *Science*, vol. 291, no. 5503, pp. 490–493, 2001.

[25] U. Schibler and P. Sassone-Corsi, "A web of circadian pacemakers," *Cell*, vol. 111, no. 7, pp. 919–922, 2002.

[26] G. Mazzoccoli, "The timing clockwork of life," *Journal of Biological Regulators and Homeostatic Agents*, vol. 25, no. 1, pp. 137–143, 2011.

[27] E. Filipski, V. M. King, M. C. Etienne et al., "Persistent twenty-four hour changes in liver and bone marrow despite suprachiasmatic nuclei ablation in mice," *American Journal of Physiology*, vol. 287, no. 4, pp. R844–R851, 2004.

[28] E. Nagoshi, C. Saini, C. Bauer, T. Laroche, F. Naef, and U. Schibler, "Circadian gene expression in individual fibroblasts: cell-autonomous and self-sustained oscillators pass time to daughter cells," *Cell*, vol. 119, no. 5, pp. 693–705, 2004.

[29] E. Filipski, V. M. King, X. Li et al., "Host circadian clock as a control point in tumor progression," *Journal of the National Cancer Institute*, vol. 94, no. 9, pp. 690–697, 2002.

[30] L. Fu and C. C. Lee, "The circadian clock: pacemaker and tumour suppressor," *Nature Reviews Cancer*, vol. 3, no. 5, pp. 350–361, 2003.

[31] J. J. Wille Jr., "Circadian rhythm of tumor promotion in the two-stage model of mouse tumorigenesis," *Cancer Letters*, vol. 190, no. 2, pp. 143–149, 2003.

[32] G. Mazzoccoli, A. Panza, M. R. Valvano et al., "Clock gene expression levels and relationship with clinical and pathological features in colorectal cancer patients," *Chronobiology International*, vol. 28, no. 10, pp. 841–851, 2011.

[33] G. Mazzoccoli, V. Pazienza, A. Panza et al., "ARNTL2 and SERPINE1: potential biomarkers for tumor aggressiveness in colorectal cancer," *Journal of Cancer Research and Clinical Oncology*, vol. 138, no. 3, pp. 501–511, 2012.

[34] G. Mazzoccoli, A. Piepoli, M. Carella et al., "Altered expression of the clock gene machinery in kidney cancer," *Biomedicine & Pharmacotherapy*, vol. 66, pp. 175–179, 2012.

[35] G. Mazzoccoli, R. B. Sothern, G. Greco et al., "Time-related dynamics of variation in core clock gene expression levels in tissues relevant to the immune system," *International Journal of Immunopathology and Pharmacology*, vol. 24, no. 4, pp. 869–879, 2011.

[36] V. Pazienza, A. Piepoli, and A. Panza, "SIRT1 and the clock gene machinery in colorectal cancer," *Cancer Investigation*, vol. 30, no. 2, pp. 98–105, 2012.

[37] M. F. Robert, S. Morin, N. Beaulieu et al., "DNMT1 is required to maintain CpG methylation and aberrant gene silencing in human cancer cells," *Nature Genetics*, vol. 33, no. 1, pp. 61–65, 2003.

[38] E. L. Deer, J. González-Hernández, J. D. Coursen et al., "Phenotype and genotype of pancreatic cancer cell lines," *Pancreas*, vol. 39, no. 4, pp. 425–435, 2010.

[39] W. Motomura, T. Okumura, N. Takahashi, T. Obara, and Y. Kohgo, "Activation of peroxisome proliferator-activated receptor γ by troglitazone inhibits cell growth through the increase of p27(Kip1) in human pancreatic carcinoma cells," *Cancer Research*, vol. 60, no. 19, pp. 5558–5564, 2000.

[40] A. Elnemr, T. Ohta, K. Iwata et al., "PPARgamma ligand (thiazolidinedione) induces growth arrest and differentiation markers of human pancreatic cancer cells," *International Journal of Oncology*, vol. 17, no. 6, pp. 1157–1164, 2000.

[41] K. Hashimoto, R. T. Ethridge, and B. M. Evers, "Peroxisome proliferator-activated receptor γ ligand inhibits cell growth and invasion of human pancreatic cancer cells," *International Journal of Gastrointestinal Cancer*, vol. 32, no. 1, pp. 7–22, 2002.

[42] H. Koga, K. Selvendiran, R. Sivakumar et al., "PPARγ potentiates anticancer effects of gemcitabine on human pancreatic cancer cells," *International Journal of Oncology*, vol. 40, no. 3, pp. 679–685, 2012.

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Review Article

Update on Ppar*γ* and Nonalcoholic Fatty Liver Disease

Gene P. Ables

Animal Science Laboratory, Orentreich Foundation for the Advancement of Science, 855 Route 301, Cold Spring, NY 10516, USA

Correspondence should be addressed to Gene P. Ables, gables@orentreich.org

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Nonalcoholic fatty liver disease (NAFLD) is the most common initial presentation of obesity and insulin resistance. Uninterrupted progression of hepatic lipid accumulation often leads to fatty liver disease and eventually cirrhosis. Insulin resistance is one of the characteristics of type 2 diabetes. Several types of treatment have been employed against type 2 diabetes some of which ameliorate NAFLD. The frequent line of treatment to improve insulin sensitivity is the use of thiazolidinediones (TZD) which activate the nuclear receptor, peroxisome proliferator activated receptor gamma (*Pparγ*). Although TZDs are proven to be very effective in promoting insulin sensitivity, its actions on *Pparγ* have been complicated, specifically on NAFLD. According to studies in different models, *Pparγ* manifests both beneficial and undesirable effects on NAFLD. This paper will focus on the current knowledge of *Pparγ* and its effect on NAFLD.

1. Introduction

Hepatic steatosis without excessive alcohol intake, called nonalcoholic fatty liver disease (NAFLD), is commonly associated with obesity and insulin resistance [1, 2]. NAFLD affects the general population and its incidence is linked with the epidemics of obesity and type 2 diabetes [3]. The metabolic pathways leading to hepatic steatosis include enhanced nonesterified fatty acid release from the adipose tissue, increased *de novo* lipogenesis, decreased β-oxidation and reduced VLDL export [4, 5]. Steatosis in the liver is characterized by a large intracytoplasmic fat droplet or well-defined droplets displacing the nucleus to the cell periphery [6]. The accumulation of hepatic lipids could be due to elevated peripheral fatty acids, *de novo* lipogenesis and defective apolipoprotein biosynthesis [7, 8]. The progression of hepatic steatosis often leads to liver inflammation or steatohepatitis and, if unchecked, will worsen liver fibrosis and cirrhosis [9]. Several mouse models are used to elucidate the mechanisms of fatty liver disease [10, 11]. The bulk of studies on NAFLD have been through the administration of high fat diets or methionine-choline-deficient diets and the use of genetically leptin-deficient (*ob/ob*) or leptin-receptor-deficient (*db/db*) mouse models [12]. These models exhibit insulin resistance which is one sequelae of NAFLD [13]. Increased insulin secretion has been directly implicated in the development of fatty liver disease [14]. The therapy used for insulin resistance frequently include the administration of TZDs which are agonists for the nuclear receptor peroxisome proliferator activated receptor gamma (*Pparγ*) [15]. However, studies have demonstrated that TZDs have also exhibited deleterious side effects that warrant their withdrawal from the market [16]. Of interest, selective *Pparγ* modulators (SPPARMs) that do not have the undesirable effects of TZDs have been recently identified [17, 18]. Since NAFLD often leads to insulin resistance, this paper focuses on the relationship between *Pparγ* and NAFLD.

2. Ppar-gamma *(Pparγ)*

Pparγ is a member of the nuclear receptor superfamily of ligand-activated transcription factors [19] highly expressed in adipocytes [20, 21] and plays a role in improving glucose homeostasis and adipocyte differentiation [22]. It increases insulin sensitivity by upregulating the glucose transporter 4 (*Glut4*) [23]. *Pparγ* also enhances the transcription factors adipocyte determination and differentiation-dependent factor 1 (*Add1*) and sterol regulatory element binding protein 1 (*Srebp1*) which results in the expression of lipogenic genes such as fatty acid synthase (*Fas*) [24]. *Pparγ* is expressed as

2 major isoforms, *γ1* and *γ2*, generated from the same gene by alternate promoter usage and RNA splicing [25]. Both isoforms could stimulate adipogenesis when introduced to fibroblasts [26]. Adipocytes treated with the *Pparγ* ligand, TZD, stimulate expression of uncoupling protein 2 (*Ucp2*) and therefore increase energy expenditure [27]. Another mechanism of action by TZDs in the adipose tissue is to upregulate the expression of AMP-activated protein kinase (*Ampk*) which increases fatty acid oxidation while decreasing lipogenesis via downregulation of *Srebp-1c* and carbohydrate response element binding protein (*Chrebp*) [28]. The upregulation of hepatic *Pparγ* is frequently observed in mice fed a high fat diet [29]. In addition, liver specific deletion of *Pparγ* in mice established its role as a prosteatotic factor in the development of NAFLD [30]. Of importance is that *Pparγ* activation by TZDs promotes efflux of free fatty acids from the liver and muscle while increasing fat mass which consequently improves insulin sensitivity [31]. Therefore, whether the upregulation of *Pparγ* causes steatosis or vice versa remains unclear.

3. The Effect of *Pparγ* Variants in the Development of NAFLD

Variants in the *Pparγ* gene found in human genotyping studies have been reported to affect hepatic steatosis. A Japanese cohort was first reported to have a polymorphism in the peroxisome proliferator activator receptor gamma coactivator 1 alpha (*Pparγc1a*) gene [32].The polymorphism in the T allele of rs2290602 was found in patients with nonalcoholic steatosis which was further confirmed by quantitative real time PCR [32]. In addition, single nucleotide polymorphisms (SNPs) in the C161T genotype in the *Pparγ* gene found in a Chinese population was associated NAFLD possibly through the adiponectin pathway [33]. Moreover, the Pro12Ala variant in the *Pparγ* gene was found to be associated with pathogenesis of NAFLD in Indian, Chinese, and North American cohorts but not in German and Italian cohorts [34–38]. Taken together, polymorphisms in the *Pparγ* gene could be useful to identify individuals that are at high risk for NAFLD but should not be considered as the main factor for the disease.

4. The Role of *Pparγ* in the Development of Hepatic Steatosis

The mode of action of *Pparγ* in liver was suggested to promote insulin sensitivity but with concomitant development of fatty liver. High-fat diet fed mice develop hepatic steatosis and have increased *Pparγ* expression [39]. This could be due to the suppression cAMP response element binding protein (*Creb*) levels, the upstream regulator of *Pparγ*, in high fat diet fed mice [39]. In hepatic overexpression studies, Yu et al. showed that *Pparγ1* leads to adipogenic hepatic steatosis [40]. This group employed the *Pparα* deleted (*Pparα*-KO) mouse model and then injected the mice with adenovirus overexpressing *Pparγ1* [40]. They showed that hepatic overexpression of *Pparγ1* induced adipocyte specific gene expression patterns in the livers of *Pparα*-KO mice [40]. Therefore, they propose that excess *Pparγ* activity can lead to the development of adipogenic hepatic steatosis [40]. In addition, hepatic adenoviral overexpression of *Pparγ2* in lean mice increased liver triglyceride content and induced hypertension [41]. This occurrence was reported to involve the target of *Pparγ*, fat specific protein 27 (Fsp27) [42] and its actions on the afferent vagal signals in the liver [41]. In a liver specific *Pparγ* deletion study, Gavrilova et al. reported that the A/ZIP/F-1 mouse model, which develops severe lipoatrophic diabetes, exhibited attenuation of hepatic steatosis but compromised triglyceride clearance [43]. The same group also showed that the liver *Pparγ* is essential for the effects of a *Pparγ* agonist, rosiglitazone, to improve glucose metabolism [43]. Moran-Salvador et al. reported that hepatocyte specific deletion of *Pparγ* in mice protected high fat diet fed mice from accumulation of lipids and, therefore, further implicated its role in the development of hepatic steatosis [30]. They also showed that the *Pparγ* in Kupffer cells might not be involved in the development of hepatic steatosis [30]. In addition, a mouse model of dyslipidemia showed that hepatic *Pparγ2* upregulation induced hepatic *de novo* lipogenesis [44]. Zhang et al. fed a western-type diet to mice that express the human apolipoprotein B and lack the brown adipose tissue (apoB/BATless) [44]. These mice are obese, insulin resistant and have hepatic steatosis [44]. They showed that hepatic *Pparγ2* expression is increased due to elevated rates of lipogenesis via the upregulation of *de novo* lipogenic genes *Fas* and acetyl-CoA carboxylase (*Acc*) [44]. Taken together, these studies strongly implicate *Pparγ* in the development of hepatic steatosis.

5. The Role of *Pparγ* in the Reduction of Hepatic Steatosis

Diet induced hepatic fibrosis mouse models that were either treated with rosiglitazone or administered with adenovirus overexpressing *Pparγ* were shown to ameliorate hepatic steatosis [45, 46]. Mice fed a methionine-choline-deficient (MCD) diet developed severe hepatic steatosis, inflammation, and fibrosis with downregulation of *Pparγ* levels [45]. Meanwhile, mice that were fed the same diet supplemented with rosiglitazone were protected from the adverse effects of the MCD diet [45]. The protection from nutritional fibrosing steatohepatitis by *Pparγ* could be due to the inhibition of hepatic stellate cell activation which is one of the main causes for fibrosis [45]. Similarly, the hepatic adenoviral overexpression of *Pparγ* in MCD diet-fed mice elicited protection from fibrotic steatohepatitis [46]. This could be explained by the genetic upregulation of adiponectin (*adipoQ*) and hemeoxygenase 1 (*Hmox1*) and the downregulation of inflammatory markers such as tumor necrosis factor alpha (*Tnfα*) and interleukin 6 (*Il-6*) [46]. In a model of hepatic steatosis involving alcohol, mice that were fed ethanol showed amelioration of hepatic steatosis following administration of rosiglitazone due to stimulation of fatty acid oxidation in the liver [47]. In addition, the Long Evans rats, which exhibit moderate obesity and

insulin resistance, were given rosiglitazone and subsequently ameliorated hepatic steatosis which could be modulated by Sirtuin 6 (*Sirt6*) and its target genes *Pparγc1a*, forkhead box protein O1 (*Foxo1*), liver kinase B1 (*Lkb1*) and 5′ adenosine monophosphate-activated protein kinase (*Ampk*) [48]. *In vitro* experiments, confirmed the function of *Sirt6* by using the free fatty acid stimulated mouse hepatocyte cell line, AML 12, which also showed the protection from hepatic steatosis following rosiglitazone treatment [48]. Similarly, Sprague-Dawley rats that were given high sucrose and high fat diet showed amelioration of hepatic steatosis following treatment with rosiglitazone [49]. The decrease in liver triglycerides in these rats could be due to the effect the *Pparγ* agonist in increasing serum adiponectin and the upregulation of fatty acid oxidation genes, carnitine palmitoyl transferase 1 (*Cpt1*) and acyl coenzyme A oxidase (*Aco*) [49]. Furthermore, dietary methionine restriction (MR) in F344 rats upregulated hepatic *Pparγ* expression, improved insulin sensitivity, and increased fatty acid oxidation [50–52]. Overall, these sets of data suggest that *Pparγ* ameliorated hepatic steatosis due to increased fatty acid oxidation.

6. The Development of Selective *Pparγ* Modulators (SPPARMS)

Although *Pparγ* agonists have direct actions to improve insulin sensitivity, this line of treatment also has undesirable side effects. For example, rosiglitazone was reported to reduce bone mass in mice [53]. In addition, mice that are obese and diabetic develop hepatic steatosis following treatment with TZDs [54]. More recently, a meta-analysis of type 2 diabetes patients showed that pioglitazone is associated with increased risk for urinary bladder cancer [55]. The development of SPPARMS could potentially reduce these negative effects. Modifications in the *Pparγ* ligands showed direct effects on insulin sensitivity but not on adipogenesis [56, 57]. The ligand FMOC-L-Leucine, a chemically distinct ligand for *Pparγ*, was reported to improve insulin sensitivity but did not affect hepatic lipid metabolism in *db/db* mice [58]. Telmisartan, an angiotensin receptor blocker that acts as a *Pparγ* ligand, enhanced insulin sensitivity and decreased body fat in high fat diet fed mice [59]. A synthetic *Pparγ* ligand, nTZDpa, ameliorated fasting hyperglycemia and hyperinsulinemia and caused decrease in weight gain and adipose tissue size in high fat diet fed mice [60]. In addition, results using a gene expression-based screening identified N-acetylfarnesylcysteine (AFC) as a full and partial agonist of *Pparγ* [61]. The compound upregulated *Pparγ* agonist target genes adipose differentiation-related protein (*Adrp*), angiopoietin-related protein 4 (*Angptl4*) and *adipoq*, but was only a partial agonist of adipocyte fatty acid binding protein 2 (*ap2*) [61]. The AFC also improved glucose homeostasis and reduced adipose tissue inflammation and expansion in diet-induced obese mice [61]. Furthermore, a synthetic *Pparγ* antagonist, SR-202, decreased expression of *Pparγ* target genes and promoted insulin sensitivity in diet-induced obese mice as well as in *ob/ob* mice [62]. Moreover, a partial *Pparγ* agonist, INT131, was designed to mitigate insulin sensitivity while minimizing the side effects of thiazolidinediones [63]. It was reported that INT131 reduced fasting plasma glucose in humans and also increased insulin sensitivity in *db/db* and diet induced obesity in mice [64, 65]. Taken together, recent data on the use of SPPARMS maintain the effects of *Pparγ* as an insulin sensitizing agent but with decreased risks for undesirable effects.

7. Conclusion

With all these data surrounding the effect of *Pparγ* on the development of NAFLD and improvements in insulin sensitivity in several models, it is still not conclusive as to whether the nuclear receptor is beneficial or detrimental. Therefore, further investigations are necessary to elucidate the effect of specific conformational and structural differences between the nuclear receptor and its ligands. The advent of the development of SPPARMS points to the direction of specifically eliciting the desirable effects of *Pparγ* activation.

Conflict of Interests

The author reports no conflict of interests.

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References

[1] J. D. Browning, L. S. Szczepaniak, R. Dobbins et al., "Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity," *Hepatology*, vol. 40, no. 6, pp. 1387–1395, 2004.

[2] H. Yki-Jarvinen, "Fat in the liver and insulin resistance," *Annals of Medicine*, vol. 37, no. 5, pp. 347–356, 2005.

[3] C. D. Williams, J. Stengel, M. I. Asike et al., "Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study," *Gastroenterology*, vol. 140, no. 1, pp. 124–131, 2011.

[4] C. Postic and J. Girard, "The role of the lipogenic pathway in the development of hepatic steatosis," *Diabetes & Metabolism*, vol. 34, no. 6, pp. 643–648, 2008.

[5] C. Postic and J. Girard, "Contribution of de novo fatty acid synthesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice," *Journal of Clinical Investigation*, vol. 118, no. 3, pp. 829–838, 2008.

[6] E. M. Brunt and D. G. Tiniakos, "Histopathology of nonalcoholic fatty liver disease," *World Journal of Gastroenterology*, vol. 16, no. 42, pp. 5286–5296, 2010.

[7] K. L. Donnelly, C. I. Smith, S. J. Schwarzenberg, J. Jessurun, M. D. Boldt, and E. J. Parks, "Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease," *Journal of Clinical Investigation*, vol. 115, no. 5, pp. 1343–1351, 2005.

[8] M. Charlton, R. Sreekumar, D. Rasmussen, K. Lindor, and K. S. Nair, "Apolipoprotein synthesis in nonalcoholic steatohepatitis," *Hepatology*, vol. 35, no. 4, pp. 898–904, 2002.

[9] S. Milic and D. Stimac, "Nonalcoholic Fatty liver disease/steatohepatitis: epidemiology, pathogenesis, clinical presentation and treatment," *Digestive Diseases*, vol. 30, no. 2, pp. 158–162, 2012.

[10] A. Koteish and A. M. Diehl, "Animal models of steatosis," *Seminars in Liver Disease*, vol. 21, no. 1, pp. 89–104, 2001.

[11] P. Nagarajan, M. J. M. Kumar, R. Venkatesan, S. S. Majundar, and R. C. Juyal, "Genetically modified mouse models for the study of nonalcoholic fatty liver disease," *World Journal of Gastroenterology*, vol. 18, no. 11, pp. 1141–1153, 2012.

[12] Q. M. Anstee, "Animal models in nonalcoholic steatohepatitis research: utility and clinical translation," *Liver International*, vol. 31, no. 4, pp. 440–442, 2011.

[13] J. Jou, S. S. Choi, and A. M. Diehl, "Mechanisms of disease progression in nonalcoholic fatty liver disease," *Seminars in Liver Disease*, vol. 28, no. 4, pp. 370–379, 2008.

[14] S. Chitturi, S. Abeygunasekera, G. C. Farrell et al., "NASH and insulin resistance: insulin hypersecretion and specific association with the insulin resistance syndrome," *Hepatology*, vol. 35, no. 2, pp. 373–379, 2002.

[15] L. Guo and R. Tabrizchi, "Peroxisome proliferator-activated receptor gamma as a drug target in the pathogenesis of insulin resistance," *Pharmacology and Therapeutics*, vol. 111, no. 1, pp. 145–173, 2006.

[16] N. Saraf, P. K. Sharma, S. C. Mondal, V. K. Garg, and A. K. Singh, "Role of PPARg2 transcription factor in thiazolidinedione-induced insulin sensitization," *Journal of Pharmacy and Pharmacology*, vol. 64, no. 2, pp. 161–171, 2012.

[17] J. P. Whitehead, "Diabetes: new conductors for the peroxisome proliferator-activated receptor gamma (PPARγ) orchestra," *International Journal of Biochemistry and Cell Biology*, vol. 43, no. 8, pp. 1071–1074, 2011.

[18] J. H. Choi, A. S. Banks, J. L. Estall et al., "Anti-diabetic drugs inhibit obesity-linked phosphorylation of PPARγ 3 by Cdk5," *Nature*, vol. 466, no. 7305, pp. 451–456, 2010.

[19] P. Cornelius, O. A. MacDougald, and M. D. Lane, "Regulation of adipocyte development," *Annual Review of Nutrition*, vol. 14, pp. 99–129, 1994.

[20] A. Chawla, E. J. Schwarz, D. D. Dimaculangan, and M. A. Lazar, "Peroxisome proliferator-activated receptor (PPAR) γ: adipose-predominant expression and induction early in adipocyte differentiation," *Endocrinology*, vol. 135, no. 2, pp. 798–800, 1994.

[21] P. Tontonoz, E. Hu, R. A. Graves, A. I. Budavari, and B. M. Spiegelman, "mPPARγ2: tissue-specific regulator of an adipocyte enhancer," *Genes and Development*, vol. 8, no. 10, pp. 1224–1234, 1994.

[22] E. R. Kallwitz, A. McLachlan, and S. J. Cotler, "Role of peroxisome proliferators-activated receptors in the pathogenesis and treatment of nonalcoholic fatty liver disease," *World Journal of Gastroenterology*, vol. 14, no. 1, pp. 22–28, 2008.

[23] Z. Wu, Y. Xie, R. F. Morrison, N. L. R. Bucher, and S. R. Farmer, "PPARγ induces the insulin-dependent glucose transporter GLUT4 in the absence of C/EBPα during the conversion of 3T3 fibroblasts into adipocytes," *Journal of Clinical Investigation*, vol. 101, no. 1, pp. 22–32, 1998.

[24] J. B. Kim and B. M. Spiegelman, "ADD1/SREBP1 promotes adipocyte differentiation and gene expression linked to fatty acid metabolism," *Genes and Development*, vol. 10, no. 9, pp. 1096–1107, 1996.

[25] L. Fajas, D. Auboeuf, E. Raspé et al., "The organization, promoter analysis, and expression of the human PPARγ gene," *Journal of Biological Chemistry*, vol. 272, no. 30, pp. 18779–18789, 1997.

[26] P. Tontonoz, E. Hu, and B. M. Spiegelman, "Stimulation of adipogenesis in fibroblasts by PPARγ2, a lipid-activated transcription factor," *Cell*, vol. 79, no. 7, pp. 1147–1156, 1994.

[27] A. Camirand, V. Marie, R. Rabelo, and J. E. Silva, "Thiazolidinediones stimulate uncoupling protein-2 expression in cell lines representing white and brown adipose tissues and skeletal muscle," *Endocrinology*, vol. 139, no. 1, pp. 428–431, 1998.

[28] J. D. Browning and J. D. Horton, "Molecular mediators of hepatic steatosis and liver injury," *Journal of Clinical Investigation*, vol. 114, no. 2, pp. 147–152, 2004.

[29] A. Vidal-Puig, M. Jimenez-Liñan, B. B. Lowell et al., "Regulation of PPAR γ gene expression by nutrition and obesity in rodents," *Journal of Clinical Investigation*, vol. 97, no. 11, pp. 2553–2561, 1996.

[30] E. Moran-Salvador, M. Lopez-Parra, V. Garcia-Alonso et al., "Role for PPARγ in obesity-induced hepatic steatosis as determined by hepatocyte- and macrophage-specific conditional knockouts," *FASEB Journal*, vol. 25, no. 8, pp. 2538–2550, 2011.

[31] T. Yamauchi, J. Kamon, H. Waki et al., "The mechanisms by which both heterozygous peroxisome proliferator-activated receptor γ (PPARγ) deficiency and PPARγ agonist improve insulin resistance," *Journal of Biological Chemistry*, vol. 276, no. 44, pp. 41245–41254, 2001.

[32] M. Yoneda, K. Hotta, Y. Nozaki et al., "Association between PPARGC1A polymorphisms and the occurrence of nonalcoholic fatty liver disease (NAFLD)," *BMC Gastroenterology*, vol. 8, p. 27, 2008.

[33] Y. Hui, L. Yu-yuan, N. Yu-qiang et al., "Effect of peroxisome proliferator-activated receptors-γ and co-activator-1α genetic polymorphisms on plasma adiponectin levels and susceptibility of non-alcoholic fatty liver disease in Chinese people," *Liver International*, vol. 28, no. 3, pp. 385–392, 2008.

[34] P. Dongiovanni, R. Rametta, A. L. Fracanzani et al., "Lack of association between peroxisome proliferator-activated receptors alpha and gamma2 polymorphisms and progressive liver damage in patients with non-alcoholic fatty liver disease: a case control study," *BMC Gastroenterology*, vol. 10, p. 102, 2010.

[35] S. Gawrieh, M.C. Marion, R. Komorowski et al., "Genetic variation in the peroxisome proliferator activated receptor-gamma gene is associated with histologically advanced NAFLD," *Digestive Diseases and Sciences*, vol. 57, no. 4, pp. 952–957, 2011.

[36] A. C. Gupta, A. K. Chaudhory, Sukriti et al., "Peroxisome proliferators-activated receptor γ2 Pro12Ala variant is associated with body mass index in non-alcoholic fatty liver disease patients," *Hepatology International*, vol. 5, no. 1, pp. 575–580, 2011.

[37] J. W. Rey, A. Noetel, A. Hardt et al., "Pro12Ala polymorphism of the peroxisome proliferatoractivated receptor γ2 in patients with fatty liver diseases," *World Journal of Gastroenterology*, vol. 16, no. 46, pp. 5830–5837, 2010.

[38] Z. Yang, J. Wen, Q. Li et al., "PPARG gene Pro12Ala variant contributes to the development of non-alcoholic fatty liver in middle-aged and older Chinese population," *Molecular and Cellular Endocrinology*, vol. 348, no. 1, pp. 255–259, 2012.

[39] M. Inoue, T. Ohtake, W. Motomura et al., "Increased expression of PPARγ in high fat diet-induced liver steatosis in mice," *Biochemical and Biophysical Research Communications*, vol. 336, no. 1, pp. 215–222, 2005.

[40] S. Yu, K. Matsusue, P. Kashireddy et al., "Adipocyte-specific gene expression and adipogenic steatosis in the mouse liver due to peroxisome proliferator-activated receptor γ1

(PPARγ1) overexpression," *Journal of Biological Chemistry*, vol. 278, no. 1, pp. 498–505, 2003.

[41] K. Uno, H. Katagiri, T. Yamada et al., "Neuronal pathway from the liver modulates energy expenditure and systemic insulin sensitivity," *Science*, vol. 312, no. 5780, pp. 1656–1659, 2006.

[42] K. Matsusue, T. Kusakabe, T. Noguchi et al., "Hepatic steatosis in leptin-deficient mice is promoted by the PPARγ target gene Fsp27," *Cell Metabolism*, vol. 7, no. 4, pp. 302–311, 2008.

[43] O. Gavrilova, M. Haluzik, K. Matsusue et al., "Liver peroxisome proliferator-activated receptor γ contributes to hepatic steatosis, triglyceride clearance, and regulation of body fat mass," *Journal of Biological Chemistry*, vol. 278, no. 36, pp. 34268–34276, 2003.

[44] Y. L. Zhang, A. Hernandez-Ono, P. Siri et al., "Aberrant hepatic expression of PPARγ2 stimulates hepatic lipogenesis in a mouse model of obesity, insulin resistance, dyslipidemia, and hepatic steatosis," *Journal of Biological Chemistry*, vol. 281, no. 49, pp. 37603–37615, 2006.

[45] Y.-M. Nan, N. Fu, W.-J. Wu et al., "Rosiglitazone prevents nutritional fibrosis and steatohepatitis in mice," *Scandinavian Journal of Gastroenterology*, vol. 44, no. 3, pp. 358–365, 2009.

[46] Y. M. Nan, F. Han, L. B. Kong et al., "Adenovirus-mediated peroxisome proliferator activated receptor gamma overexpression prevents nutritional fibrotic steatohepatitis in mice," *Scandinavian Journal of Gastroenterology*, vol. 46, no. 3, pp. 358–369, 2011.

[47] X. Sun, Y. Tang, X. Tan et al., "Activation of peroxisome proliferator-activated receptor-γ by rosiglitazone improves lipid homeostasis at the adipose tissue-liver axis in ethanol-fed mice," *American Journal of Physiology-Gastrointestinal and Liver Physiology*, vol. 302, no. 5, pp. G548–G557, 2012.

[48] S. J. Yang, J. M. Choi, S. W. Chae et al., "Activation of peroxisome proliferator-activated receptor gamma by rosiglitazone increases Sirt6 expression and ameliorates hepatic steatosis in rats," *PLoS ONE*, vol. 6, no. 2, Article ID e17057, 2011.

[49] M. Berthiaume, M. Laplante, W. T. Festuccia, J. P. Berger, R. Thieringer, and Y. Deshaies, "Additive action of 11beta-HSD1 inhibition and PPAR-gamma agonism on hepatic steatosis and triglyceridemia in diet-induced obese rats," *International Journal of Obesity*, vol. 33, no. 5, pp. 601–604, 2009.

[50] A. K. Elshorbagy, M. Valdivia-Garcia, D. A. L. Mattocks et al., "Cysteine supplementation reverses methionine restriction effects on rat adiposity: significance of stearoyl-coenzyme a desaturase," *Journal of Lipid Research*, vol. 52, no. 1, pp. 104–112, 2011.

[51] V. L. Malloy, R. A. Krajcik, S. J. Bailey, G. Hristopoulos, J. D. Plummer, and N. Orentreich, "Methionine restriction decreases visceral fat mass and preserves insulin action in aging male Fischer 344 rats independent of energy restriction," *Aging Cell*, vol. 5, no. 4, pp. 305–314, 2006.

[52] E. P. Plaisance, F. L. Greenway, A. Boudreau et al., "Dietary methionine restriction increases fat oxidation in obese adults with metabolic syndrome," *Journal of Clinical Endocrinology and Metabolism*, vol. 96, no. 5, pp. E836–E840, 2011.

[53] P. D. Broulik, L. Sefc, and M. Haluzik, "Effect of PPAR-gamma agonist rosiglitazone on bone mineral density and serum adipokines in C57BL/6 male mice," *Folia Biologica*, vol. 57, no. 4, pp. 133–138, 2011.

[54] M. Bedoucha, E. Atzpodien, and U. A. Boelsterli, "Diabetic KKAy mice exhibit increased hepatic PPARγ1 gene expression and develop hepatic steatosis upon chronic treatment with antidiabetic thiazolidinediones," *Journal of Hepatology*, vol. 35, no. 1, pp. 17–23, 2001.

[55] I. N. Colmers, S. L. Bowker, S. R. Majumdar, and J. A. Johnson, "Use of thiazolidinediones and the risk of bladder cancer among people with type 2 diabetes: a meta-analysis," *Canadian Medical Association Journal*. In press.

[56] S. M. Rangwala and M. A. Lazar, "The dawn of the SPPARMs?" *Sciences STKE*, vol. 2002, no. 121, p. PE9, 2002.

[57] M. Wang and S. Tafuri, "Modulation of PPARgamma activity with pharmaceutical agents: treatment of insulin resistance and atherosclerosis," *Journal of Cellular Biochemistry*, vol. 89, no. 1, pp. 38–47, 2003.

[58] S. Rocchi, F. Picard, J. Vamecq et al., "A unique PPARγ ligand with potent insulin-sensitizing yet weak adipogenic activity," *Molecular Cell*, vol. 8, no. 4, pp. 737–747, 2001.

[59] M. Schupp, M. Clemenz, R. Gineste et al., "Molecular characterization of new selective peroxisome proliferator-activated receptor γ modulators with angiotensin receptor blocking activity," *Diabetes*, vol. 54, no. 12, pp. 3442–3452, 2005.

[60] J. P. Berger, A. E. Petro, K. L. Macnaul et al., "Distinct properties and advantages of a novel peroxisome proliferator-activated protein γ selective modulator," *Molecular Endocrinology*, vol. 17, no. 4, pp. 662–676, 2003.

[61] K. Bhalla, B. J. Hwang, J. H. Choi et al., "N-acetylfarnesylcysteine is a novel class of peroxisome proliferator-activated receptor γ ligand with partial and full agonist activity in vitro and in vivo," *Journal of Biological Chemistry*, vol. 286, no. 48, pp. 41626–41635, 2011.

[62] J. Rieusset, F. Touri, L. Michalik et al., "A new selective peroxisome proliferator-activated receptor γ antagonist with antiobesity and antidiabetic activity," *Molecular Endocrinology*, vol. 16, no. 11, pp. 2628–2644, 2002.

[63] L. S. Higgins and C. S. Mantzoros, "The development of INT131 as a selective PPARgamma modulator: approach to a safer insulin sensitizer," *PPAR Research*, vol. 2008, Article ID 936906, 2008.

[64] D. H. Lee, H. Huang, K. Choi, C. Mantzoros, and Y. B. Kim, "Selective PPARgamma modulator INT131 normalizes insulin signaling defects and improves bone mass in diet-induced obese mice," *American Journal of Physiology-Endocrinology and Metabolism*, vol. 302, no. 5, pp. 552–560, 2012.

[65] F. L. Dunn, L. S. Higgins, J. Fredrickson, and A. M. Depaoli, "Selective modulation of PPARγ activity can lower plasma glucose without typical thiazolidinedione side-effects in patients with Type 2 diabetes," *Journal of Diabetes and its Complications*, vol. 25, no. 3, pp. 151–158, 2011.

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Review Article

PPARs and HCV-Related Hepatocarcinoma: A Mitochondrial Point of View

Francesca Agriesti,[1] Tiziana Tataranni,[1] Vitalba Ruggieri,[1] Nazzareno Capitanio,[2] and Claudia Piccoli[1,2]

[1] *Laboratory of Pre-Clinical and Translational Research, IRCCS, Centro di Riferimento Oncologico della Basilicata, 85028 Rionero in Vulture (Pz), Italy*
[2] *Department of Clinical and Experimental Medicine, University of Foggia, 71100 Foggia, Italy*

Correspondence should be addressed to Claudia Piccoli, c.piccoli@unifg.it

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Hepatitis-C-virus-related infective diseases are worldwide spread pathologies affecting primarily liver. The infection is often asymptomatic, but when chronically persisting can lead to liver scarring and ultimately to cirrhosis, which is generally apparent after decades. In some cases, cirrhosis will progress to develop liver failure, liver cancer, or life-threatening esophageal and gastric varices. HCV-infected cells undergo profound metabolic dysregulation whose mechanisms are yet not well understood. An emerging feature in the pathogenesis of the HCV-related disease is the setting of a pro-oxidative condition caused by dysfunctions of mitochondria which proved to be targets of viral proteins. This causes deregulation of mitochondria-dependent catabolic pathway including fatty acid oxidation. Nuclear receptors and their ligands are fundamental regulators of the liver metabolic homeostasis, which are disrupted following HCV infection. In this contest, specific attention has been focused on the peroxisome proliferator activated receptors given their role in controlling liver lipid metabolism and the availability of specific pharmacological drugs of potential therapeutic utilization. However, the reported role of PPARs in HCV infection provides conflicting results likely due to different species-specific contests. In this paper we summarize the current knowledge on this issue and offer a reconciling model based on mitochondria-related features.

1. PPARs and Cancer

The peroxisome proliferator-activated receptors (PPARs) are transcription factors that translate nutritional signals into specific gene-expression patterns that control cellular differentiation, development, metabolism (carbohydrate, lipid, protein), and tumorigenesis. There are three members of the PPAR family: PPARα, γ, and δ (aka PPARβ), which have tissue-specific distributions [1]. Each PPAR initially binds a ligand and then heterodimerizes with the retinoid X receptor (RXR) before the complex binds to DNA sequences referred to as peroxisome proliferator hormone-response elements (PPREs), which are generally found in the promoter region of PPAR-targeted genes [2]. This action of heterodimerization and binding to PPREs is further modulated by the presence of coactivator and corepressor proteins. The ligands for the PPARs consist in a range of metabolites, including certain free fatty acids, eicosanoids, and xenobiotics [1], referred as to peroxisome proliferators (PP) able to differentially modulate PPAR-regulatory activities.

PPARα is the most abundant nuclear receptor in the liver especially in hepatocytes [3], and it has been identified as the master regulator of hepatic metabolism [4]. When activated, PPARα upregulates β-oxidation and thus promotes lipid clearance. PPARα has mostly been linked to fatty acid metabolism but it plays a role also in glucose metabolism. PPAR γ is expressed particularly in adipose tissue where it initiates the differentiation cascade in preadipocytes. Among its known target genes are adipocyte fatty acid-binding protein and fatty acid synthase, which are effectors of lipid accumulation during adipogenesis. Even if mainly expressed in adipocytes, also PPARγ is involved in metabolism of

hepatic cells. In contrast to PPARα and γ, the function of PPARδ is relatively unknown. PPARδ, also known as PPARβ, NUC1, and FAAR, is expressed in a wide range of tissues, but progress in understanding the function of this protein has been hampered by the lack of selective ligands. PPARδ has recently been implicated in a variety of physiological and pathophysiological processes such as embryonic implantation, wound healing, inflammation, cancer, and osteoporosis. Exposure of rodents to PPs leads to hepatomegaly, peroxisome proliferation, and an increase of fatty acid catabolism as a result of enhanced expression of genes involved in lipid transport and fatty acid β-oxidation [5, 6]. Accordingly, PPARs are involved in regulating other physiological processes such as cell proliferation, apoptosis, inflammation, oxidative stress, and differentiation. Although all these functions might contribute to the influence of PPARs in carcinogenesis, whether PPARs function as tumor suppressor or as oncogenes in cancer is still unclear.

Long-term administration of PPARα ligands causes liver cancer in mice and rodents, an effect that is dependent on PPARα, as PPARα-null mice are refractory to the hepatocarcinogenic effect of PPARα agonists [7]. Moreover, chronic exposure to synthetic PPARs agonists results in sustained activation of PPARα and transcriptional activation of its responsive genes that affect intermediary liver metabolism leading to oxidative stress-induced DNA damage in liver [8, 9]. In response to ligand activation, the induction of the peroxisomal β-oxidation enzyme acyl CoA oxidase (ACO), as a result of peroxisome proliferation, increases intracellular levels of H_2O_2 leading to oxidative stress and/or generation of lipid peroxides or free radicals that could damage macromolecules [8]. In addition, it has been reported that mitochondrial dysfunction is also responsible for the oxidative stress induced by PPARα ligands activity. Those ligands could disrupt mitochondrial electron respiratory chain at the level of the NADH cytochrome c reductase causing a compensatory shift in the metabolic state, which results in (i) preferential use of lipids through glycerol catabolism via mitochondrial FAD-dependent glycerol-3-phosphate dehydrogenase and (ii) stimulation of fatty acid β-oxidation via electron-transferring flavoprotein. The increase in free radical oxygen species resulting from stimulated peroxisomal β-oxidation may further increase the oxidative stress that results from complex I inhibition and thereby contributes significantly to the observed carcinogenic properties of PPAR ligand in rodents, particularly in liver [10]. Hypoxia in the tumour microenvironment is a common feature of solid tumours and known to stimulate mitochondrial release of reactive species of oxygen (ROS) able to function as important secondary messengers in signalling transduction. The increased ROS response can promote tumor growth and cell survival through activation of the hypoxia inducible factor 1α (HIF-1α) [11]. Interestingly, HIF-1α increases the expression of GLUT1 and other genes encoding glycolytic enzymes [12]. It has been demonstrated that hepatoma cell growth is dependent on the cellular redox state and that ROS could regulate glycolysis through HIF-1α. In fact, ROS levels directly regulate the hexokinase II (HKII) protein expression and lactate dehydrogenase (LDH) activity [13]. As redox level is able to modulate the tumour glycolysis in hepatoma cells, this mechanism could be exploited to selectively kill tumour cells through interference in energy pathways.

Liver cancer, as well as other solid tumours, shows an upregulation of the glycolytic activity in order to escape from the severe hypoxia characterizing the tumour microenvironment. Moreover, even under normoxic condition most transformed cells exhibit a robust dependence on glycolysis for energy production. This property, although long known as "Warburg effect," still remains to be fully elucidated [14, 15]. The predominance of anaerobic/aerobic glycolysis leads to conversion of its end-product pyruvate to lactate, which is secreted into the blood, instead of completing oxidation [16]. Increased glucose uptake and metabolism, due to increased levels of glucose transporters (Gluts) and of HKII, correlate with poor prognosis of many tumor types [17], supporting the notion that metabolic alterations may contribute to the malignant phenotype [18].

Kroemer and Pouyssegur [17] showed a significant correlation between ^{18}F-fluoro-2-deoxy-D-glucose (^{18}F- FDG) uptake, evaluated through positron emission tomography (PET), and the expression of Glut2 and HKII. Another clinical study [19] demonstrated a high correlation between GLUT1 expression and Ki-67, a prognostic marker of proliferation. Moreover, GLUT1, absent in normal liver as well as in most of human HCC tissues [20], is upregulated in HCC with specific high proliferative activity and over expressed especially in hypoxic regions [19].

Recently, a DNA damage response-signaling network has been proposed as novel mechanism for PP-induced hepatocyte proliferation and hepatocellular carcinoma [21]. Accordingly several genes involved in cell cycle or DNA damage repair, such as *Chek1*, *Prkdc*, *Mcm*, and *Rad51*, were significantly induced in a PPARα-dependent manner. It is postulated that PPARα-induced-DNA damage repair is due to oxidative stress. Treatment of rodents with PPs induces expression of genes encoding enzymes involved in peroxisomal and mitochondrial fatty acid β-oxidation, which produces ROS as byproducts [8, 22, 23]. Previous studies also reported that several oxidative stress-related genes were upregulated upon PP challenge, such as Txnip, Sod2, Gpx2, and Cat [22, 23].

Collectively, these studies revealed the involvement of oxidative stress in the multiple effects of PP induced hepatocarcinomas. Interestingly, oxidative stress can activate a variety of transcription factors including PPAR genes [24] and this could be a novel link explaining their role in hepatic glucose homeostasis as well in liver carcinogenesis.

Activation of PPARα also leads to increased proliferation and inhibition of apoptosis and when this occurs in a DNA-damaged cells, it is thought to lead to proliferation of initiated cells that ultimately progress to a liver tumor. This effect is supported by observation in PPARα-null mice that are refractory to all these changes in response to long-term ligand-feeding studies [25, 26]. Whereas it is clear that PPARα-agonists lead to increased cell proliferation and inhibition of apoptosis, the specific target genes mediating these events remain unidentified. Increased cell proliferation

and inhibition of apoptosis are clearly causally linked to PPARα agonist treatment and hepatocarcinogenesis. Since increased cell proliferation can influence both initiation and promotion events, the precise role of these changes is less clear. However, strong evidences causally link changes in cell proliferation and apoptosis to PPARα agonist-induced hepatocarcinogenesis [27].

Thus, the metabolic changes along with the anti-apoptotic effects of PPARs activation contribute to oxidative DNA damage and increase hepatocellular proliferation leading to liver cancer development [2, 28].

Although the mode of action for the hepatocarcinogenic effect of PPARα agonists has been determine in mice and rodents model, it is not clear if chronic administration of PPARα ligands leads to tumorigenesis in humans. Different levels of regulation of PPARα-induced response may account for this discrepancy among species. First of all, PPARs ligands show an intrinsic difference in their capability to induce maximal PPARα activation and peroxisome proliferation whose induction, in part, can be dose dependent, but not a species-specific event. Furthermore, tissue levels of expression of the PPARα receptor may explain the differences seen among animals and humans. PPARα levels appear to be lower in human livers as compared to rodent livers and have been proposed to account for reduced response of human liver to peroxisome proliferation and tumors development. Being the PPARα transactivational response also regulated by the nature of its recognition sequence presents in the promoter region of responsive genes, species differences in these promoters regions may account for some of the differences observed in response to treatment with PPARα ligands. Finally, a differential expression of certain coactivator proteins necessary for PPARα-mediated transactivation and different expression levels and activity of PPARα target genes may contribute to the variable response among different species to treatment with PPARα ligands. When the transcription coactivator complex PBP/Med is disrupted in mouse, liver, hepatocyte population fails to show cell proliferation and induction of peroxisomal β-oxidation enzymes exhibiting abrogation of peroxisome proliferation and other pleiotropic effects of treatment with PPARα ligands. These data indicate that PBP is essential for PPARα ligand-induced hepatocyte proliferation and tumorigenesis [29].

Recent data from studies using PPARα-humanized mice (that express a human PPARα gene in a PPAR null background) offer a new explanation for the species difference between rodents and humans in response to peroxisome proliferators (PPs) mediated by peroxisome proliferator-activated receptor PPARα. It has been shown that activation of PPARs by its agonists, although causes overexpression of genes involved in lipid catabolism in both wild type and humanized mice, determines tumors development and hepatocellular proliferation only in wild-type mice [28, 29]. Moreover, mice expressing the human PPARα protein do not exhibit a downregulation of the let7C miRNA, which in turn regulates the repression of c-Myc expression [30]. Thus the induced stability of Myc protein (in mice, but not in humans) might contribute to increased mitogenic signaling that causes hepatocyte proliferation in mouse model.

Despite the above-mentioned role of PPARα in liver tumorigenesis, the role of PPARγ in the onset and treatment of cancer has been focus of recent attention. Ligand activation of PPARγ is associated with differentiation and inhibition of proliferation in the normal and malignant cells. PPARγ agonists inhibit the proliferative activity of neoplastic cells, suppress the growth of human tumor xenografts in nude mice [31, 32] and reduce the frequency of spontaneous and carcinogen induced pre-neoplastic and neoplastic lesions in animals [31, 33], which is indicative of the tumor suppressor effects of PPARγ [31]. The antitumorigenic effect of PPARγ agonists in several liver cancer cell lines has been previously demonstrated [34, 35] although there have been no studies to mechanistically define the role of PPARγ in hepatocarcinogenesis. In a recent work, by using a diethylnitrosamine (DEN)-induced murine model of HCC, it has been demonstrated that the loss of one PPARγ allele significantly enhanced liver carcinogenesis. Accordingly, previous studies have been reported that human HCC displays impaired PPARγ expression [34]. Moreover PPARγ suppresses tumor cell growth through reducing cell proliferation and inducing G2/M phase arrest, apoptosis, and upregulating the putative suppressor gene, growth differentiation factor-15. Thus, PPARγ has been proposed as a tumor suppressor gene in the liver [34].

In striking contrast another study unveils the regulation of TGF-β signaling (well known to inhibit hepatocyte proliferation and induce apoptosis) by cPLA2α and PPARγ as an important mechanism for control hepatic cell growth and hepatocarcinogenesis [36]. In particular it has been described that PPARγ signaling pathways counteracts TGF-β mediated inhibition of primary and transformed hepatocyte growth. The study has shown that TGF-β regulates the growth of primary and transformed hepatocytes through concurrent activation of Smad-mediated gene transcription and phosphorylation of cPLA2α suggesting that the level and activation status of cPLA2α/PPAR-γ signaling in hepatic cells likely represents a key factor that determines the cellular response to TGF-β. It is possible that activation of cPLA2α/PPAR-γ signaling may in part explain the loss of responsiveness of neoplastic cells to the anti proliferative actions of TGF-β (due to suppression of Smad2/3 activity) [36].

Agonists for PPAR isoforms induce many physiological changes and their oncogenicity seems to depend on the oxidative stress caused by peroxisome proliferators as well as on their ability to alter balance between cell proliferation and death. These are good reasons to suggest that PPARs agonists could be potential candidates for treating and preventing cancer. PPARα remains a viable target for the treatment and prevention of cancer because of evidence indicating that humans are refractory to the hepatocarcinogenic effects of PPARα agonists. PPARγ also remains a potential target for the treatment and prevention of cancer, in particular for PPARγ agonists with good safety profiles.

However, the complexity of PPAR regulation and the effects resulting from receptor activation impose considerable research and drug discovery efforts to fully delineate the potential of targeting PPARs for the treatment and

prevention of cancer. Figure 1 illustrates schematically the main points discussed in this paragraph. The main mitochondrial alterations in in Figure 1(b) are highlighted in terms of intramitochondrial Ca^{2+} level ($mtCa^{2+}$), reactive oxygen species level (ROS), membrane potential ($\Delta\Psi_m$), and oxidative phosphorylation efficiency (OXPHOS). The tight physical interactions of mitochondria with the endoplasmic reticulum (ER) and the therein HCV proteins localization are shown. An attempt is made to reconcile the conflicting species-specific results reported in literature concerning the role of PPARs in the HCV-related development of steatosis and hepatocellularcarcinoma in infected subjects. The evidence in human is a reduction/increase of PPARα/PPARγ activities (black arrows starting from PPARs) whereas in mouse the opposite holds (red arrows starting from PPARs). This causes in infected humans enhanced lipogenesis with accumulation of triglycerides (TGs) in form of lipid droplets, which are essential for stabilization of the viral replication complex and consequent viral particle maturation. To note, under this condition a proinflammatory state is also developed because of the derepressive effect of PPARα upregulation on NF$\kappa\beta$ expression (not shown, but see text). In mouse the deregulation of the PPARs reciprocal activities would lead to enhanced uptake and oxidation of fatty acid (FA) as well as of lipoproteins (not shown). However, the set mitochondrial dysfunctions hamper the mitochondrial β-oxidation fostering ER- and peroxisome-mediated β- and ω-oxidation. This causes enhanced oxidative stress which adds to that of mitochondria. Such a constitutive pro-oxidative setting might provide mutagenic hits and genome instability leading to cell transformation and HCC development. Moreover, also in mouse the low efficient extramitochondrial FA oxidation leads to intracellular accumulation of lipids and progressive steatosis. Consistently, both PPARS control the expression of the FA transporters across the cell membranes. The possible direct involvement of the HCV proteins in controlling the PPARs pathways and the potential therapeutic utilization of PPARs agonist/antagonist to correct PPARs deregulation following HCV infection is also illustrated. For clarity co-transcription/corepressor factors recruited by PPARs have been omitted (but see text). The contribution of the hypoxia inducible factor (HIF-1α) to the metabolic/bioenergetic adaptation by (normoxic) up-regulation of the glycolytic pathway is also shown.

2. HCV and Liver Cancer

Hepatitis C virus (HCV) infects hundreds of millions of people persistently, and induces a spectrum of chronic liver disease worldwide [37]. Chronic infection with the hepatitis C virus (HCV) is a major risk factor for the development of hepatocellular carcinoma (HCC). The pathogenesis of HCC in HCV infection has extensively been analyzed. However, it remains controversial in the pathogenesis of HCC associated with HCV as to whether the virus plays a direct or an indirect role. HCV is a positive strand RNA virus with a 9.6 kb genome encoding a single approximately 3000 aminoacid polyprotein. This is translated on the endoplasmic reticulum (ER) and is co- and post-translationally cleaved by host and viral proteases into 10 individual membrane-associated proteins comprising structural (Core, E1, E2) and non structural (p7, NS2-NS5B) proteins [38]. It has been demonstrated that the core protein of HCV has an oncogenic potential, indicating that HCV is directly involved in hepatocarcinogenesis, albeit other factors such as continued cell death and regeneration associated with inflammation would also play a role.

It is known that the core protein is able to induce ROS increase in liver. Thus, oxidative stress production in the absence of inflammation by the core protein would partly contribute to the development of HCC. Accordingly it has been reported an augmented production of oxidative stress along with the activation of scavenging system, including catalase and glutathione, in the putative pre-neoplastic stage with steatosis in the liver. HCV infection can induce a state of oxidative stress that is more pronounced than that observed in many other inflammatory diseases. Many different oxidative stress markers have been reported in hepatitis C patients, including lipid peroxidation products [39] and oxidized protein and lipid derivatives in the liver [40]. Consistently a greater degree of oxidative stress markers correlates with a severer disease [39] and successful eradication of HCV decreases oxidative stress markers [41].

The generation of hepatic oxidative stress is assessed to originate from mitochondrial dysfunction in HCV-infected hepatocytes [42] and see Figure 1(b). Numerous studies have, particularly, shown that the expression of the HCV core protein can increase ROS production at the mitochondrial level. Although the synthesis and maturation of HCV proteins occur at level of ER [43], a number of studies unveiled partial localization of some HCV proteins, notably core and NS3/4a, to the outer mitochondrial membrane [44, 45]. A specific sequence in the C-terminal portion of the molecule serves as a targeting sequence to the mitochondrial outer membrane [44, 46]. At the mitochondria level, a chain of events is initiated by core binding, which consists of increased Ca^{2+} uptake, increased mitochondrial superoxide production, oxidation of the mitochondrial glutathione pool, inhibition of the electron transport complex I activity, and sensitization of mitochondria to Ca^{2+}- and ROS-induced membrane permeability transition. These effects have been observed in isolated mitochondria [47], cells line inducibly expressing the entire HCV open reading frame (U-2 OS human osteosarcoma human derived cells) [43, 48] and liver mitochondria derived from HCV transgenic mice [49, 50]. In addition to these direct effects on mitochondria, core protein has been shown to cause a state of ER stress and an increase in the efficiency of ER to mitochondria Ca^{2+} transfer. The resulting oxidized redox state has a number of potential consequences for liver function: it interferes with the antiviral innate immune response and potentiates fibrosis and carcinogenesis. By using both HCV-induced U-2 OS derived cells and HCV-infected Huh-7 cells, our group has shown that the expression of all HCV proteins causes indirect Ca^{2+} mediated deregulation of the mitochondrial oxidative metabolism. Our studies unveiled a marked intramitochondrial Ca^{2+} increase as the causative event leading

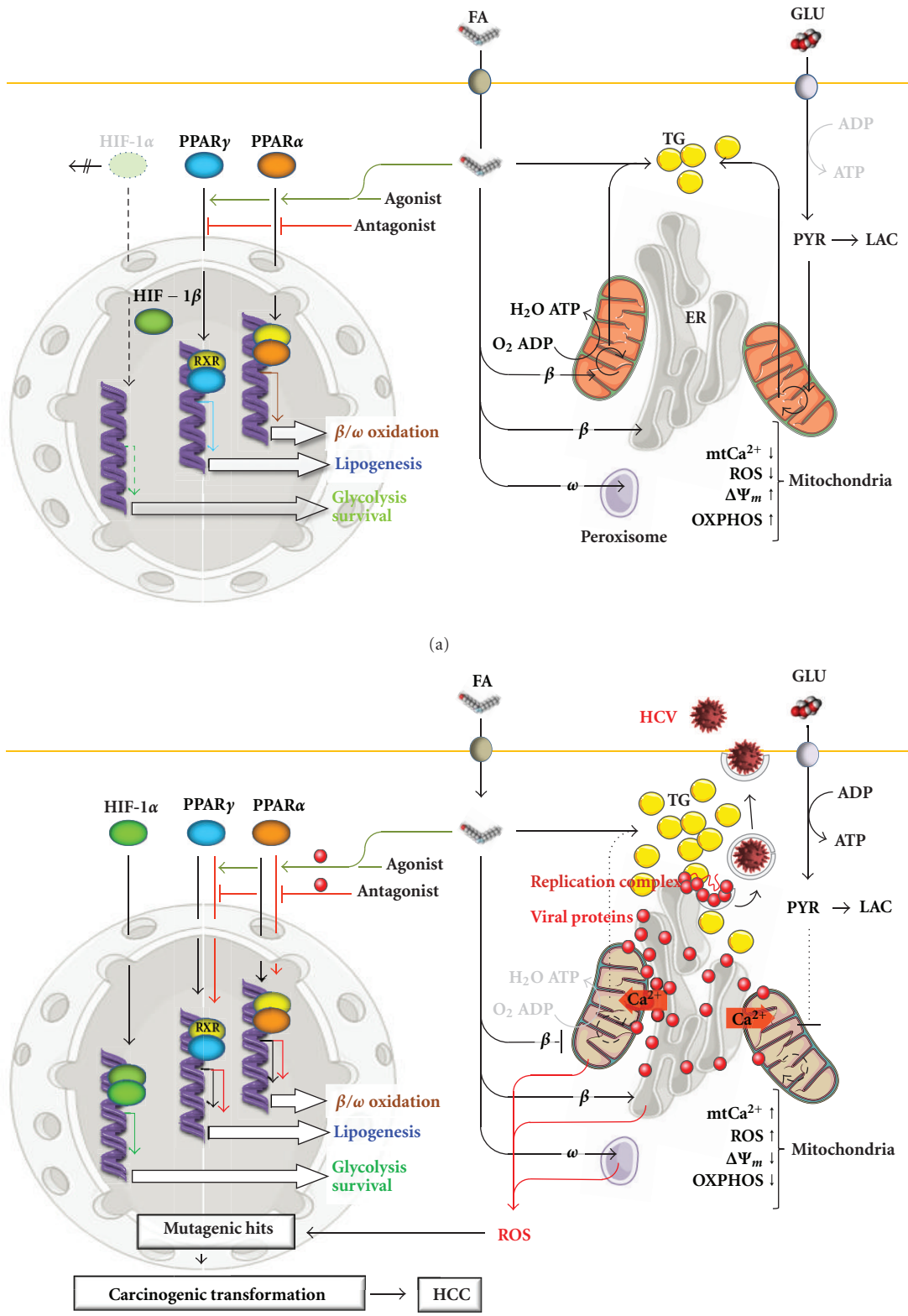

Figure 1: Schematic representation of the interplay between deregulation of the PPARs pathways and the HCV-mediated dysfunctions of mitochondria. (a) Normal noninfected condition. (b) HCV-infected condition Glu, glucose; Pyr, pyruvate; Lac, lactate; HIF-1β, hypoxia inducible factor 1β; RXR, retinoid X receptor.

to profound mitochondrial oxidative metabolism alteration following coordinate HCV protein expression [48].

High level of calcium can detach cytochrome *c* from the cardiolipin inner membrane [51] and activate the mitochondrial nitric oxide synthase with production of nitric oxide (NO) [52, 53] that is known to be an inhibitor of complex IV [54, 55] as well as of complex I [56] although by a different mechanism. The combination of these possible effects would result in an overload of harmful reducing equivalents throughout the respiratory chain complexes and in an extraproduction of ROS with respect to their basal level [57, 58]. Once exhausted the buffering antioxidant capacity of glutathione and other redox buffers, a self-fuelling cycle can be activated with further enhancement of reactive oxygen/nitrogen species and alteration of the mitochondrial homeostasis [59, 60]. In summary, increased Ca^{2+}, ROS ($O^{\bullet -}$, H_2O_2), and RNS ($NO^{\bullet}$, $ONOO^{-}$) can trigger PTP opening and cytochrome c release across the outer membrane, culminating in the actuation of the apoptotic program, [56, 61] thus favoring diffusion of virus infection. Alternatively, ROS may act as redox modulators in pro-survival signaling (i.e., the NF-κB/JNK/STAT3 pathway), resulting in carcinogenic priming of the host cell [56, 62]. Pharmacological treatment with compounds able to restore mitochondrial Ca^{2+} homeostasis might prevent or even reverse the effects of HCV [48].

Using a transgenic mouse model in which HCC develops late in life after the preneoplastic steatosis stage, it has been further demonstrated that HCV shrewdly exacerbates oxidative stress by modulating both production and scavenging of reactive oxygen species. Accordingly,the core protein of HCV was shown to induce overproduction of ROS in the liver. Under excessive generation of ROS, HCV affects the steady-state levels of a mitochondrial protein chaperone, that is, prohibitin, leading to an impaired function of the mitochondrial respiratory chain with the production of further ROS. On the other hand, HCV compromises some of the antioxidant systems, including heme oxygenase-1 and NADH dehydrogenase quinone 1. Thus, HCV infection not only induces ROS but also hampers the antioxidant system in the liver, thereby exacerbating oxidative stress that would facilitate hepatocarcinogenesis.

Another study confirmed the accumulation of an ROS-mediated oxidative DNA damage in the progression of chronic liver damage to HCC and suggested that this correlates with induction of telomerase activity and, as a novel finding, with overexpression of miR-92, a microRNA that plays a role in both the apoptotic process and the cellular proliferation pathways [63].

Recently a new strategy by which HCV promotes development of hepatocellular carcinoma has been proposed. It has been shown that the core protein overcomes premature senescence provoked by the ROS inducer, H_2O_2, in human liver cells. For this effect, core protein downregulates the level of p16 via promoter hypermethylation and subsequently induces phosphorylation of Rb in the presence of H_2O_2. The potential of core to inactivate Rb and suppress H_2O_2-mediated cellular senescence was abolished when levels of p16 were recovered by either exogenous complementation or inhibition of DNA methylation [64].

The core protein has also been found to interact with some cellular proteins, such as the retinoid X receptor (RXR)-α, which play pivotal roles in cell proliferation and metabolism [65]. The mitogen-activated protein kinase (MAPK) cascade is also activated in the liver of the core gene transgenic mouse model. The MAPK pathway, which consists of three routes, c-Jun N-terminal kinase (JNK), p38, and extracellular signal-regulated kinase (ERK), is involved in numerous cellular events including cell proliferation. In the liver of the core gene transgenic mouse model prior to HCC development, only the JNK route is activated. Downstream of the JNK activation, transcription factor activating factor (AP)-1 activation is markedly enhanced [65, 66]. Far downstream, both the mRNA and protein levels of cyclin D1 and CDK4 are increased. The suppression by HCV core protein of the inhibitor of cytokine signaling (*SOCS*)-*1*, a tumor suppressor gene, may also contribute to hepatocarcinogenesis. Thus, the HCV core protein modulates the intracellular signaling pathways and gives an advantage to hepatocytes for cell proliferation. Such an effect of the core protein on the MAPK pathway, combined with that on oxidative stress, may explain the extremely high incidence of HCC development in chronic hepatitis C.

Interestingly, it has been found that HCV core protein enhances Wnt/β-catenin signaling activity (in core-expressing hepatoma cells), whose overactivation is considered a major factor in oncogenesis. HCV core protein significantly enhances T-cell factor- (Tcf-) dependent transcriptional activity induced by Wnt3A in HCC cell lines. Additionally, core protein increases and stabilizes β-catenin levels in hepatoma cell line Huh7 through inactivation of GSK-3β, which contributes to the upregulation of downstream target genes, such as c-Myc, cyclin D1, WISP2, and CTGF. Also, core protein increases cell proliferation rate and promotes Wnt3A-induced tumor growth in the xenograft tumor model of human HCC [67]. The HCV 3a Core protein has also been found to downregulate the gene expression of caspases (3, 8–10) and p53, which are involved in apoptosis. Moreover, HCV-3a Core gene showed a stronger effect in regulating protein level of p-Akt as compared to HCV 1a Core accompanied by enhanced cell proliferation in Huh-7 cell line. Thus, it has been concluded that reduced expression of cellular genes involved in apoptosis, increased p-Akt (cell survival gene), and enhanced cell proliferation in response to HCV 3a core confirms antiapoptotic effect of HCV 3a Core gene in Huh-7 that may lead to HCC [68].

Novel insights into the pathogenesis of chronic hepatitis C and, possibly, the HCV-related development of hepatocellular carcinoma were provided by the observation that HCV induces normoxic stabilization and trans-activating upregulation of the hypoxia inducible factor (HIF) [69]. This result was obtained in different *in vitro* cell system and in human HCV-infected patient. The stabilization of HIF seemingly did not relate to the action of a specific viral protein but was rather due to accumulation of intermediates of the dysregulated mitochondrial metabolism. HIF plays a recognized role

in adapting the cell to stressing conditions by upregulating the glycolytic pathway and providing pro-surviving features.

The combination of these pathways, collective with HCV-associated alterations in lipid and glucose metabolism (see below and Figure 1(b)), would lead to the frequent development of HCC in persistent HCV infection.

3. HCV and PPARs

PPARs are master regulators of lipid and glucose homeostasis, inflammation, cell differentiation, and proliferation, processes intricately involved in HCV infection, and progression. HCV-mediated dysregulation of these processes influences the replicative efficiency of the virus [70–74]. PPARs are natural targets of HCV-related studies because of their abundant occurrence in the liver and involvement in processes known to be dysregulated by HCV. Because they regulate processes essential to the progression of chronic hepatitis-C, it is important to understand the interaction between HCV and PPARs pathways signaling.

Studies on humans report impaired PPARα activity in the livers of chronic hepatitis C patients [75]. Consequently, PPARα mRNA and protein levels are significantly decreased in steatotic hepatitis C-infected livers as compared with non steatotic livers [76]. The HCV core protein contains RNA binding domains capable of suppressing the transcriptional activity of PPARα [75] and, therefore has been indicated as a primary cause in the HCV-mediated downregulation of PPARα. In addition to effects on metabolism, the decreased expression of PPARα may be involved in the pathogenesis of HCV infection through an alteration of the protective effects of nuclear receptors against hepatic inflammation and fibrosis [77]. PPARα downregulation observed in humans may also exacerbate HCV-induced inflammation [75, 78]. For example, the HCV core protein negatively regulates the inhibitory effect of PPARα on nuclear factor kappa B (NFκB) activity [78], thus activating NFκB. From this point of view, PPARα may represent new potential therapeutic targets in HCV infection.

Conversely, studies in HCV core transgenic mice showed that the expression of core protein is associated with PPARα activation. The core serves as a coactivator and nuclear stabilizer of PPARα and may trans-activate PPARα through ERK1/2 activation and p38 MAPK phosphorylation [79]. Although apparently counterintuitive, PPARα upregulates genes involved in the generation of ROS through activation and induction of acyl CoA oxidase (AOX) and cytochrome P450 4A1. This causes increased microsomal and peroxisomal β and ω oxidation, respectively, which can cause oxidative damage of the mitochondrial membrane thereby impairing the mitochondrial β-oxidation and leading to fatty acid accumulation in hepatocytes [79, 80]. PPARα also increases expression of fatty acid transporters, promoting fatty acid influx and leading to further PPARα activation by acting as PPAR ligands [79, 80]; this helps to explain the role of PPARα in HCV-induced steatosis in the animal model.

PPARα is also involved in the development of HCV-related HCC in animal models. PPAR$\alpha^{+/+}$/HCV core transgenic mice develop HCC at a rate of about 30% higher than PPAR$\alpha^{+/-}$/HCV core or PPAR$\alpha^{-/-}$/HCV core transgenic mice [79, 81]. This may be due to the involvement of PPARα in ROS generation that subsequently leads to increases in oxidative DNA damage, predisposing hepatocytes to malignant transformation, and indicates that not the presence but the persistent activation of PPARα would be important in hepatocarcinogenesis by HCV core protein. Moreover, PPARα activation in mice also leads to increase in cell division by altering cyclins and cyclin-dependent kinases (CDK) expression without subsequent increase in apoptosis [79]. In general, PPAR-α acts to ameliorate steatosis but in the presence of mitochondrial dysfunction, which is also provoked by the core protein, the core-activated PPAR-α may exacerbate steatosis. Persistent activation of PPAR-α with strong ligands such as the core protein of HCV could be carcinogenic in humans, although the low-affinity fibrate ligands are not likely associated with human cancers.

Conflicting evidences have also emerged concerning the role of PPAR α in HCV replication upon study of its ligands. It has been shown that both PPARα agonists and antagonists inhibit HCV replication [82, 83]. Bezafibrate, a PPARα activator, is widely used to treat hyperlipidemia by reducing serum LDL, VLDL, and triglycerides. Fibrates may decrease HCV RNA titers in patients who were previously unresponsive to IFN therapy [72, 84]. This effect is attributable to its reduction of HCV RNA bound to LDL. It is also possible that PPARα-mediated suppression of NFκB is involved in HCV repression [72]. The repressive effects of an agonist are logical due to the anti-inflammatory as well as antilipogenic properties of PPARα. However, PPARα antagonists also make sense given the environment needed for viral replication. HCV replication takes place in membranous ER-derived complexes that associate with lipid droplets. HCV core induces changes in lipid metabolism [85, 86] as well as the formation and redistribution of these droplets [87, 88]. PPARα antagonist 2-chloro-5-nitro-N-(pyridyl) benzamide causes hyperlipidemia and consequent disruption in the membranous structures and in the composition of lipid droplets (notably an increase in triglyceride content) in Huh7 cells. This causes changes in the localization of HCV RNA and disruption of the replication complex [79, 82]. However, similar effects (i.e., inhibition of HCV replication) have also been described following treatment with PPARa agonists [89]. Evidences are accumulating suggesting caution in interpreting effects of drugs which appear to have pleiotropic effects and that cannot be ascribed solely to PPARs as specific targets [10]. Moreover, it should be taken into account that contradictory results might stem from differences in the cellular environment altering directly or indirectly the effects on PPARs as through phosphorylation of the receptors or availability of cofactors.

As with PPARα, there is some controversy also about the interaction between PPARγ and HCV. Several in vitro studies using the human HCC cell line Huh7 have linked HCV (specifically viral protein NS5A) with increased transcriptional activity of PPARγ [90, 91] as well as increased recruitment of PPARγ coactivator-1α (PGC1α) to the peroxisome proliferator response element [91]. Increased expression of PPARγ mRNA has also been observed in human livers

infected with HCV, with the highest levels in patients with HCV-associated steatosis [92]. PPARγ-mediated up regulation of lipogenic genes is a relatively simple mechanism for HCV-related steatosis. However, HCV genotype 3a-mediated down regulation of PPARγ in Huh7 cells has also been observed, leading to induction of suppressor of cytokine signaling 7 (SOCS-7), which is normally repressed by PPARγ, and helping the virus to inhibit cytokine signaling and to escape the immune system [74]. SOCS-7 also plays a role in the development of insulin resistance, a well-known result of chronic HCV infection, by degrading insulin receptor substrate 1 in Huh7 cells [74, 93].

There is sufficient evidence that HCV infection affects PPARs-mediated pathways thus affecting the hepatic metabolism. The relationship between hepatitis C and nuclear receptors is undoubtedly complex and it is difficult to assemble the often-discordant findings into a comprehensive picture (Figure 1). However, what emerges clearly is the profound impact of nuclear receptor-regulated pathways on the critical steps of the viral life-cycle. In-depth understanding of the interaction may prove a crucial step in the development of treatment and prevention strategies.

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References

[1] Z. Ament, M. Masoodi, and J. L. Griffin, "Applications of metabolomics for understanding the action of peroxisome proliferator-activated receptors (PPARs) in diabetes, obesity and cancer," *Genome Medicine*, vol. 4, no. 4, Article ID 32, 2012.

[2] J. M. Peters, C. Cheung, and F. J. Gonzalez, "Peroxisome proliferator-activated receptor-α and liver cancer: where do we stand?" *Journal of Molecular Medicine*, vol. 83, no. 10, pp. 774–785, 2005.

[3] D. Auboeuf, J. Rieusset, L. Fajas et al., "Tissue distribution and quantification of the expression of mRNAs of peroxisome proliferator-activated receptors and liver X receptor-α in humans: no alteration in adipose tissue of obese and NIDDM patients," *Diabetes*, vol. 46, no. 8, pp. 1319–1327, 1997.

[4] I. Issemann and S. Green, "Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators," *Nature*, vol. 347, no. 6294, pp. 645–650, 1990.

[5] P. B. Lazarow and C. De Duve, "A fatty acyl CoA oxidizing system in rat liver peroxisomes; enhancement by clofibrate, a hypolipidemic drug," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 73, no. 6, pp. 2043–2046, 1976.

[6] J. K. Reddy and T. P. Krishnakantha, "Hepatic peroxisome proliferation: induction by two novel compounds structurally unrelated to clofibrate," *Science*, vol. 190, no. 4216, pp. 787–789, 1975.

[7] J. M. Peters, R. C. Cattley, and F. J. Gonzalez, "Role of PPARα in the mechanism of action of the nongenotoxic carcinogen and peroxisome proliferator Wy-14,643," *Carcinogenesis*, vol. 18, no. 11, pp. 2029–2033, 1997.

[8] A. V. Yeldandi, M. S. Rao, and J. K. Reddy, "Hydrogen peroxide generation in peroxisome proliferator-induced oncogenesis," *Mutation Research*, vol. 448, no. 2, pp. 159–177, 2000.

[9] J. K. Reddy and T. Hashimoto, "Peroxisomal β-oxidation and peroxisome proliferator—activated receptor α: an adaptive metabolic system," *Annual Review of Nutrition*, vol. 21, pp. 193–230, 2001.

[10] R. Scatena, P. Bottoni, G. E. Martorana et al., "Mitochondrial respiratory chain dysfunction, a non-receptor-mediated effect of synthetic PPAR-ligands: biochemical and pharmacological implications," *Biochemical and Biophysical Research Communications*, vol. 319, no. 3, pp. 967–973, 2004.

[11] J. P. Fruehauf and F. L. Meyskens, "Reactive oxygen species: a breath of life or death?" *Clinical Cancer Research*, vol. 13, no. 3, pp. 789–794, 2007.

[12] G. L. Semenza, "Targeting HIF-1 for cancer therapy," *Nature Reviews Cancer*, vol. 3, no. 10, pp. 721–732, 2003.

[13] D. Y. Shi, Y. R. Deng, S. L. Liu, Y. D. Zhang, and L. Wei, "Redox stress regulates cell proliferation and apoptosis of human hepatoma through Akt protein phosphorylation," *FEBS Letters*, vol. 542, no. 1-3, pp. 60–64, 2003.

[14] J. P. Bayley and P. Devilee, "The Warburg effect in 2012.," *Current opinion in oncology*, vol. 24, no. 1, pp. 62–67, 2012.

[15] S. J. Bensinger and H. R. Christofk, "New aspects of the Warburg effect in cancer cell biology," *Seminars in Cell and Developmental Biology*, vol. 23, no. 4, pp. 352–361, 2012.

[16] M. G. V. Heiden, L. C. Cantley, and C. B. Thompson, "Understanding the warburg effect: the metabolic requirements of cell proliferation," *Science*, vol. 324, no. 5930, pp. 1029–1033, 2009.

[17] G. Kroemer and J. Pouyssegur, "Tumor cell metabolism: cancer's Achilles' Heel," *Cancer Cell*, vol. 13, no. 6, pp. 472–482, 2008.

[18] R. G. Jones and C. B. Thompson, "Tumor suppressors and cell metabolism: a recipe for cancer growth," *Genes and Development*, vol. 23, no. 5, pp. 537–548, 2009.

[19] K. Kitamura, E. Hatano, T. Higashi et al., "Proliferative activity in hepatocellular carcinoma is closely correlated with glucose metabolism but not angiogenesis," *Journal of Hepatology*, vol. 55, no. 4, pp. 846–857, 2011.

[20] T. Amann, U. Maegdefrau, A. Hartmann et al., "GLUT1 expression is increased in hepatocellular carcinoma and promotes tumorigenesis," *American Journal of Pathology*, vol. 174, no. 4, pp. 1544–1552, 2009.

[21] A. Qu, Y. M. Shah, T. Matsubara, Q. Yang, and F. J. Gonzalez, "PPARα-dependent activation of cell cycle control and DNA repair genes in hepatic nonparenchymal cells," *Toxicological Sciences*, vol. 118, no. 2, Article ID kfq259, pp. 404–410, 2010.

[22] C. G. Woods, A. M. Burns, B. U. Bradford et al., "WY-14,643-induced cell proliferation and oxidative stress in mouse liver are independent of NADPH oxidase," *Toxicological Sciences*, vol. 98, no. 2, pp. 366–374, 2007.

[23] Q. Yang, S. Ito, and F. J. Gonzalez, "Hepatocyte-restricted constitutive activation of PPARα induces hepatoproliferation but not hepatocarcinogenesis," *Carcinogenesis*, vol. 28, no. 6, pp. 1171–1177, 2007.

[24] S. Reuter, S. C. Gupta, M. M. Chaturvedi, and B. B. Aggarwal, "Oxidative stress, inflammation, and cancer: how are they linked?" *Free Radical Biology and Medicine*, vol. 49, no. 11, pp. 1603–1616, 2010.

[25] T. Hays, I. Rusyn, A. M. Burns et al., "Role of peroxisome proliferator-activated receptor-α (PPARα) in bezafibrate-induced hepatocarcinogenesis and cholestasis," *Carcinogenesis*, vol. 26, no. 1, pp. 219–227, 2005.

[26] R. A. Roberts, N. H. James, S. C. Hasmall et al., "Apoptosis and proliferation in nongenotoxic carcinogenesis: species differences and role of PPARα," *Toxicology Letters*, vol. 112-113, pp. 49–57, 2000.

[27] J. E. Klaunig, M. A. Babich, K. P. Baetcke et al., "PPARα agonist-induced rodent tumors: modes of action and human relevance," *Critical Reviews in Toxicology*, vol. 33, no. 6, pp. 655–780, 2003.

[28] H. H. Chen, Y. M. Sue, C. H. Chen et al., "Peroxisome proliferator-activated receptor alpha plays a crucial role in l-carnitine anti-apoptosis effect in renal tubular cells," *Nephrology Dialysis Transplantation*, vol. 24, no. 10, pp. 3042–3049, 2009.

[29] K. Matsumoto, S. Yu, Y. Jia et al., "Critical role for transcription coactivator Peroxisome Proliferator- activated Receptor (PPAR)-binding protein/TRAP220 in liver regeneration and PPARα ligand-induced liver tumor development," *Journal of Biological Chemistry*, vol. 282, no. 23, pp. 17053–17060, 2007.

[30] Y. M. Shah, K. Morimura, Q. Yang, T. Tanabe, M. Takagi, and F. J. Gonzalez, "Peroxisome proliferator-activated receptor α regulates a microRNA-mediated signaling cascade responsible for hepatocellular proliferation," *Molecular and Cellular Biology*, vol. 27, no. 12, pp. 4238–4247, 2007.

[31] H. P. Koeffer, "Peroxisome proliferators-activated receptor gamma and carcinomas," *Clinical Cancer Research*, vol. 9, pp. 1–9, 2003.

[32] P. Sertznig, M. Seifert, W. Tilgen, and J. Reichrath, "Present concepts and future outlook: function of peroxisome proliferator-activated receptors (PPARs) for pathogenesis, progression, and therapy of cancer," *Journal of Cellular Physiology*, vol. 212, no. 1, pp. 1–12, 2007.

[33] C. Grommes, G. E. Landreth, and M. T. Heneka, "Antineoplastic effects of peroxisome proliferator-activated receptor γ agonists," *Lancet Oncology*, vol. 5, no. 7, pp. 419–429, 2004.

[34] J. Yu, L. Qiao, L. Zimmermann et al., "Troglitazone inhibits tumor growth in hepatocellular carcinoma in vitro and in vivo," *Hepatology*, vol. 43, no. 1, pp. 134–143, 2006.

[35] M. C. Hsu, C. C. Huang, H. C. Chang, T. H. Hu, and W. C. Hung, "Overexpression of Jab1 in hepatocellular carcinoma and its inhibition by peroxisome proliferator-activated receptorγ ligands in vitro and in vivo," *Clinical Cancer Research*, vol. 14, no. 13, pp. 4045–4052, 2008.

[36] C. Han, W. C. Bowen, G. Li, A. J. Demetris, G. K. Michalopoulos, and T. Wu, "Cytosolic phospholipase A2α and peroxisome proliferator-activated receptor γ signaling pathway counteracts transforming growth factor β-mediated inhibition of primary and transformed hepatocyte growth," *Hepatology*, vol. 52, no. 2, pp. 644–655, 2010.

[37] C. W. Shepard, L. Finelli, and M. J. Alter, "Global epidemiology of hepatitis C virus infection," *Lancet Infectious Diseases*, vol. 5, no. 9, pp. 558–567, 2005.

[38] K. E. Reed and C. M. Rice, "Overview of hepatitis C virus genome structure, polyprotein processing, and protein properties," *Current Topics in Microbiology and Immunology*, vol. 242, pp. 55–84, 2000.

[39] G. Vendemiale, I. Grattagliano, P. Portincasa, G. Serviddio, G. Palasciamo, and E. Altomare, "Oxidative stress in symptom-free HCV carriers: relation with ALT flare-up," *European Journal of Clinical Investigation*, vol. 31, no. 1, pp. 54–63, 2001.

[40] V. Paradis, M. Kollinger, M. Fabre, A. Holstege, T. Poynard, and P. Bedossa, "In situ detection of lipid peroxidation by-products in chronic liver diseases," *Hepatology*, vol. 26, no. 1, pp. 135–142, 1997.

[41] F. Serejo, I. Emerit, P. M. Filipe et al., "Oxidative stress in chronic hepatitis C: the effect of interferon therapy and correlation with pathological features," *Canadian Journal of Gastroenterology*, vol. 17, no. 11, pp. 644–650, 2003.

[42] C. Piccoli, R. Scrima, A. D'Aprile et al., "Mitochondrial dysfunction in hepatitis C virus infection," *Biochimica et Biophysica Acta*, vol. 1757, no. 9-10, pp. 1429–1437, 2006.

[43] D. Moradpour, F. Penin, and C. M. Rice, "Replication of hepatitis C virus," *Nature Reviews Microbiology*, vol. 5, no. 6, pp. 453–463, 2007.

[44] G. Mottola, G. Cardinali, A. Ceccacci et al., "Hepatitis C virus nonstructural proteins are localized in a modified endoplasmic reticulum of cells expressing viral subgenomic replicons," *Virology*, vol. 293, no. 1, pp. 31–43, 2002.

[45] Y. Nomura-Takigawa, M. Nagano-Fujii, L. Deng et al., "Non-structural protein 4A of Hepatitis C virus accumulates on mitochondria and renders the cells prone to undergoing mitochondria-mediated apoptosis," *Journal of General Virology*, vol. 87, no. 7, pp. 1935–1945, 2006.

[46] B. Schwer, S. Ren, T. Pietschmann et al., "Targeting of hepatitis C virus core protein to mitochondria through a novel C-terminal localization motif," *Journal of Virology*, vol. 78, no. 15, pp. 7958–7968, 2004.

[47] M. Korenaga, T. Wang, Y. Li et al., "Hepatitis C virus core protein inhibits mitochondrial electron transport and increases reactive oxygen species (ROS) production," *Journal of Biological Chemistry*, vol. 280, no. 45, pp. 37481–37488, 2005.

[48] C. Piccoli, R. Scrima, G. Quarato et al., "Hepatitis C virus protein expression causes calcium-mediated mitochondrial bioenergetic dysfunction and nitro-oxidative stress," *Hepatology*, vol. 46, no. 1, pp. 58–65, 2007.

[49] K. Moriya, K. Nakagawa, T. Santa et al., "Oxidative stress in the absence of inflammation in a mouse model for hepatitis C virus-associated hepatocarcinogenesis," *Cancer Research*, vol. 61, no. 11, pp. 4365–4370, 2001.

[50] M. Okuda, K. Li, M. R. Beard et al., "Mitochondrial injury, oxidative stress, and antioxidant gene expression are induced by hepatitis C virus core protein," *Gastroenterology*, vol. 122, no. 2, pp. 366–375, 2002.

[51] M. Ott, J. D. Robertson, V. Gogvadze, B. Zhivotovsky, and S. Orrenius, "Cytochrome c release from mitochondria proceeds by a two-step process," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 3, pp. 1259–1263, 2002.

[52] W. K. Alderton, C. E. Cooper, and R. G. Knowles, "Nitric oxide synthases: structure, function and inhibition," *Biochemical Journal*, vol. 357, no. 3, pp. 593–615, 2001.

[53] P. Ghafourifar and E. Cadenas, "Mitochondrial nitric oxide synthase," *Trends in Pharmacological Sciences*, vol. 26, no. 4, pp. 190–195, 2005.

[54] G. C. Brown, "Regulation of mitochondrial respiration by nitric oxide inhibition of cytochrome c oxidase," *Biochimica et Biophysica Acta*, vol. 1504, no. 1, pp. 46–57, 2001.

[55] S. Shiva and V. M. Darley-Usmar, "Control of the nitric oxide-cytochrome C oxidase signaling pathway under pathological and physiological conditions," *IUBMB Life*, vol. 55, no. 10-11, pp. 585–590, 2003.

[56] A. Jekabsone, L. Ivanoviene, G. C. Brown, and V. Borutaite, "Nitric oxide and calcium together inactivate mitochondrial complex I and induce cytochrome c release," *Journal of Molecular and Cellular Cardiology*, vol. 35, no. 7, pp. 803–809, 2003.

[57] P. S. Brookes, Y. Yoon, J. L. Robotham, M. W. Anders, and S. S. Sheu, "Calcium, ATP, and ROS: a mitochondrial love-hate

triangle," *American Journal of Physiology. Cell Physiology*, vol. 287, no. 4, pp. C817–C833, 2004.

[58] K. D. Tardif, G. Waris, and A. Siddiqui, "Hepatitis C virus, ER stress, and oxidative stress," *Trends in Microbiology*, vol. 13, no. 4, pp. 159–163, 2005.

[59] S. Papa and V. P. Skulachev, "Reactive oxygen species, mitochondria, apoptosis and aging," *Molecular and Cellular Biochemistry*, vol. 174, no. 1-2, pp. 305–319, 1997.

[60] M. Inoue, E. F. Sato, M. Nishikawa et al., "Mitochondrial generation of reactive oxygen species and its role in aerobic life," *Current Medicinal Chemistry*, vol. 10, no. 23, pp. 2495–2505, 2003.

[61] K. Machida, K. T. H. Cheng, C. K. Lai, K. S. Jeng, V. M. H. Sung, and M. M. C. Lai, "Hepatitis C virus triggers mitochondrial permeability transition with production of reactive oxygen species, leading to DNA damage and STATS activation," *Journal of Virology*, vol. 80, no. 14, pp. 7199–7207, 2006.

[62] P. Storz, "Reactive oxygen species in tumor progression," *Frontiers in Bioscience*, vol. 10, no. 2, pp. 1881–1896, 2005.

[63] R. Cardin, M. Piciocchi, A. Sinigaglia, E. Lavezzo, M. Bortolami, A. Kotsafti et al., "Oxidative DNA damage correlates with cell immortalization and mir-92 expression in hepatocelllular carcinoma," *BMC Cancer*, vol. 12, no. 1, article 177, 2012.

[64] J. S. Lim, S. H. Park, and K. L. Jang, "Hepatitis C virus Core protein overcomes stress-induced premature senescence by down-regulating p16 expression via DNA methylation," *Cancer Letters*, vol. 321, no. 2, pp. 154–161, 2012.

[65] T. Tsutsumi, T. Suzuki, K. Moriya et al., "Hepatitis C virus core protein activates ERK and p38 MAPK in cooperation with ethanol in transgenic mice," *Hepatology*, vol. 38, no. 4, pp. 820–828, 2003.

[66] T. Tsutsumi, T. Suzuki, K. Moriya et al., "Alteration of intrahepatic cytokine expression and AP-1 activation in transgenic mice expressing hepatitis C virus core protein," *Virology*, vol. 304, no. 2, pp. 415–424, 2002.

[67] J. Liu, X. Ding, J. Tang, Y. Cao, P. Hu, F. Zhou et al., "Enhancement of canonical Wnt/β-catenin signaling activity by HCV core protein promotes cell growth of hepatocellular carcinoma cells," *PLoS One*, vol. 6, no. 11, Article ID e27496, 2011.

[68] S. Jahan, S. Khaliq, M. H. Siddiqi, B. Ijaz, W. Ahmad, U. A. Ashfaq et al., "Anti-apoptotic effect of HCV core gene of genotype 3a in Huh-7 cell line," *Virology Journal*, vol. 8, article 522, 2011.

[69] M. Ripoli, A. D'Aprile, G. Quarato et al., "Hepatitis C virus-linked mitochondrial dysfunction promotes hypoxia-inducible factor 1α-mediated glycolytic adaptation," *Journal of Virology*, vol. 84, no. 1, pp. 647–660, 2010.

[70] K. Watashi, M. Hijikata, A. Tagawa, T. Doi, H. Marusawa, and K. Shimotohno, "Modulation of retinoid signaling by a cytoplasmic viral protein via sequestration of sp110b, a potent transcriptional corepressor of retinoic acid receptor, from the nucleus," *Molecular and Cellular Biology*, vol. 23, no. 21, pp. 7498–7509, 2003.

[71] M. Morbitzer and T. Herget, "Expression of gastrointestinal glutathione peroxidase is inversely correlated to the presence of hepatitis C virus subgenomic RNA in human liver cells," *Journal of Biological Chemistry*, vol. 280, no. 10, pp. 8831–8841, 2005.

[72] N. Fujita, M. Kaito, M. Kai et al., "Effects of bezafibrate in patients with chronic hepatitis C virus infection: combination with interferon and ribavirin," *Journal of Viral Hepatitis*, vol. 13, no. 7, pp. 441–448, 2006.

[73] K. O. Chang and D. W. George, "Bile acids promote the expression of hepatitis C virus in replicon-harboring cells," *Journal of Virology*, vol. 81, no. 18, pp. 9633–9640, 2007.

[74] V. Pazienza, M. Vinciguerra, A. Andriulli, and A. Mangia, "Hepatitis C virus core protein genotype 3a increases SOCS-7 expression through PPAR-γ in Huh-7 cells," *Journal of General Virology*, vol. 91, no. 7, pp. 1678–1686, 2010.

[75] S. Dharancy, M. Malapel, G. Perlemuter et al., "Impaired expression of the peroxisome proliferator-activated receptor alpha during hepatitis C virus infection," *Gastroenterology*, vol. 128, no. 2, pp. 334–342, 2005.

[76] K. Yasui, Y. Harano, H. Mitsuyoshi et al., "Steatosis and hepatic expression of genes regulating lipid metabolism in Japanese patients infected with hepatitis C virus," *Journal of Gastroenterology*, vol. 45, no. 1, pp. 95–104, 2010.

[77] S. Dharancy, M. Lemoine, P. Mathurin, L. Serfaty, and L. Dubuquoy, "Peroxisome proliferator-activated receptors in HCV-related infection," *PPAR Research*, vol. 2009, Article ID 357204, 5 pages, 2009.

[78] Z. H. Li, Q. B. Tang, J. Wang et al., "Hepatitis C virus core protein induces malignant transformation of biliary epithelial cells by activating nuclear factor-κB pathway," *Journal of Gastroenterology and Hepatology*, vol. 25, no. 7, pp. 1315–1320, 2010.

[79] N. Tanaka, K. Moriya, K. Kiyosawa, K. Koike, F. J. Gonzalez, and T. Aoyama, "PPARα activation is essential for HCV core protein-induced hepatic steatosis and hepatocellular carcinoma in mice," *Journal of Clinical Investigation*, vol. 118, no. 2, pp. 683–694, 2008.

[80] N. Tanaka, K. Moriya, K. Kiyosawa, K. Koike, and T. Aoyama, "Hepatitis C virus core protein induces spontaneous and persistent activation of peroxisome proliferator-activated receptor α in transgenic mice: implications for HCV-associated hepatocarcinogenesis," *International Journal of Cancer*, vol. 122, no. 1, pp. 124–131, 2008.

[81] K. Koike, "Steatosis, liver injury, and hepatocarcinogenesis in hepatitis C viral infection," *Journal of Gastroenterology*, vol. 44, no. 19, pp. 82–88, 2009.

[82] B. Rakic, S. M. Sagan, M. Noestheden et al., "Peroxisome proliferator-activated receptor α antagonism inhibits hepatitis C virus replication," *Chemistry and Biology*, vol. 13, no. 1, pp. 23–30, 2006.

[83] Y. Nishimura-Sakurai, N. Sakamoto, K. Mogushi et al., "Comparison of HCV-associated gene expression and cell signaling pathways in cells with or without HCV replicon and in replicon-cured cells," *Journal of Gastroenterology*, vol. 45, no. 5, pp. 523–536, 2010.

[84] N. Fujita, M. Kaito, H. Tanaka, S. Horiike, and Y. Adachi, "Reduction of serum HCV RNA titer by bezafibrate therapy in patients with chronic hepatitis C," *American Journal of Gastroenterology*, vol. 99, no. 11, p. 2280, 2004.

[85] S. B. Kapadia and F. V. Chisari, "Hepatitis C virus RNA replication is regulated by host geranylgeranylation and fatty acids," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 7, pp. 2561–2566, 2005.

[86] C. Piccoli, G. Quarato, M. Ripoli et al., "HCV infection induces mitochondrial bioenergetic unbalance: causes and effects," *Biochimica et Biophysica Acta*, vol. 1787, no. 5, pp. 539–546, 2009.

[87] Y. Miyanari, K. Atsuzawa, N. Usuda et al., "The lipid droplet is an important organelle for hepatitis C virus production," *Nature Cell Biology*, vol. 9, no. 9, pp. 1089–1097, 2007.

[88] S. Boulant, M. W. Douglas, L. Moody, A. Budkowska, P. Targett-Adams, and J. McLauchlan, "Hepatitis C virus core

protein induces lipid droplet redistribution in a microtubule- and dynein-dependent manner," *Traffic*, vol. 9, no. 8, pp. 1268–1282, 2008.

[89] J. Goldwasser, P. Y. Cohen, W. Lin et al., "Naringenin inhibits the assembly and long-term production of infectious hepatitis C virus particles through a PPAR-mediated mechanism," *Journal of Hepatology*, vol. 55, pp. 963–971, 2011.

[90] K. H. Kim, S. P. Hong, K. Kim, M. J. Park, K. J. Kim, and J. Cheong, "HCV core protein induces hepatic lipid accumulation by activating SREBP1 and PPARγ," *Biochemical and Biophysical Research Communications*, vol. 355, no. 4, pp. 883–888, 2007.

[91] K. Kim, K. H. Kim, E. Ha, J. Y. Park, N. Sakamoto, and J. Cheong, "Hepatitis C virus NS5A protein increases hepatic lipid accumulation via induction of activation and expression of PPARgamma," *FEBS Letters*, vol. 583, no. 17, pp. 2720–2726, 2009.

[92] E. Lima-Cabello, M. V. García-Mediavilla, M. E. Miquilena-Colina et al., "Enhanced expression of pro-inflammatory mediators and liver X-receptor-regulated lipogenic genes in non-alcoholic fatty liver disease and hepatitis C," *Clinical Science*, vol. 120, no. 6, pp. 239–250, 2011.

[93] V. Pazienza, S. Clément, P. Pugnale et al., "The hepatitis C virus core protein of genotypes 3a and 1b downregulates insulin receptor substrate 1 through genotype-specific mechanisms," *Hepatology*, vol. 45, no. 5, pp. 1164–1171, 2007.

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Review Article

The Role of PPARγ in *Helicobacter pylori* Infection and Gastric Carcinogenesis

Jong-Min Lee, Sung Soo Kim, and Young-Seok Cho

Department of Internal Medicine, Uijeongbu St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Uijeongbu 480717, Republic of Korea

Correspondence should be addressed to Young-Seok Cho, yscho@catholic.ac.kr

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Peroxisome proliferator-activated receptor γ (PPARγ) is a nuclear receptor that is important in many physiological and pathological processes, such as lipid metabolism, insulin sensitivity, inflammation, cell proliferation, and carcinogenesis. Several studies have shown that PPARγ plays an important role in gastric mucosal injury due to *Helicobacter pylori* (*H. pylori*). As *H. pylori* infection is the main etiologic factor in chronic gastritis and gastric cancer, understanding of the potential roles of PPARγ in *H. pylori* infection may lead to the development of a therapeutic target. In this paper, the authors discuss the current knowledge on the role of PPARγ in *H. pylori* infection and its related gastric carcinogenesis.

1. Introduction

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors and members of the nuclear hormone receptor superfamily. To date, three isoforms of PPARs (PPARα, PPARδ/β, and PPARγ) have been identified in mammals. PPAR forms a heterodimer with its preferential binding partner—retinoid X receptor (RXR). The function of PPAR/RXR heterodimer depends on its interactions with cofactor complexes (coactivators or corepressors). After activation by ligand, the PPAR/RXR heterodimer binds to specific DNA response elements called peroxisome proliferator response elements (PPREs) of the target genes. This results in transcription regulation of these genes (Figure 1) [1]. PPARs play a significant role in regulation of fatty acid oxidation and glucose utilization [2]. PPARγ was originally identified as a differentiation transcription factor for adipose tissue [3]. In addition, PPARγ is involved in the control of inflammation and glucose metabolism and participates in the processes of cellular proliferation, differentiation, and apoptosis [4]. Natural ligands for PPARγ are 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) and various polyunsaturated fatty acids [5, 6]. The insulin sensitizing thiazolinediones, which are selective ligands of the nuclear transcription factor PPARγ, were the first drugs used to treat insulin resistance in patients with type II diabetes [7].

Helicobacter pylori (*H. pylori*) infection is the main etiologic agent in gastric inflammation, and longstanding infection with this organism is linked to gastric cancer [8]. Based on epidemiological studies, the risk of gastric cancer conferred by *H. pylori* has been estimated to be 75% [9]. Although the mechanism of *H. pylori*-induced carcinogenesis is still being investigated, inflammation is the strongest risk factor in the carcinogenic process [10], because it affects host responses such as epithelial cell proliferation and apoptosis [9]. PPARγ may be involved in the regulation of gene expression associated with inflammation and cancer. This paper reviews current knowledge of the role of PPARγ in *H. pylori* infection and its related gastric carcinogenesis.

2. PPARγ Expression in *H. pylori* Infection

PPARγ is predominantly expressed in adipose tissue, intestinal epithelium, monocytes and macrophages, the retina, skeletal muscle, and lymphoid organs [1]. Braissant et al. demonstrated PPARγ expression in the adult rat gastric mucosa by *in situ* hybridization and immunohistochemistry

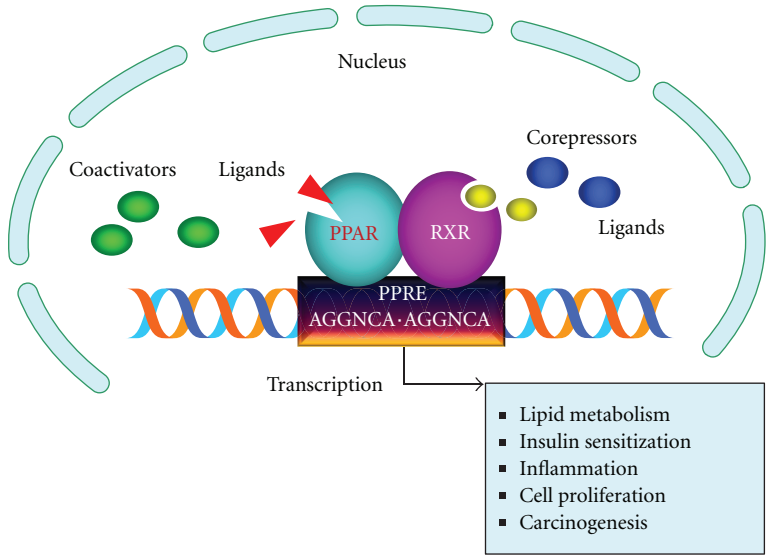

Figure 1: A basic mechanism of PPAR signaling. Following ligand binding, PPAR forms a heterodimer with RXR, which binds to the PPRE of target genes and regulates the transcription of these genes.

[11]. Several studies have found that PPARγ expression increases during *H. pylori* infection [12–14]. First, Konturek et al. demonstrated that PPARγ gene and protein expression were significantly higher in the gastric mucosa of *H. pylori*-positive gastric cancer patients than in *H. pylori*-negative healthy controls [12]. In addition, *H. pylori* eradication significantly reduced PPARγ expression. We demonstrated previously that PPARγ expression, identified by immunohistochemistry, was mostly detected in the nucleus of the foveolar epithelial cells in gastric mucosa and the intensity of PPARγ expression was significantly higher in the 18 patients with *H. pylori*-associated chronic gastritis than in the 21 *H. pylori*-negative patients (Figure 2) [13]. However, there was no correlation between the numbers of neutrophils and PPARγ expression in the two groups. Haruna et al. reported results similar to ours [14]. In this study, cyclooxygenase-2 (COX-2) and PPARγ mRNA expression in the gastric mucosa of children were found to be increased with *H. pylori* infection. The expression of COX-2, which plays an important role in inflammation, carcinogenesis, and development, is regulated by a negative feedback loop mediated through PPARγ [15]. Overexpression of both PPARγ and COX-2 was detected in the gastric mucosa of Mongolian gerbils infected with *H. pylori* [16]. Taking these findings together, enhanced PPARγ expression in gastric mucosa infected with *H. pylori* may have anti-inflammatory and cytoprotective effects.

3. The Role of PPARγ Activation in *H. pylori* Infection

Several studies have demonstrated that PPARγ has an overall anti-inflammatory effect [2, 17]. Molecular mechanisms include inhibition of signaling pathways regulating expression of proinflammatory genes (e.g., nuclear factor (NF)-κB) and stress-kinase pathways [17]. Gupta et al. demonstrated that PPARγ activation suppresses *H. pylori*-induced apoptosis in gastric epithelial cells and that this effect is mediated by direct inhibition of *H. pylori*-induced NF-κB activation [18]. *H. pylori* lipopolysaccharide (LPS), a component of the outer membrane, is a potent virulence factor for mucosal inflammatory changes, and its mechanism is mediated by increased proinflammatory cytokine production, excessive nitric oxide (NO) and PG generation, and epithelial cell apoptosis [19, 20]. B. L. Slomiany and A. Slomiany reported that PPARγ activation by ciglitazone inhibits gastric mucosal inflammation and that this effect is mediated by reduced apoptosis, mucosal PGE2 generation, expression of COX-2, and inducible nitric oxide synthase (NOS-2) [20]. In addition, ciglitazone impedes the inhibition of *H. pylori* LPS on gastric mucin synthesis, an effect likely dependent on the activation of the extracellular signal-related kinase (ERK) pathway by phosphatidylinositol 3-kinase (PI3K) [21]. These findings suggest that PPARγ activation may provide therapeutic benefits in *H. pylori*-associated gastric inflammation.

The transactivation of epidermal growth factor receptor (EGFR) is strongly linked to *H. pylori* infection, gastric epithelial hyperplasia, and gastric atrophy [10]. It depends on genes in the *cag* pathogenicity island, secreted proteins, and host factors such as TLR4 and NOD1 [22]. The biological responses to EGFR transactivation include increased proliferation, reduced apoptosis, altered cell polarity, and enhanced migration [10]. Although the underlying mechanism involved in the differential activation by ciglitazone of the EGFR/Erk mitogen activated protein kinases (MAPK) pathway is not well understood, this effect can be mediated by activation of Erk, an event requiring Src-kinase-dependent EGFR transactivation [23]. Ciglitazone has been shown to suppress *H. pylori* LPS inhibition of gastric mucin synthesis mediated by Src-kinase-dependent EGFR transactivation [24].

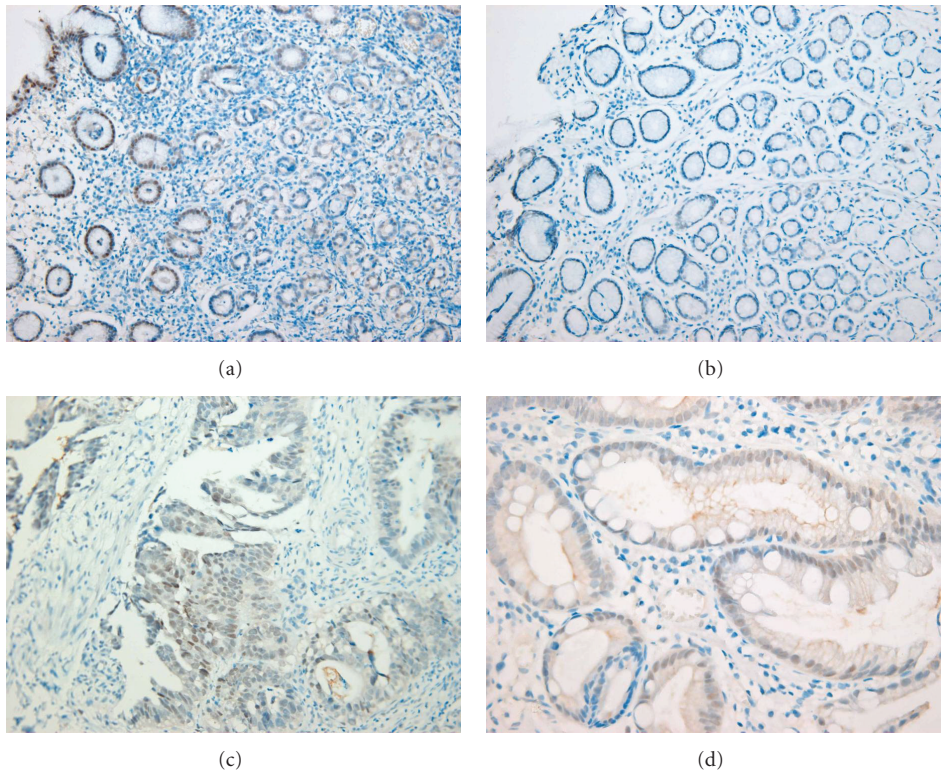

Figure 2: PPARγ protein expression in various tissues. Strong nuclear staining in gastric epithelial cells of *H. pylori*-infected patients (a) and weak nuclear staining in *H. pylori*-negative subjects (b). PPARγ protein is also expressed in the nucleus of tumor cells in gastric adenocarcinoma (c) and in noncancerous tissue with intestinal metaplasia adjacent to cancer tissue (d).

4. The Role of PPARγ in *H. pylori*-Related Gastric Carcinogenesis

Although the incidence of gastric adenocarcinoma is decreasing, it remains the second most common cause of cancer-related mortality worldwide [25]. There are large variations in the incidence and death rates among racial and ethnic groups, and the gastric cancer incidence and death rate are twice as high as in Asian American/Pacific islanders compared with Caucasians, reflecting an increased prevalence of chronic *H. pylori* infection [26]. Although gastric cancer treatments are continuously improving, the prognosis for this disease is poor and the survival rate is less than 40% even after curative resection and adjuvant chemotherapy [27].

Although the involvement of PPARγ in the development of cancer in various tissues remains controversial, PPARγ activation has antitumorigenic effects due to its antiproliferative and prodifferentiation activities [1]. Several *in vitro* studies have found that PPARγ activation results in cell cycle arrest and/or apoptosis of gastric cancer cells [28–32]. First, Takahashi et al. demonstrated that a human gastric cancer cell line, MKN45, expressed PPARγ mRNA and protein, and that PPARγ activation inhibited cell growth and induced apoptosis in gastric cancer cells [28]. Sato et al. used immunohistochemistry to show that the PPARγ protein is expressed in surgically resected specimens from well-, moderately, and poorly differentiated gastric adenocarcinomas as well as in noncancerous gastric mucosa with intestinal metaplasia [29]. The results of our study of PPARγ protein expression in gastric adenocarcinoma and normal mucosa with intestinal metaplasia adjacent to cancer were consistent with Sato's results (Figure 2) [13]. However, recent studies have reported that redistribution of PPARγ expression occurs in human gastric adenocarcinoma [33–35]. The immunohistochemical staining pattern of PPARγ is nuclear in the normal gastric mucosa but primarily cytoplasmic in intestinal metaplasia (IM) [33]. The high cytoplasmic-to-nuclear expression ratio of PPARγ decreases as the differentiation stage changes from IM to adenoma, and to well-, moderately-, and poorly-differentiated cancers. PPARγ is expressed primarily in the nucleus in metastatic gastric cancer [34]. Burgermeister et al. demonstrated that the molecular mechanisms of PPARγ redistribution include interaction with Ras/mitogen activated protein kinases (MAPKs) such as caveolin-1 (Cav1) and docking protein 1 (Dok1) [35].

The inhibitory effect of PPARγ on gastric cancer may be attributed to various mechanisms. Ligand-induced activation of PPARγ was found to inhibit c-MET (an important protooncogene-encoding receptor for hepatocyte growth factor) [36] and expression of cyclin D1 [37] and COX-2 [31], induce expression of p27 [38], p21 [32], and p53 [39], and suppress gastrin (a potent cancer cell growth promoting

factor) [40]. An *in vivo* animal study determined that heterozygous (PPAR$\gamma^{+/-}$) knockout mice were more susceptible to N-methyl-N-nitrosourea-induced gastric carcinoma than homozygotes (PPAR$\gamma^{+/+}$), but troglitazone only reduced the incidence of gastric cancer in homozygotes [41].

Konturek et al. reported that expression of tissue PPARγ, tissue levels of proinflammatory cytokines (IL-1β and IL-8), and plasma gastrin concentrations were significantly higher in *H. pylori*-positive gastric cancer compared to *H. pylori*-negative controls, but *H. pylori* eradication reduced these parameters [12]. These findings suggest that these parameters could be implicated in *H. pylori*-related gastric carcinogenesis. An *in vitro* study found that *H. pylori* infection downregulates the expression of p27 in gastric epithelial cells even in the absence of inflammation [42]. Reduced expression of p27 is found in *H. pylori*-associated intestinal metaplasia and *H. pylori* eradication reverses the aberrant expression of p27 [43]. Low p27 protein expression has been reported to be associated with increased expression of p27-specific F-box protein Skp2 and *H. pylori* eradication reverses the aberrant expression of p27 and Skp2 protein [44]. However, p27 and Skp2 mRNA levels were unaffected by *H. pylori* eradication, suggesting that *H. pylori* may influence cell cycle progression and carcinogenesis post-translational effects on specific gene expression. We have shown that rosiglitazone inhibited the growth of *H. pylori*-infected gastric epithelial cells [45]. These effects of rosiglitazone were associated with decreased Skp2 expression, thereby promoting p27 accumulation in *H. pylori*-infected gastric epithelial cells.

5. PPARγ Polymorphism in *H. pylori*-Related Gastric Carcinogenesis

A common PPARγ polymorphism, a C to G substitution in exon B, results in a proline to alanine exchange at codon 12 (Pro12Ala) [46]. Functionally, this Ala variant has been reported to show decreased binding to the response element and a lower capacity for activating target genes [47]. PPARγ polymorphism (Pro12Ala) has been found to be associated with various diseases including type II diabetes, cardiovascular disease, and several types of cancer [48]. Pro12Ala polymorphism lowers the risk of diseases in colorectal cancer and type II diabetes [49]. These results could be partly explained by the etiological link between type II diabetes and colorectal cancer. On the contrary, several studies have demonstrated that the Pro12Ala polymorphism is associated with the high risk of gastric adenocarcinoma [48, 50–53]. Liao et al. reported that the carriage of G phenotype or Ala allele in codon 12 of PPARγ was associated with a 2.5-fold increase in the risk of noncardia gastric cancer in Chinese, and that the risk was higher when this polymorphism was combined with *H. pylori* infection [50]. A recent meta-analysis suggested that carriers of Pro12Ala have a 2.31-fold (95% CI = 1.59–3.36, $P_{\text{heterogeneity}} = 0.941$) increased gastric cancer risk [48]. Considering that PPARγ activation inhibits the growth of gastric cancer cells, these results suggest that gastric carcinogenesis may have a different genetic background than colorectal carcinogenesis.

The role of Pro12Ala polymorphism in peptic ulcer disease remains controversial. Prasad et al. showed that patients with Pro12Ala polymorphism were susceptible to peptic ulcer disease in the presence of *H. pylori* infection [52]. Meanwhile, Bazargani et al. found no significant increase in the risk of peptic ulcer formation among patients with Pro12Ala polymorphism [53]. This discrepancy may be due to the role of other bacterial virulence factors and/or host factors.

6. Conclusions

In this paper, we focused on the role of PPARγ in *H. pylori* infection and its related gastric carcinogenesis. PPARγ suppresses inflammation in *H. pylori* infection and tumor growth in gastric cancer. Emerging evidence indicates that mutations in PPARγ may play a crucial role in the development of noncardia gastric cancer in *H. pylori*-infected patients. Therefore, further studies are needed to investigate modulation of PPARγ as an effective therapy for chemoprevention and treatment of inflammation in *H. pylori* infection and gastric cancer.

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References

[1] L. Michalik, B. Desvergne, and W. Wahli, "Peroxisome-proliferator-activated receptors and cancers: complex stories," *Nature Reviews Cancer*, vol. 4, no. 1, pp. 61–70, 2004.

[2] S. Kersten, B. Desvergne, and W. Wahli, "Roles of PPARS in health and disease," *Nature*, vol. 405, no. 6785, pp. 421–424, 2000.

[3] P. Tontonoz, E. Hu, and B. M. Spiegelman, "Stimulation of adipogenesis in fibroblasts by PPARγ2, a lipid-activated transcription factor," *Cell*, vol. 79, no. 7, pp. 1147–1156, 1994.

[4] P. Delerive, J. C. Fruchart, and B. Staels, "Peroxisome proliferator-activated receptors in inflammation control," *Journal of Endocrinology*, vol. 169, no. 3, pp. 453–459, 2001.

[5] B. M. Forman, P. Tontonoz, J. Chen, R. P. Brun, B. M. Spiegelman, and R. M. Evans, "15-Deoxy-Δ12, 14-prostaglandin J2 is a ligand for the adipocyte determination factor PPARγ," *Cell*, vol. 83, no. 5, pp. 803–812, 1995.

[6] S. A. Kliewer, J. M. Lenhard, T. M. Willson, I. Patel, D. C. Morris, and J. M. Lehmann, "A prostaglandin J2 metabolite binds peroxisome proliferator-activated receptor γ and promotes adipocyte differentiation," *Cell*, vol. 83, no. 5, pp. 813–819, 1995.

[7] J. M. Lehmann, L. B. Moore, T. A. Smith-Oliver, W. O. Wilkison, T. M. Willson, and S. A. Kliewer, "An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ (PPARγ)," *The Journal of Biological Chemistry*, vol. 270, no. 22, pp. 12953–12956, 1995.

[8] R. M. Peek Jr. and M. J. Blaser, "*Helicobacter pylori* and gastrointestinal tract adenocarcinomas," *Nature Reviews Cancer*, vol. 2, no. 1, pp. 28–37, 2002.

[9] V. Herrera and J. Parsonnet, "*Helicobacter pylori* and gastric adenocarcinoma," *Clinical Microbiology and Infection*, vol. 15, no. 11, pp. 971–976, 2009.

[10] D. B. Polk and R. M. Peek Jr., "*Helicobacter pylori*: gastric cancer and beyond," *Nature Reviews Cancer*, vol. 10, no. 6, pp. 403–414, 2010.

[11] O. Braissant, F. Foufelle, C. Scotto, M. Dauça, and W. Wahli, "Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-α, -β, and -γ in the adult rat," *Endocrinology*, vol. 137, no. 1, pp. 354–366, 1996.

[12] P. C. Konturek, J. Kania, V. Kukharsky et al., "Implication of peroxisome proliferator-activated receptor γ and proinflammatory cytokines in gastric carcinogenesis: link to *Helicobacter pylori*-infection," *Journal of Pharmacological Sciences*, vol. 96, no. 2, pp. 134–143, 2004.

[13] S. H. Son, H. K. Kim, J. S. Ji et al., "Expression of peroxisome proliferator-activated receptor (PPAR) gamma in *Helicobacter pylori*-infected gastric epithelium," *The Korean Journal of Gastroenterology*, vol. 49, no. 2, pp. 72–78, 2007, in Korean.

[14] H. Haruna, T. Shimizu, Y. Ohtsuka et al., "Expression of COX-1, COX-2, and PPAR-γ in the gastric mucosa of children with *Helicobacter pylori* infection," *Pediatrics International*, vol. 50, no. 1, pp. 1–6, 2008.

[15] H. Inoue, T. Tanabe, and K. Umesono, "Feedback control of cyclooxygenase-2 expression through PPARγ," *The Journal of Biological Chemistry*, vol. 275, no. 36, pp. 28028–28032, 2000.

[16] P. C. Konturek, J. Kania, J. W. Konturek, A. Nikiforuk, S. J. Konturek, and E. G. Hahn, "*H. pylori* infection, atrophic gastritis, cytokines, gastrin, COX-2, PPARγ and impaired apoptosis in gastric carcinogenesis," *Medical Science Monitor*, vol. 9, no. 7, pp. SR53–SR66, 2003.

[17] L. Dubuquoy, S. Dharancy, S. Nutten, S. Pettersson, J. Auwerx, and P. Desreumaux, "Role of peroxisome proliferator-activated receptor γ and retinoid X receptor heterodimer in hepatogastroenterological diseases," *The Lancet*, vol. 360, no. 9343, pp. 1410–1418, 2002.

[18] R. A. Gupta, D. B. Polk, U. Krishna et al., "Activation of peroxisome proliferator-activated receptor γ suppresses nuclear factor κB-mediated apoptosis induced by *Helicobacter pylori* in gastric epithelial cells," *The Journal of Biological Chemistry*, vol. 276, no. 33, pp. 31059–31066, 2001.

[19] J. Piotrowski, H. Piotrowski, D. Skrodzka, A. Slomiany, and B. L. Slomiany, "Induction of acute gastritis and epithelial apoptosis by *Helicobacter pylori* lipopolysaccharide," *Scandinavian Journal of Gastroenterology*, vol. 32, no. 3, pp. 203–211, 1997.

[20] B. L. Slomiany and A. Slomiany, "Suppression of gastric mucosal inflammatory responses to *Helicobacter pylori* lipopolysaccharide by peroxisome proliferator-activated receptor γ activation," *IUBMB Life*, vol. 53, no. 6, pp. 303–308, 2002.

[21] B. L. Slomiany and A. Slomiany, "Impedance of *Helicobacter pylori* lipopolysaccharide interference with gastric mucin synthesis by peroxisome proliferator-activated receptor γ activation involves phosphatidylinositol 3-kinase/ERK pathway," *IUBMB Life*, vol. 55, no. 2, pp. 97–102, 2003.

[22] S. Basu, S. K. Pathak, G. Chatterjee, S. Pathak, J. Basu, and M. Kundu, "*Helicobacter pylori* protein HP0175 transactivates epidermal growth factor receptor through TLR4 in gastric epithelial cells," *The Journal of Biological Chemistry*, vol. 283, no. 47, pp. 32369–32376, 2008.

[23] O. S. Gardner, B. J. Dewar, H. S. Earp, J. M. Samet, and L. M. Graves, "Dependence of peroxisome proliferator-activated receptor ligand-induced mitogen-activated protein kinase signaling on epidermal growth factor receptor transactivation," *The Journal of Biological Chemistry*, vol. 278, no. 47, pp. 46261–46269, 2003.

[24] B. L. Slomiany and A. Slomiany, "Role of epidermal growth factor receptor transactivation in PPARγ-dependent suppression of *Helicobacter pylori* interference with gastric mucin synthesis," *Inflammopharmacology*, vol. 12, no. 2, pp. 177–188, 2004.

[25] K. D. Crew and A. I. Neugut, "Epidemiology of gastric cancer," *World Journal of Gastroenterology*, vol. 12, no. 3, pp. 354–362, 2006.

[26] A. Jemal, R. Siegel, J. Xu, and E. Ward, "Cancer statistics, 2010," *CA: A Cancer Journal for Clinicians*, vol. 60, no. 5, pp. 277–300, 2010.

[27] J. S. Macdonald, S. R. Smalley, J. Benedetti et al., "Chemoradiotherapy after surgery compared with surgery alone for adenocarcinoma of the stomach or gastroesophageal junction," *The New England Journal of Medicine*, vol. 345, no. 10, pp. 725–730, 2001.

[28] N. Takahashi, T. Okumura, W. Motomura, Y. Fujimoto, I. Kawabata, and Y. Kohgo, "Activation of PPARγ inhibits cell growth and induces apoptosis in human gastric cancer cells," *FEBS Letters*, vol. 455, no. 1-2, pp. 135–139, 1999.

[29] H. Sato, S. Ishihara, K. Kawashima et al., "Expression of peroxisome proliferator-activated receptor (PPAR)γ in gastric cancer and inhibitory effects of PPARγ agonists," *British Journal of Cancer*, vol. 83, no. 10, pp. 1394–1400, 2000.

[30] Y.-X. Chen, X.-Y. Zhong, Y.-F. Qin, W. Bing, and L.-Z. He, "15d-PGJ_2 inhibits cell growth and induces apoptosis of MCG-803 human gastric cancer cell line," *World Journal of Gastroenterology*, vol. 9, no. 10, pp. 2149–2153, 2003.

[31] W. K. Leung, A. H. C. Bai, V. Y. W. Chan et al., "Effect of peroxisome proliferator activated receptor γ ligands on growth and gene expression profiles of gastric cancer cells," *Gut*, vol. 53, no. 3, pp. 331–338, 2004.

[32] C. W. Cheon, D. H. Kim, D. H. Kim, Y. H. Cho, and J. H. Kim, "Effects of ciglitazone and troglitazone on the proliferation of human stomach cancer cells," *World Journal of Gastroenterology*, vol. 15, no. 3, pp. 310–320, 2009.

[33] S. Nomura, A. Nakajima, S. Ishimine, N. Matsuhashi, T. Kadowaki, and M. Kaminishi, "Differential expression of peroxisome proliferator-activated receptor in histologically different human gastric cancer tissues," *Journal of Experimental and Clinical Cancer Research*, vol. 25, no. 3, pp. 443–448, 2006.

[34] Q. He, J. Chen, H.-L. Lin, P.-J. Hu, and M.-H. Chen, "Expression of peroxisome proliferator-activated receptor γ, E-cadherin and matrix metalloproteinases-2 in gastric carcinoma and lymph node metastases," *Chinese Medical Journal*, vol. 120, no. 17, pp. 1498–1504, 2007.

[35] E. Burgermeister, T. Friedrich, I. Hitkova et al., "The Ras inhibitors caveolin-1 and docking protein 1 activate peroxisome proliferator-activated receptor γ through spatial relocalization at helix 7 of its ligand-binding domain," *Molecular and Cellular Biology*, vol. 31, no. 16, pp. 3497–3510, 2011.

[36] S. Kitamura, Y. Miyazaki, S. Hiraoka et al., "PPARγ inhibits the expression of c-MET in human gastric cancer cells through the suppression of Ets," *Biochemical and Biophysical Research Communications*, vol. 265, no. 2, pp. 453–456, 1999.

[37] K. Yoshida, K. Tanabe, D. Fujii, N. Oue, W. Yasui, and T. Toge, "Induction mechanism of apoptosis by troglitazone through peroxisome proliferator-activated receptor-γ in gastric carcinoma cells," *Anticancer Research*, vol. 23, no. 1, pp. 267–273, 2003.

[38] S. Takeuchi, T. Okumura, W. Motomura, M. Nagamine, N. Takahashi, and Y. Kohgo, "Troglitazone induces G1 arrest by $p27^{Kip1}$ induction that is mediated by inhibition of proteasome in human gastric cancer cells," *Japanese Journal of Cancer Research*, vol. 93, no. 7, pp. 774–782, 2002.

[39] M. Nagamine, T. Okumura, S. Tanno et al., "PPARγ ligand-induced apoptosis through a p53-dependent mechanism in human gastric cancer cells," *Cancer Science*, vol. 94, no. 4, pp. 338–343, 2003.

[40] P. C. Konturek, J. Kania, V. Kukharsky, S. Ocker, E. G. Hahn, and S. J. Konturek, "Influence of gastrin on the expression of cyclooxygenase-2, hepatocyte growth factor and apoptosis-related proteins in gastric epithelial cells," *Journal of Physiology and Pharmacology*, vol. 54, no. 1, pp. 17–32, 2003.

[41] J. Lu, K. Imamura, S. Nomura et al., "Chemopreventive effect of peroxisome proliferator-activated receptor γ on gastric carcinogenesis in mice," *Cancer Research*, vol. 65, no. 11, pp. 4769–4774, 2005.

[42] H. Shirin, E. M. Sordillo, T. K. Kolevska et al., "Chronic *Helicobacter pylori* infection induces an apoptosis-resistant phenotype associated with decreased expression of $p27^{kip1}$," *Infection and Immunity*, vol. 68, no. 9, pp. 5321–5328, 2000.

[43] J. Yu, W. K. Leung, E. K. W. Ng et al., "Effect of *Helicobacter pylori* eradication on expression of cyclin D2 and p27 in gastric intestinal metaplasia," *Alimentary Pharmacology and Therapeutics*, vol. 15, no. 9, pp. 1505–1511, 2001.

[44] S. S. Kim, P. Meitner, T. A. Konkin, Y. S. Cho, M. B. Resnick, and S. F. Moss, "Altered expression of Skp2, c-Myc and p27 proteins but not mRNA after *H. pylori* eradication in chronic gastritis," *Modern Pathology*, vol. 19, no. 1, pp. 49–58, 2006.

[45] S. S. Kim, Y. S. Cho, H. K. Kim et al., "The effect of rosiglitazone on the cell proliferation and the expressions of p27 and skp2 in *Helicobacter pylori* infected human gastric epithelial cells," *The Korean Journal of Gastroenterology*, vol. 55, no. 4, pp. 225–231, 2010, in Korean.

[46] Y. Chung-Jen, B. A. Beamer, C. Negri et al., "Molecular scanning of the human peroxisome proliferator activated receptor γ (hPPARγ) gene in diabetic Caucasians: identification of a Pro12Ala PPARγ2 missense mutation," *Biochemical and Biophysical Research Communications*, vol. 241, no. 2, pp. 270–274, 1997.

[47] S. S. Deeb, L. Fajas, M. Nemoto et al., "A Pro12Ala substitution in PPARγ2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity," *Nature Genetics*, vol. 20, no. 3, pp. 284–287, 1998.

[48] W. Xu, Y. Li, X. Wang et al., "PPARγ polymorphisms and cancer risk: a meta-analysis involving 32,138 subjects," *Oncology Reports*, vol. 24, no. 2, pp. 579–585, 2010.

[49] W.-P. Koh, J. M. Yuan, D. Van Den Berg, S. A. Ingles, and M. C. Yu, "Peroxisome proliferator-activated receptor (PPAR) γ gene polymorphisms and colorectal cancer risk among Chinese in Singapore," *Carcinogenesis*, vol. 27, no. 9, pp. 1797–1802, 2006.

[50] S.-Y. Liao, Z.-R. Zeng, W. K. Leung et al., "Peroxisome proliferator-activated receptor-gamma Pro12Ala polymorphism, *Helicobacter pylori* infection and non-cardia gastric carcinoma in Chinese," *Alimentary Pharmacology and Therapeutics*, vol. 23, no. 2, pp. 289–294, 2006.

[51] T. Tahara, T. Arisawa, T. Shibata et al., "Influence of peroxisome proliferator-activated receptor (PPAR)γ Plo12Ala polymorphism as a shared risk marker for both gastric cancer and impaired fasting glucose (IFG) in Japanese," *Digestive Diseases and Sciences*, vol. 53, no. 3, pp. 614–621, 2008.

[52] K. N. Prasad, A. Saxena, U. C. Ghoshal, M. R. Bhagat, and N. Krishnani, "Analysis of Pro12Ala PPAR gamma polymorphism and *Helicobacter pylori* infection in gastric adenocarcinoma and peptic ulcer disease," *Annals of Oncology*, vol. 19, no. 7, pp. 1299–1303, 2008.

[53] A. Bazargani, S. S. Khoramrooz, E. Kamali-Sarvestani, S. A. Taghavi, and M. Saberifiroozi, "Association between peroxisome proliferator-activated receptor-γ gene polymorphism (Pro12Ala) and *Helicobacter pylori* infection in gastric carcinogenesis," *Scandinavian Journal of Gastroenterology*, vol. 45, no. 10, pp. 1162–1167, 2010.

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Research Article

Correlations among PPARγ, DNMT1, and DNMT3B Expression Levels and Pancreatic Cancer

Valerio Pazienza,[1] Francesca Tavano,[2] Giorgia Benegiamo,[1] Manlio Vinciguerra,[3] Francesca Paola Burbaci,[2] Massimiliano Copetti,[4] Fabio Francesco di Mola,[2] Angelo Andriulli,[1] and Pierluigi di Sebastiano[2]

[1] *Division and Laboratory of Gastroenterology Unit, "Casa Sollievo della Sofferenza" IRCCS Hospital, Viale dei Cappuccini n.1, 71013 San Giovanni Rotondo, Italy*
[2] *Division of Abdominal Surgery, "Casa Sollievo della Sofferenza" IRCCS Hospital, 71013 San Giovanni Rotondo, Italy*
[3] *The Foundation for Liver Research, c/o The Institute of Hepatology, 69-75 Chenies Mews, London WC1E 6HX, UK*
[4] *Biostatistic Unit, "Casa Sollievo della Sofferenza" IRCCS Hospital, 71013 San Giovanni Rotondo, Italy*

Correspondence should be addressed to Valerio Pazienza, v.pazienza@operapadrepio.it

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Emerging evidence indicates that peroxisome proliferator-activated receptor γ (PPARγ) and DNA methyltransferases (DNMTs) play a role in carcinogenesis. In this study we aimed to evaluate the expression of PPARγ, DNMT1, and DNMT3B and their correlation with clinical-pathological features in patients with pancreatic cancer (PC), and to define the effect of PPARγ activation on DNMTs expression in PC cell lines. qRT-PCR analysis showed that DNMT3B expression was downregulated in tumors compared to normal tissues ($P = 0.03$), whereas PPARγ and DNMT1 levels did not show significant alterations in PC patients. Expression levels between PPARγ and DNMT1 and between DNMT1 and DNMT3B were highly correlated ($P = 0.008$ and $P = 0.05$ resp.). DNMT3B overexpression in tumor tissue was positively correlated with both lymph nodes spreading ($P = 0.046$) and resection margin status ($P = 0.04$), and a borderline association with perineural invasion ($P = 0.06$) was found. Furthermore, high levels of DNMT3B expression were significantly associated with a lower mortality in the whole population (HR = 0.485; 95% CI = 0.262–0.895, $P = 0.02$) and in the subgroup of patients without perineural invasion (HR = 0.314; 95%CI = 0.130–0.758; $P = 0.01$), while such association was not observed in patients with tumor invasion into perineural structures ($P = 0.70$). In conclusion, *in vitro* and *in vivo* PPARγ and DNMTs appear interrelated in PC, and this interaction might influence cell phenotype and disease behavior.

1. Introduction

Pancreatic cancer (PC) is ranked as the fourth leading cause of cancer-related deaths worldwide [1, 2]. It is highly aggressive and resistant to chemotherapy, and our inability to detect it at an early stage and the lack of effective systemic therapies are responsible for nearly identical incidence and mortality rates [3, 4]. More effective treatments and/or development of novel strategies are needed to improve the prognosis for patients with PC.

The peroxisome proliferator-activated receptors (PPARs) belong to the nuclear receptor superfamily and are considered master regulators of lipid and glucose metabolism by transducing metabolic and nutritional signals into transcriptional responses [5, 6]. Three subtypes of PPARs are known: PPARα, PPARδ, and PPARγ [7]. The latter has been implicated in the pathology of numerous diseases including obesity, diabetes, atherosclerosis, and cancer. PPARγ ligands induce differentiation of liposarcoma cells and have a variety of antitumor effects also in pancreatic cancer cells [8]. The availability of such high-affinity ligands has facilitated the study of the signalling pathways through which PPARγ regulates metabolic processes, which are regulated also by epigenetic events. The mechanisms underlying epigenetic modulation mediated by PPARs remain to be fully explored. DNA methyltransferases (DNMTs) are critical in epigenetic

Table 1: Clinical and pathological features of patients with pancreatic ductal adenocarcinoma (PDAC).

	PDAC $n = 30$
Age at diagnosis, median (*Q*1–*Q*3)	69 (42–81)
Duration of followup, median (*Q*1–*Q*3)	18.48 (8.16–53.11)
Gender, male/female (% male)	25/5 (83)
Tumour localization, *n* (%)	
Head	28 (93)
Body-tail	2 (7)
Tumour type, *n* (%)	
Adenocarcinoma	24 (80)
Adenocarcinoma mucinous	6 (20)
Tumour grading, *n* (%)	
G1: well differentiated	4 (13)
G2: moderately differentiated	16 (54)
G3: poorly differentiated	10 (33)
T: tumour size, *n* (%)	
T2	5 (17)
T3	25 (83)
N: regional lymph nodes, *n* (%)	
N0	7 (23)
N1	23 (77)
Lymph nodes ratio, median (*Q*1–*Q*3)	0.13 (0.00–0.80)
Tumour stage, *n* (%)	
IIA	7 (23)
IIB	23 (77)
Perineural invasion, *y*/*n* (%*y*)	9/21 (30)
Margins of resection , *n* (%)	
*R*0: negative rection margins	22 (27)
*R*1: microscopic positive resection margins	8 (73)

Pancreatic cancer staging. "Exocrine and endocrine pancreas," in [25].

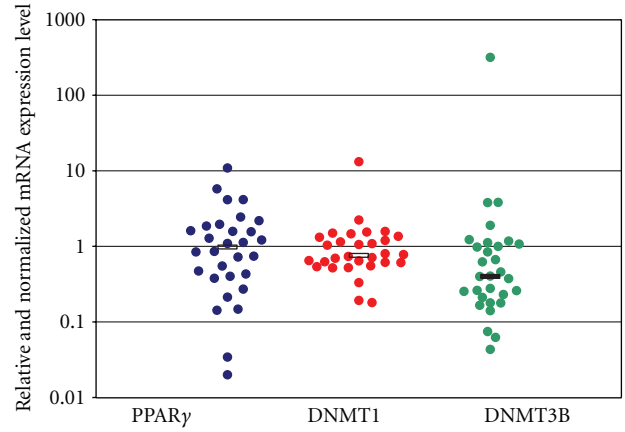

Figure 1: PPARγ, DNMT1, and DNMT3b mRNA expression levels in tissues from 30 patients with pancreatic ductal Adenocarcinoma (PDAC). Each blot indicates the relative expression of genes in tumour compared to normal tissue, after normalization to the endogenous GAPDH. Values greater than 1 indicate gene overexpression in inflamed tissue. For each gene, the median expression levels observed in patients group were indicated by the horizontal black bars. Relative expression values are reported in log scale (y-axis).

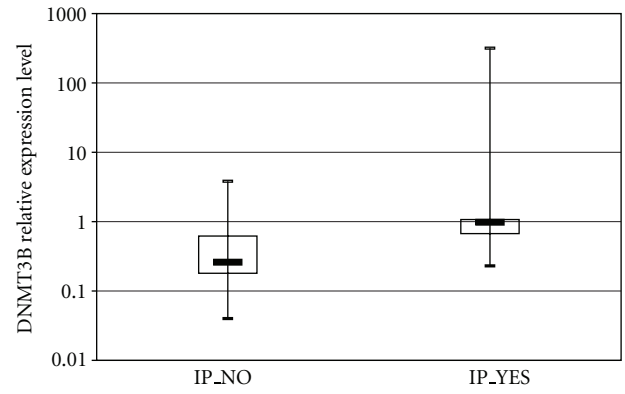

Figure 2: Association of DNMT3b expression levels with perineural invasion (IP) in patients with pancreatic ductal adenocarcinoma (PDAC). Patients were stratified according to IP status (No versus Yes). Each box highlights median, interquartile range (*Q*1–*Q*3) and lower and upper adjacent values (vertical bars) for each subjects group. The upper and lower boundaries of the boxes define the quartiles, 75% and 25% percentiles, respectively, and the black bar represents the median value. Relative expression values are reported in log scale (y-axis).

events through the addition of methyl groups to DNA [9, 10]. Maintenance of methylation pattern is achieved by DNMT1 function [11] during DNA replication while new or *de novo* methylation is primarily catalyzed by DNMT3a and DNMT3b [12]. Whether and how PPARs modulate epigenetic events remain to be fully explored. In this paper, we sought to examine mRNA levels of PPARγ and DNMT1 and 3B in a cohort of PC patients and to correlate the findings with clinical-pathologic features, including patient survival, and to evaluate whether pharmacological modulation of PPARγ could influence the expression of DNMTs in PC cell lines.

2. Material and Methods

2.1. Patients and Tissues Samples Preparation. A cohort of 30 matched pairs of tumour and adjacent normal tissue samples were collected from patients undergoing pancreatic resection at the Department of Surgery, "Casa Sollievo della Sofferenza" Hospital, IRCCS, San Giovanni Rotondo, Italy between October 2007 and June 2011. Written informed consent was obtained before collection of tissues from patients. The final diagnosis of pancreatic ductal adenocarcinoma was ascertained in all patients by histological examinations. At the last followup, 18 (60%) patients were still alive and 12 (40%) patients had died. Demographics and clinical characteristics of patients are shown in Table 1.

Tissue specimens were immediately frozen in liquid nitrogen, and stored at −80°C until RNA extraction. Cancer

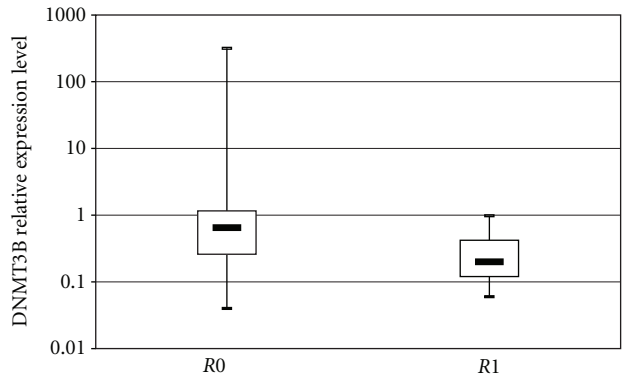

Figure 3: Association of DNMT3b expression levels with resection margins in patients with pancreatic ductal adenocarcinoma (PDAC). Patients were stratified according to resection margin status (*R*0 versus *R*1). Each box highlights median, interquartile range (*Q*1–*Q*3) and lower and upper adjacent values (vertical bars) for each subjects group. The upper and lower boundaries of the boxes define the quartiles, 75% and 25% percentiles, respectively, and the black bar represents the median value. Relative expression values are reported in log scale (*y*-axis).

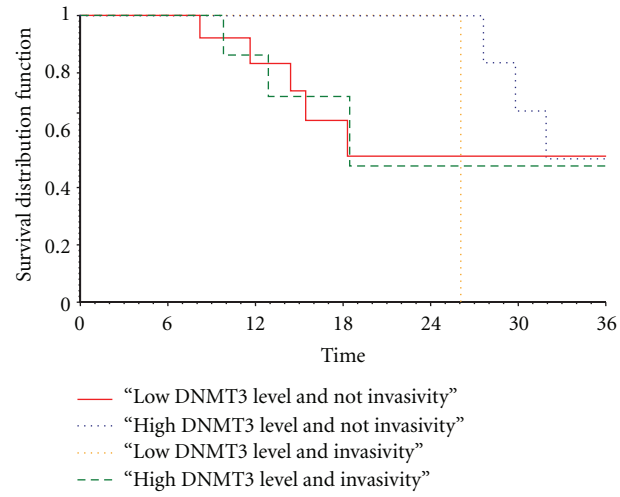

Figure 4

cellularity was enriched by cryostat sectioning and dissection of most cellular areas.

2.2. Cell Culture and Treatment. BxPC3, CF-PAC, MiaPaca, and Panc1 cells were cultured at 37°C in 5% CO_2 atmosphere in DMEM medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 ng/mL streptomycin (Invitrogen Life Technologies, Milan, Italy) while CFPAC and MiaPaca were maintained in RPMI medium (Invitrogen Life Technologies, Milan, Italy). Treatment with rosiglitazone (purchased from Cayman Chemicals) was performed at different time points (24 hours and 48 hours) and at different concentration (5 μM and 15 μM).

2.3. Quantitative Real-Time PCR (qRT-PCR). Total RNA was extracted from 30 PC fresh frozen specimens and from different pancreatic cancer cells (BxPC3, CFPAC, MiaPaca, and Panc1) using the RNeasy Mini Kit (Qiagen S.P.A. Milano Italy) and subsequently digested by DNase I. cDNA was synthesized from 50 ng total RNA and quantitative real-time PCR was performed using QuantiFast Sybr Green PCR kit following the one-step protocol. For real-time RT-PCR, we used the following SYBR Green QuantiTect Primer purchased from Qiagen: human PPARγ (QT00029841) DNMT1 (QT00034335) and DNMT3B (QT00032067). Reactions were set up in 96-well plates using a 7700 real-time PCR System (Applied Biosystems, Foster City, CA) and all samples were assayed in triplicate. Optical data obtained were analyzed using the default and variable parameters available in the SDS software package (version 1.9.1; Applied Biosystems, Foster City, CA). Expression levels of target gene were normalized using the housekeeping control genes: TATA binding protein (TBP, QT00000721) as previously performed [14].

2.4. Statistics. Demographic, clinical, and genetic characteristics were reported as median and interquartile range (*Q*1–*Q*3). Gene expression up- or downregulation was tested by using the one-sample Wilcoxon signed-rank test. Group comparisons were performed using the Pearson, chi-square test and the Mann-Whitney *U* test for categorical and continuous variables, respectively. Correlations between continuous variables were assessed using the *r* Spearman coefficient. Time-to-death analyses were performed using Cox proportional hazards regression models and risks were reported as hazards ratios (HR) along with their 95% confidence intervals (95% CI). Overall survival was defined as the time elapsed between surgery and death. For subjects who did not experience the event, time variable was censored at the time of the last available follow-up time. In the time to death analysis, genes' expressions were logarithm-transformed to respect Cox model's linearity assumption. Kaplan-Meier curves were also reported for display purposes. All statistical analyses were performed using SAS version 9.1.3 (SAS Institute, Cary, NC, USA). As for cell experiments, results were expressed as means ± SD. For statistical comparison, significance was evaluated using the Student *t* test. Values of $P < 0.05$(*) and $P < 0.005$(**) or $P < 0.001$ (***) were considered statistically significant.

3. Results

3.1. PPARγ, DNMT1 and 3B Expression in Pancreatic Cancer Biopsies. Relative expression levels of PPARγ, DNMT1, and DNMT3B mRNA in tissue samples from 30 PC patients are presented in Figure 1. Looking at median levels of gene expression in tumor compared to adjacent nontumor tissues, the DNMT3B mRNA expression was downregulated (median = 0.4, *Q*1–*Q*3 = 0.22–1.05, $P = 0.03$), while PPARγ and DNMT1 mRNA levels were not significantly altered (PPARγ: median = 0.98, *Q*1–*Q*3 = 0.41–1.8, $P = 0.54$; DNMT1: median = 0.76; *Q*1–*Q*3 = 0.61–1.29, $P = 0.4$).

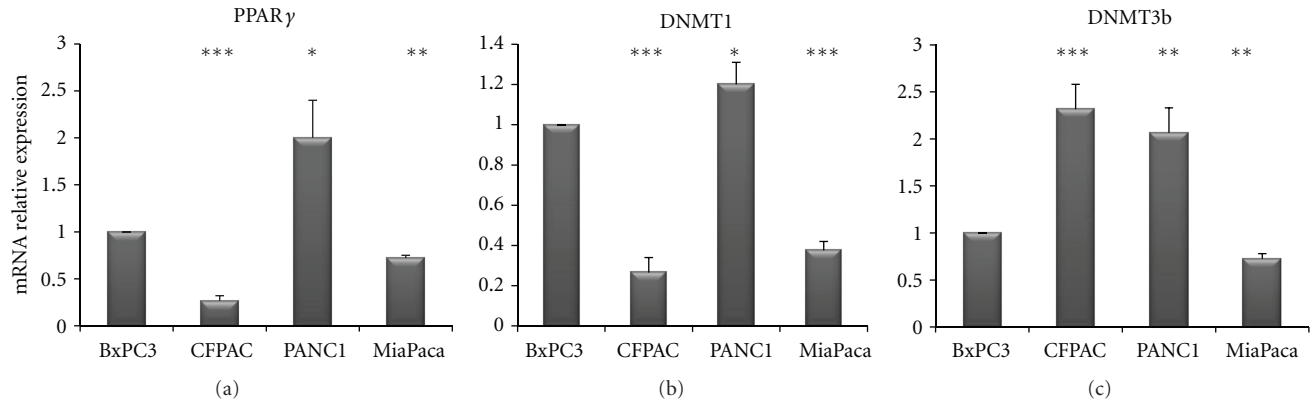

Figure 5: Quantitative real-time PCR. mRNA relative expression levels of PPARγ, DNMT1, and 3B in four different pancreatic cancer cell lines.

Analysis of the association among PPARγ, DNMT1, and DNMT3B mRNA levels showed that PPARγ expression levels were positively correlated with DNMT1 expression levels in PC patients ($r = 0.48$, $P = 0.008$), but not with DNMT3B expression levels ($r = -0.20$, $P = 0.30$). A significant correlation between DNMT1 and DNMT3B expression levels in patients with PC was observed, ($r = 0.36$, $P = 0.053$).

3.2. Correlation between PPARγ, DNMT1, and DNMT3B mRNA Levels with Clinical and Pathological Features. When PC patients were stratified according to their clinical phenotypes, PPARγ expression levels were unrelated to the considered demographic and clinical features, and DNMT1 showed only a trend of increased expression towards tumors of lower grades of differentiation (*G1*: median = 0.55, *Q*1–*Q*3 = 0.45–0.57; *G2*: median = 0.87, *Q*1–*Q*3 = 0.62–1.47; *G3*: median = 0.94, *Q*1–*Q*3 = 0.75–1.29; $P = 0.06$). Conversely, DNMT3B expression levels were associated with important prognostic variables in PC patients. In details, expression of DNMT3B in tumor tissues was directly related with lymph node ratio, expressed as total involved lymph nodes over total number of resected lymph nodes ($r = 0.37$, $P = 0.046$). Furthermore, DNMT3B expression levels showed a borderline association with perineural invasion in PC patients: DNMT3B levels were higher in patients with evidence of perineural invasion (median = 0.98, *Q*1–*Q*3 = 0.67–1.07) than in those without tumor invasion into neural structures (median = 0.26, *Q*1–*Q*3 = 0.18–0.62), $P = 0.06$ (Figure 2). In addition, DNMT3B expression was higher in patients with resection margins free of tumor cells (*R*0: median = 0.65, *Q*1–*Q*3 = 0.26–1.16), than in those with evidence of tumor infiltration on resected margins (*R*1: median = 0.20, *Q*1–*Q*3 = 0.12–0.42), $P = 0.04$ (Figure 3).

3.3. Survival Analysis. In the time-to-death analysis, DNMT3B expression levels were logarithmic-transformed to accomplish with the linearity assumption of the Cox model. At univariate analysis of the 30 PC patients, DNMT3B high expression levels were associated with lower mortality with an HR= 0.485 (95% CI = 0.262–0.895, $P = 0.02$). In addition, a significant interaction between DNMT3B expression levels and perineural invasion was also observed ($P = 0.05$). In details, in the subgroup of 21 patients without evidence of perineural invasion high DNMT3B expression levels were related with longer survival (HR = 0.314; 95% CI = 0.130–0.758; $P = 0.01$). Conversely, such an effect was not observed in the subgroup of 9 patients with tumour invasion into perineural structures (HR = 0.879; 95%CI = 0.466–1.655; $P = 0.70$) (Figure 4).

3.4. PPARγ, DNMT1, and 3b in Pancreatic Cancer Cell Lines. In order to corroborate previous findings, we then analyzed PPARγ, DNMT1, and 3B mRNA levels in four different pancreatic cancer cell lines. As shown in Figure 5, PANC-1 cells displayed higher levels of PPARγ as compared to the other cell lines, whilst CFPAC and MiaPaca cells presented lower mRNA levels of PPARγ. A similar pattern was observed for DNMT1 expression. DNMT3B display higher levels in CFPAC and PANC-1cells, while it was downregulated in BxPC3 and MiaPaca cells.

3.5. Effect of Rosiglitazone Treatment on DNMTs Expression in Pancreatic Cancer Cell Lines. In order to test whether PPARγ and DNMTs were correlated each other, we used a pharmacological approach by treating PC cells with rosiglitazone (a PPARγ agonist): upon rosiglitazone challenge different expression patterns in different PC cell lines were observed. As shown in Figure 6, in BxPC3 cells rosiglitazone treatment at 15 μM concentration for 48 h decreased DNMT1 expression ($P < 0.0001$) while DNMT3B resulted downregulated after treatment with rosiglitazone at 5 μM for 48 h as compared to vehicle treatment ($P = 0.003$). As for CFPAC cells (Figure 6), rosiglitazone challenge did not influence DNMT1 expression, whereas DNMT3B resulted increased upon treatment with rosiglitazone at 5 μM for 24 h and for 48 h ($P = 0.0007$ and $P = 0.03$, resp.) and after treatment with rosiglitazone at 15 μM for 48 h

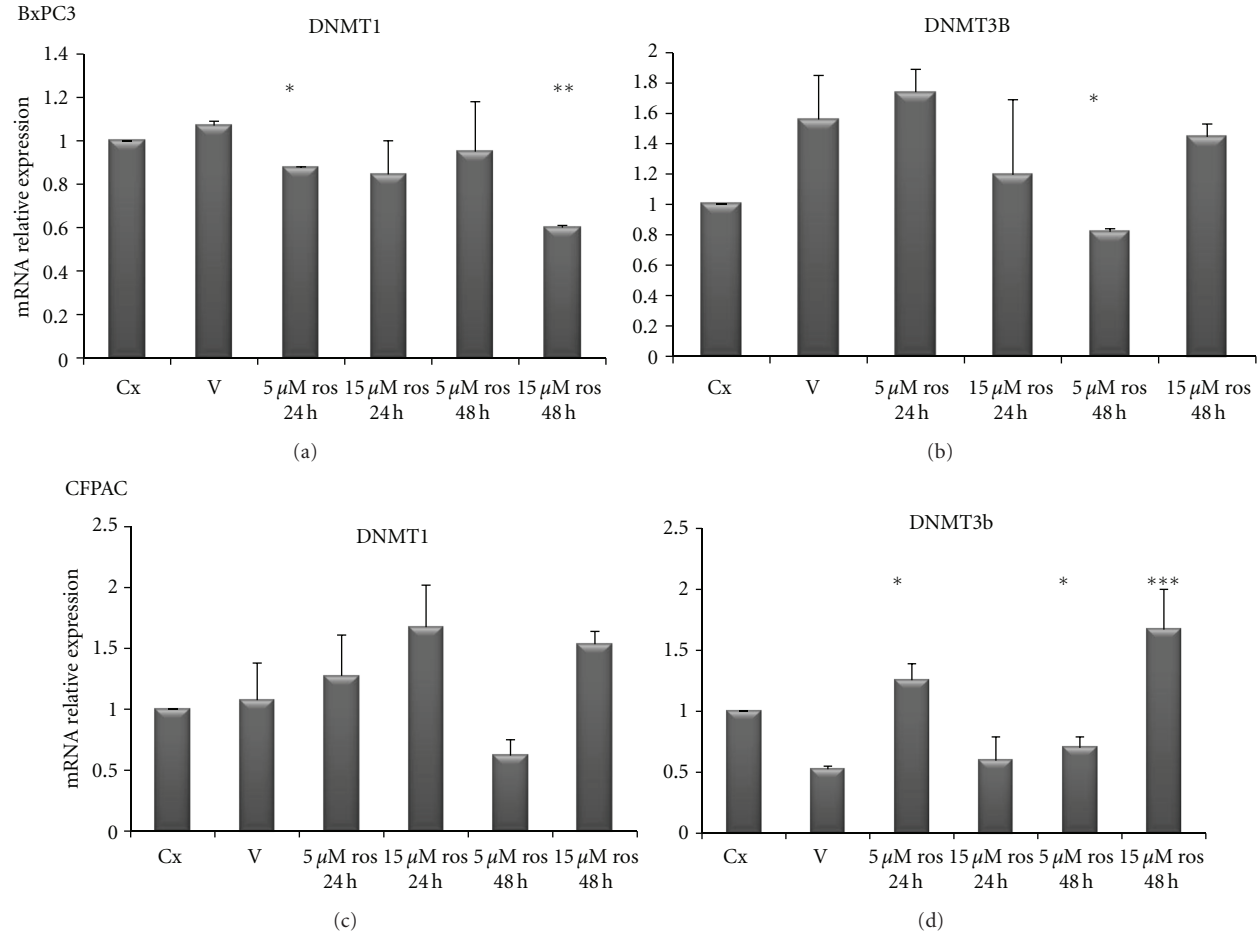

Figure 6: Quantitative real-time PCR. mRNA relative expression levels of DNMT1 and 3B in BxPC3 and CFPAC pancreatic cancer cell lines upon treatment with rosiglitazone at the indicated concentrations and time points.

($P = 0.03$). In PANC1 cells (Figure 7) DNMT1 was decreased after rosiglitazone treatment at 5 μM for 24 h ($P = 0.047$) and 48 h ($P = 0.07$), and at 15 μM for 24 h ($P = 0.008$) and 48 h ($P = 0.006$). Regarding DNMT3B, rosiglitazone treatment induced a significant upregulation at 5 μM and 15 μM for 24 h ($P = 0.03$ and $P = 0.02$) but not significant at 5 μM and 15μM for 48 h ($P = 0.139$ and $P = 0.153$ resp.).

As for MiaPaca cells (Figure 7), DNMT1 resulted increased after ros treatment at 5 μM and 15 μM for 24 h ($P = 0.02$ and $P = 0.039$ resp.), but this overexpression was not evident after 48 h treatment 5 μM ($P = 0.21$) being still present after 48 h at 15 μM concentration ($P = 0.003$). As for DNMT3B we observed a reduced expression after 48 h treatment with 5 μM of rosiglitazone ($P = 0.02$).

4. Discussion

Emerging evidence indicates that PPARγ plays a role in the pathogenesis of several pathological processes such as diabetes, obesity, atherosclerosis, and cancer [15]. Recent scientific reports suggest that the modulation of PPARγ activity may be of therapeutic value in PC [16–19]. PPARs activators might be an ideal combination partner in therapeutic settings where the inhibition of tumour-protecting proteins may be relevant to overcome treatment resistance [20]. Kristiansen et al. [21] demonstrated that PPARγ mRNA and protein expression were upregulated in pancreatic ductal adenocarcinoma and might serve as prognostic marker for this disease. In the present investigation we analyzed mRNA levels of PPARγ, DNMT1, and DNMT3B in PC patients in order to assess correlations among these factors and their associations with some clinical and pathological features of patients. We did not find an altered PPARγ expression in tumor as compared to normal matched tissues, but PPARγ expression showed a positive correlation with DNMT1 but not with DNMT3B expression. In addition, mRNA expression of the latter two genes was highly related. DNMT3B expression was significantly downregulated in tumors as compared to normal matched tissue, and positively associated to lymph node ratio and margin status. Peng et al. [22] suggested that increased DNMT1 protein expression participates in multistage pancreatic carcinogenesis from a precancerous stage to the malignant transition of ductal carcinomas and may be a biological predictor of poor prognosis. In our cohort, we found that DNMT3B expression

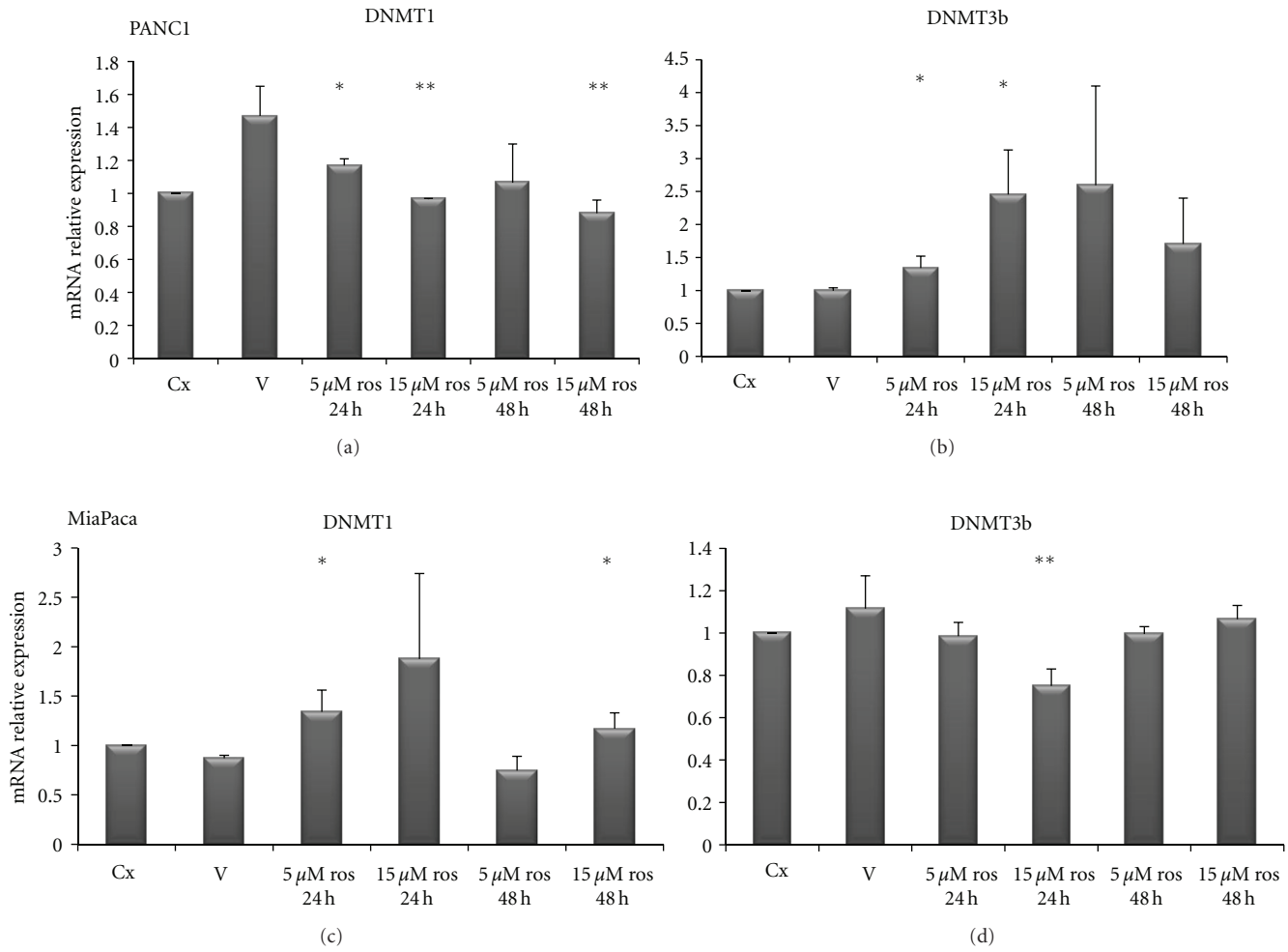

Figure 7: Quantitative real-time PCR. mRNA relative expression levels of DNMT1 and 3B in PANC1 and MiaPaca pancreatic cancer cell lines upon treatment with rosiglitazone at the indicated concentrations and time points.

displayed lower levels in noninvasive compared to invasive tumors. These data were in agreement with the *in vitro* findings showing that DNMT3B levels were higher in PANC1 and CFPAC cells whose superior invasive ability has been already demonstrated [23–25] as compared to BxPC3 and MiaPaca cells.

In PC patients, the univariate analysis showed a protective role, in terms of reduced mortality, of high DNMT3B expression levels in noninvasive tumours, whereas in invasive tumours such an effect for DNMT3B overexpression was not further observed.

Moreover, in our *in vitro* models of PC cells, PPARγ displayed higher levels in PANC-1 cells as compared to the other cell lines whilst CFPAC and MiaPaca cells presented the lower PPARγ mRNA levels. DNMT1 showed the same trend of PPARγ corroborating the positive correlation between PPARγ expression with DNMT1 in PC tissue.

When treated with PPARγ ligand (rosiglitazone), pancreatic cancer cells responded in different manner suggesting a dose-dependent and time-dependent effect. The observed difference of PPARγ and DNMTs levels and the differences in rosiglitazone response in the different cell lines could be due to their different genetic background [23]. Moreover, DNMTs alterations triggered by a low dose of rosiglitazone after 24 hours, which surprisingly was not observed upon incubation with a higher dose for 48 hours, indicate the existence of cell adaptative mechanisms to enhanced and perhaps saturated PPAR signaling.

5. Conclusion

Our study demonstrated that PPARγ positively correlates with DNMT1, but not with DNMT3B, and that higher DNMT3B mRNA levels in presence of noninvasive tumor predict longer survival in pancreatic cancer patients whereas in presence of invasive tumour higher DNMT3B mRNA levels were associated with a poor prognosis.

Authors' Contribution

A. Andriulli and P. di Sebastiano have contributed equally to this work.

Acknowledgments

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References

[1] A. Jemal, R. Siegel, E. Ward, Y. Hao, J. Xu, and M. J. Thun, "Cancer statistics, 2009," *CA: Cancer Journal for Clinicians*, vol. 59, no. 4, pp. 225–249, 2009.

[2] S. H. Landis, T. Murray, S. Bolden, and P. A. Wingo, "Cancer Statistics, 1999," *CA: Cancer Journal for Clinicians*, vol. 49, no. 1, pp. 8–31, 1999.

[3] A. L. Warshaw and C. Fernández-del Castillo, "Pancreatic carcinoma," *The New England Journal of Medicine*, vol. 326, no. 7, pp. 455–465, 1992.

[4] D. Hariharan, A. Saied, and H. M. Kocher, "Analysis of mortality rates for pancreatic cancer across the world," *HPB*, vol. 10, no. 1, pp. 58–62, 2008.

[5] P. Ferré, "The biology of peroxisome proliferator-activated receptors: relationship with lipid metabolism and insulin sensitivity," *Diabetes*, vol. 53, supplement 1, pp. S43–S50, 2004.

[6] M. H. Hsu, U. Savas, K. J. Griffin, and E. F. Johnson, "Identification of peroxisome proliferator-responsive human genes by elevated expression of the peroxisome proliferator-activated receptor α in HepG2 cells," *Journal of Biological Chemistry*, vol. 276, no. 30, pp. 27950–27958, 2001.

[7] S. A. Kliewer, B. M. Forman, B. Blumberg et al., "Differential expression and activation of a family of murine peroxisome proliferator-activated receptors," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 91, pp. 7355–7359, 1994.

[8] B. Farrow, K. L. O'Connor, K. Hashimoto, T. Iwamura, and B. M. Evers, "Selective activation of PPARγ inhibits pancreatic cancer invasion and decreases expression of tissue plasminogen activator," *Surgery*, vol. 134, no. 2, pp. 206–212, 2003.

[9] X. Cheng, "DNA modification by methyltransferases," *Current Opinion in Structural Biology*, vol. 5, no. 1, pp. 4–10, 1995.

[10] S. S. Smith, B. E. Kaplan, L. C. Sowers, and E. M. Newman, "Mechanism of human methyl-directed DNA methyltransferase and the fidelity of cytosine methylation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 89, no. 10, pp. 4744–4748, 1992.

[11] M. F. Robert, S. Morin, N. Beaulieu et al., "DNMT1 is required to maintain CpG methylation and aberrant gene silencing in human cancer cells," *Nature Genetics*, vol. 33, no. 1, pp. 61–65, 2003.

[12] I. Rhee, K. E. Bachman, B. H. Park et al., "DNMT1 and DNMT3b cooperate to silence genes in human cancer cells," *Nature*, vol. 416, no. 6880, pp. 552–556, 2002.

[13] American Joint Committee on Cancer, "Exocrine and endocrine pancreas," in *AJCC Cancer Staging Manual*, S. B. Edge, D. R. Byrd, C. C. Compton et al., Eds., pp. 241–246, Springer, New York, NY, USA, 7th edition, 2010.

[14] G. Benegiamo, M. Vinciguerra, G. Mazzoccoli, A. Piepoli, A. Andriulli, and V. Pazienza, "DNA methyltransferases 1 and 3b expression in Huh-7 cells expressing HCV core protein of different genotypes," *Digestive Disease Science*, vol. 57, no. 6, pp. 1598–1603, 2012.

[15] P. Lefebvre, G. Chinetti, J. C. Fruchart, and B. Staels, "Sorting out the roles of PPARα in energy metabolism and vascular homeostasis," *Journal of Clinical Investigation*, vol. 116, no. 3, pp. 571–580, 2006.

[16] M. Tsujie, S. Nakamori, J. Okami et al., "Thiazolidinediones inhibit growth of gastrointestinal, biliary, and pancreatic adenocarcinoma cells through activation of the peroxisome proliferator-activated receptor γ/retinoid X receptor α pathway," *Experimental Cell Research*, vol. 289, no. 1, pp. 143–151, 2003.

[17] K. Hashimoto, R. T. Ethridge, and B. M. Evers, "Peroxisome proliferator-activated receptor γ ligand inhibits cell growth and invasion of human pancreatic cancer cells," *International Journal of Gastrointestinal Cancer*, vol. 32, no. 1, pp. 7–22, 2002.

[18] A. Galli, E. Ceni, D. W. Crabb et al., "Antidiabetic thiazolidinediones inhibit invasiveness of pancreatic cancer cells via PPARγ independent mechanisms," *Gut*, vol. 53, no. 11, pp. 1688–1697, 2004.

[19] W. Motomura, M. Nagamine, S. Tanno et al., "Inhibition of cell invasion and morphological change by troglitazone in human pancreatic cancer cells," *Journal of Gastroenterology*, vol. 39, no. 5, pp. 461–468, 2004.

[20] H. Koga, K. Selvendiran, R. Sivakumar et al., "PPARγ potentiates anticancer effects of gemcitabine on human pancreatic cancer cells," *International Journal of Oncology*, vol. 40, no. 3, pp. 679–685, 2012.

[21] G. Kristiansen, J. Jacob, A. C. Buckendahl et al., "Peroxisome proliferator-activated receptor γ is highly expressed in pancreatic cancer and is associated with shorter overall survival times," *Clinical Cancer Research*, vol. 12, no. 21, pp. 6444–6451, 2006.

[22] D. F. Peng, Y. Kanai, M. Sawada et al., "Increased DNA methyltransferase 1 (DNMT1) protein expression in precancerous conditions and ductal carcinomas of the pancreas," *Cancer Science*, vol. 96, no. 7, pp. 403–408, 2005.

[23] E. L. Deer, J. González-Hernández, J. D. Coursen et al., "Phenotype and genotype of pancreatic cancer cell lines," *Pancreas*, vol. 39, no. 4, pp. 425–435, 2010.

[24] K. Hashimoto, B. J. Farrow, and B. M. Evers, "Activation and role of MAP kinases in 15d-PGJ2-induced apoptosis in the human pancreatic cancer cell Line MIA PaCa-2," *Pancreas*, vol. 28, no. 2, pp. 153–159, 2004.

[25] M. Gaviraghi, P. Tunici, S. Valensin et al., "Pancreatic cancer spheres are more than just aggregates of stem marker-positive cells," *Bioscience Reports*, vol. 31, no. 1, pp. 45–55, 2011.