Review Article

PPARs and Mitochondrial Metabolism: From NAFLD to HCC

Tommaso Mello,[1] Maria Materozzi,[1] and Andrea Galli[1,2]

[1]*Clinical Gastroenterology Unit, Department of Biomedical Clinical and Experimental Sciences "Mario Serio",*
 University of Florence, Viale Pieraccini 6, 50129 Florence, Italy
[2]*Careggi University Hospital, Florence, Italy*

Correspondence should be addressed to Tommaso Mello; tommaso.mello@unifi.it

Metabolic related diseases, such as type 2 diabetes, metabolic syndrome, and nonalcoholic fatty liver disease (NAFLD), are widespread threats which bring about a significant burden of deaths worldwide, mainly due to cardiovascular events and cancer. The pathogenesis of these diseases is extremely complex, multifactorial, and only partially understood. As the main metabolic organ, the liver is central to maintain whole body energetic homeostasis. At the cellular level, mitochondria are the metabolic hub connecting and integrating all the main biochemical, hormonal, and inflammatory signaling pathways to fulfill the energetic and biosynthetic demand of the cell. In the liver, mitochondria metabolism needs to cope with the energetic regulation of the whole body. The nuclear receptors PPARs orchestrate lipid and glucose metabolism and are involved in a variety of diseases, from metabolic disorders to cancer. In this review, focus is placed on the roles of PPARs in the regulation of liver mitochondrial metabolism in physiology and pathology, from NAFLD to HCC.

1. Introduction

Liver cancer is a major challenge in contemporary medicine. It represents the fifth most common cancer in men, the ninth in women, and the second most frequent cause of mortality among oncological patients. It was responsible for nearly 746,000 deaths in 2012, with an estimated incidence of over 780,000 new cases yearly worldwide [1]. The prognosis for liver cancer is extremely poor (overall ratio of mortality to incidence of 0.95), reflecting the absence of effective treatments. The most frequent type of primary liver cancer is hepatocellular carcinoma (HCC), accounting for up to 85% of total cancers [2].

Major risk factors include HBV or HCV infection, alcoholic liver disease, and most likely nonalcoholic liver disease (NAFLD) [2]. These and other chronic liver diseases lead to cirrhosis, which is found in 80–90% of HCC patients [2]. NAFLD is now the most common liver disease worldwide [3], with a global prevalence of about 25%. NAFLD is closely associated with other metabolic disorders such as obesity, metabolic syndrome, and type 2 diabetes [3]. Indeed, obesity and diabetes are now definitively recognized as independent risk factors for the development of HCC [4, 5]. NAFLD is

histologically classified into nonalcoholic fatty liver (NALF), defined as the presence of steatosis in the absence of causes for secondary hepatic fat accumulation (i.e., alcohol consumption, steatogenic drugs, or genetic disorders), and nonalcoholic steatohepatitis (NASH), in which steatosis is complicated by inflammation and hepatocellular damage (ballooning hepatocytes), with or without fibrosis [6]. A relatively small portion of NAFL patients evolve into NASH, a progressive type of liver disease with the potential of evolving into cirrhosis and HCC. The cumulative incidence of HCC in NASH cirrhosis ranges from 2.4% to 12.8%, and although it is lower than in HCV cirrhotic patients, the absolute burden of NASH related HCC is higher due to the epidemic spread of NAFLD [7]. Moreover, NAFLD greatly increases the risk of HCC from other aetiologies, especially HCV and HBV. While the vast majority of HCC arise in cirrhotic livers, it can also occur in noncirrhotic patients [2]. Of notice, a significant amount of new HCC cases is diagnosed in patients with noncirrhotic NASH [4, 8]. The global incidence of HCC among NAFLD patients was recently estimated to be 0.44 per 1,000 person-year [3], which combined with the epidemic spread of metabolic disorders results in an enormous burden. The recent meta-analysis by Younossi et al. raised the question

whether NAFLD could even precede the onset of metabolic syndrome rather than just being the hepatic manifestation of it [3].

The mechanisms that promote HCC development in NASH/NAFLD patients are complex and still poorly understood. A number of molecular mechanisms have been linked to obesity and related metabolic disorders that may accelerate the development of HCC, such as adipose-derived inflammation, lipotoxicity, and insulin resistance. These and other pathological events in obesity have complex interactions while their relative contribution to hepatocarcinogenesis in various stages of NAFLD progression remains to be determined.

Mitochondria can be seen as the energetic hub of the cell. As such, beyond their role in energy production, they play a central role in coordinating the cell anabolic and catabolic processes, in balancing the cell energetic demands in response to internal and external stimuli, and in the regulation of several cell signaling pathways. Deregulation of mitochondrial activity is a common trait to several human diseases, including cancer. Since Warburg, it has long been known that cancer cells undergo a radical metabolic shift toward glycolysis, irrespective of the oxygen availability (aerobic glycolysis) [9]. However, the actual significance of this metabolic remodeling, its consequences on cancer cell biology, and its plasticity have begun to be grasped only in recent years. The initial perception of the Warburg effects was that cancer cells rely primarily on glycolysis for energy production due to a defective mitochondrial respiration [10]. On the contrary, it is now clear that cancer cells hijack their mitochondria metabolism toward massive anabolic processes, in order to cope with the cell fast-growing rates [11]. In this line of view, exacerbate biosynthesis, in particular lipid biosynthesis, rather than glycolysis dependence, emerges as cancer metabolic hallmark.

Peroxisome proliferator activated receptors (PPARs) are master regulators of whole body and liver metabolism. Despite a similar structure, the three PPAR isotypes α, β/δ, and γ vary greatly in tissue distribution, pharmacological and endogenous ligands, and biological effects. In the past decades PPARs have been the focus of massive research effort that helped uncovering their contribution to cancer, metabolic, and cardiovascular diseases. The different PPAR isotypes regulate lipid metabolism by a number of mechanisms: (i) controlling the rate of FA disposal through mitochondrial and peroxysomal β-oxidation (FAO), (ii) regulating lipid biosynthesis via de novo lipogenesis, (iii) regulating FA uptake in peripheral tissue and in the liver, (iv) regulating whole body lipid trafficking through apolipoproteins, (v) interacting in complex regulatory network with other nuclear receptors (LXR, FXR), coactivators (PGC-1α and β, SREBP), or corepressors (NCOR) involved in the metabolic homeostasis. As liver is primarily a metabolic organ, PPARs-regulated processes are involved virtually in any liver disease.

This review summarizes current notions on the roles of PPARs in the regulation of liver mitochondrial metabolism in physiology and pathology, from NAFLD to HCC.

2. PPARs and Mitochondrial Metabolism in the Liver

2.1. PPARα. Peroxisome proliferator activated receptor α (PPARα) is the main PPAR isotype expressed in the liver and plays a major role in energy homeostasis, by regulating lipid metabolism and ketone body formation [12]. In mice but not in humans, PPARα also controls the glycolysis-gluconeogenesis pathway [13]. PPARα natural ligands are endogenous lipids such as fatty acids (FA) and their derivatives (eicosanoids, oxidized phospholipids) [14], while synthetic ligands include the class of hypolipidemic drugs fibrates, xenobiotic agents, and plasticizers.

Despite the fact that FA and derivatives can bind and activate PPARα in the liver, not all FA are created equal. Indeed, it has been now recognized that FA released in the bloodstream by the adipose tissue (i.e., during fasting or intense physical exercise) have little role as PPARα agonist [15], while preferentially activating PPARβ/δ, whereas fatty acids derived from dietary intake or de novo lipogenesis are efficient PPARα activators [15–18]. However, PPARα is absolutely required for the metabolic adaptation to fasting, since PPAR$\alpha^{-/-}$ mice, either full body [19] or liver-specific [20], develop steatosis with prolonged fasting. Moreover, the time course activation of PPARα in the liver mimics the kinetics of circulating FFA during fasting, and liver transcriptomic profiling revealed that the fasted state (versus fed or refed) triggered the broader PPARα-dependent response, strengthening the functional link between hepatic PPARα and adipose tissue-FA disposal [20]. Since activation of hepatic PPARα requires de novo lipogenesis [15, 21], the mechanisms that fine-tune PPARα activation in different metabolic conditions are still unclear and possibly involve separate pools of PPARα that can be activated in a context-dependent manner.

Moreover, the adipose tissue cross-talk with the hepatic PPARs can occur via adiponectin-induced FAO, which is dependent upon AdipoR2 subtype and requires PPARα induction [22], and via FGF21, produced mainly in the liver in a PPARα-dependent manner [20], which promotes both glucose uptake and lipolysis in the adipocytes [23], as well as hepatic lipid disposal [24].

In hepatocytes, PPARα promotes the expression of several genes involved in FA uptake, activation to acyl-CoA, and transport to the mitochondria or peroxisomes and subsequent β- or ω-oxidation, ketogenesis, and lipoprotein trafficking [25, 26] (Figure 1).

Many of the PPARα regulated genes directly modulate mitochondrial metabolism. Interestingly, among the many PPARα-regulated genes in hepatocytes, those involved in mitochondrial metabolic functions, especially in fatty acid oxidation, are consistently dependent upon PPARα regardless of the nutritional condition [20]. PPARα target genes are also carnitine palmitoyl transferase 1 (CPT-1) and carnitine palmitoyltransferase 2 (CPT-2) [19, 25], which mediate transport of long-chain fatty acids through the outer and inner mitochondrial membrane, respectively, to initiate their degradation in the β-oxidative pathway (Figure 1). The
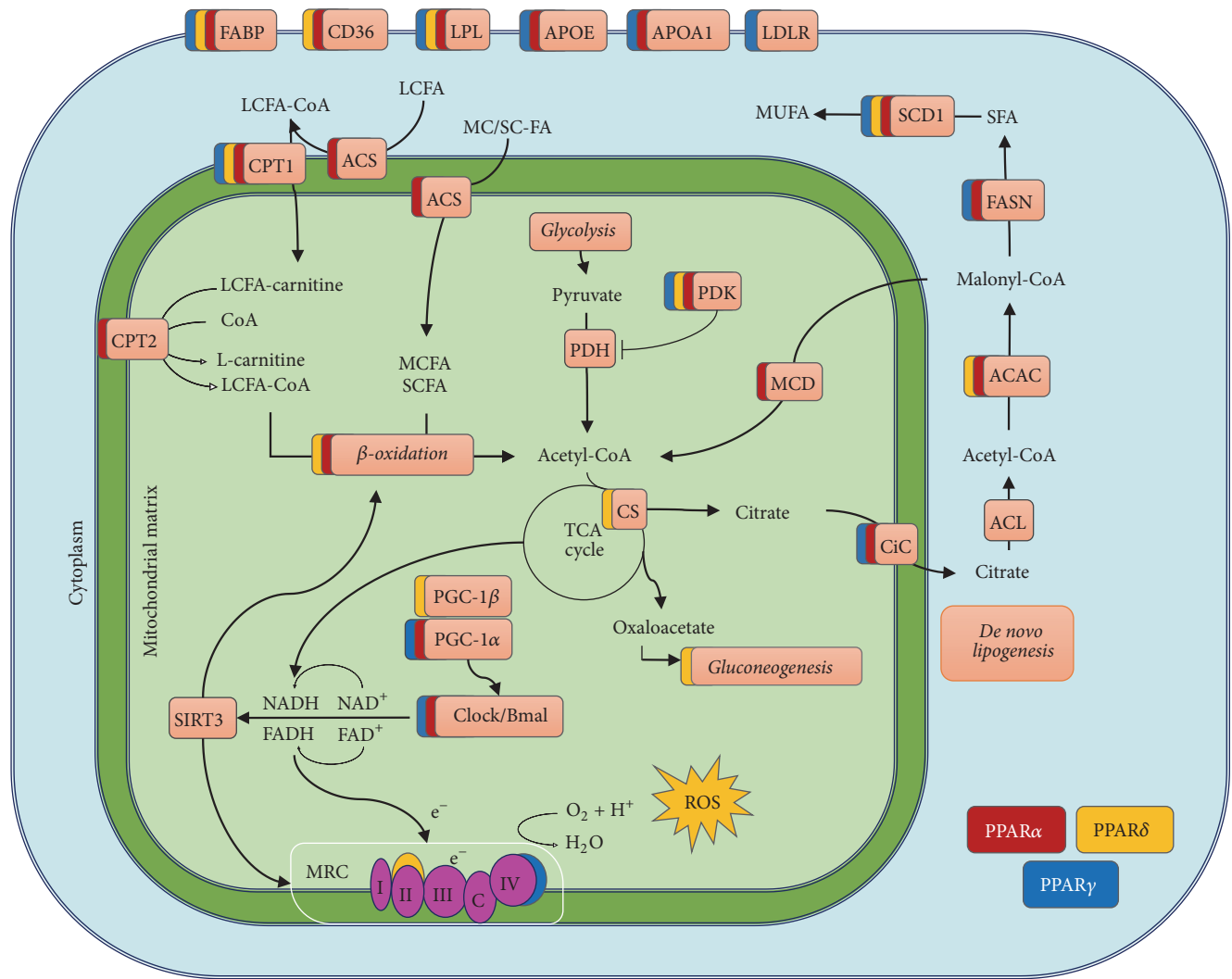
FIGURE 1: Role of hepatic PPARs in mitochondrial metabolism: fatty acid oxidation, circadian control of NAD+ dependent SIRT activity, de novo lipogenesis, and gluconeogenesis. Color-coding depicts PPAR isotypes-dependency of target genes.

β-oxidation cycle consists of four reactions, catalyzed by acyl-CoA dehydrogenases (ACADs), enoyl-CoA hydratases, L-3-hydroxyacyl-CoA dehydrogenase, and 3-ketoacyl-CoA thiolase, that sequentially remove two carbons—one acetyl-CoA molecule, until the acyl-CoA is completely converted to acetyl-CoA. The initial step of the β-oxidation cycle is catalyzed by length specific acyl-CoA dehydrogenases (such as ACADM, ACADS, and ACADVL), all of which are PPARα target genes [26]. The last three steps are carried on by the mitochondrial trifunctional protein (MTP), a large complex of four α and four β subunits. The expression of both subunits (encoded by genes HADHA, HADHB) as well as the MTP protein 3-ketoacyl-CoA thiolase (encoded by ACAA2) is regulated by PPARα [26].

The acetyl-CoA produced during FAO is then used to produce ketone bodies (acetoacetate and β-hydroxybutyrate) via mitochondrial HMG-CoA synthase, another PPARα regulated gene [27]. Ketone bodies are released in the blood

stream and, after conversion to citrate, fuel the TCA cycle in peripheral tissues (mostly heart, muscle, and brain).

FAO is functionally and physically linked to OXPHOS: the reducing equivalents produced by FAO are directly used by the electron transport chain (ETC); moreover, the two pathways are likely happening in large mitochondrial supercomplexes containing both FAO and ETC complexes [28]. Therefore, an unbalance in FAO or ETC directly affects the other pathway.

PPARα, as well as β/δ and γ, also induces the expression of all uncoupling protein (UCP-1, UCP-2, and UCP-3), of which UCP-2 is the main type expressed in liver [29, 30]. Uncoupling proteins allow protons to reenter the mitochondrial matrix without production of adenosine triphosphate, thus promoting energy expenditure and FA oxidation.

Paralleling its role in promoting energy expenditure through FA disposal, PPARα also inhibits the lipogenic pathway by induction of the malonyl-CoA decarboxylase

which degrades malonyl-CoA, a precursor of FA biosynthesis and inhibitor of the mitochondrial transporter CPT-1 [31] (Figure 1).

2.2. PPARβ/δ. PPARβ/δ is ubiquitously expressed, often at higher level than PPARα or γ. Overall, PPARβ/δ role in lipid metabolism appears to be largely overlapping with PPARα in most tissues. Indeed, PPARβ/δ stimulates FAO in muscle and heart, the latter organ being extremely dependent on PPARβ/δ function [32].

Several PPARα target genes are thus not surprisingly induced also by PPPARβ/δ (UCP-1, UCP-2, and UCP-3, FABP, FAT/CD36, LPL, ACS, and CPT-1) [33, 34] and loss of PPARα in muscle is efficiently compensated by PPARβ/δ [33]. Indeed, numerous studies have shown that PPARβ/δ overexpression or activation in muscle dramatically improves FA utilization as energy source, reduces hyperlipidemia, improves endurance, and decreases insulin secretion from β-cells [32, 35–37].

However, in the liver PPARβ/δ seems to play a different role than PPARα. Adenoviral-mediated overexpression of PPARβ/δ in the liver enhanced glucose utilization either to increase glycogen storage or to promote de novo lipogenesis, rather than inducing FAO [38] (Figure 1). PPARβ/δ induced the expression of several genes involved in glucose metabolism (GLUT2, GK, and pyruvate kinase) and lipogenesis (FAS, ACC1, ACC2, SCD1, SREBP-1c, and PGC-1β) [38]. Conversely, gluconeogenesis genes (PEPCK, HNF-4) were inhibited by PPARβ/δ expression in hepatocytes. Importantly, the levels of PPARα and its target (CPT-1, acyl-CoA oxidase, and MCAD) were unaffected; therefore PPARβ/δ seems not to overlap with PPARα function in the liver [38]. Consistently, whole transcriptome profiling and liver metabolites analysis of PPARα$^{-/-}$ and PPARβ/δ$^{-/-}$ mice revealed clearly divergent roles [39]. Very interestingly, liver PPARβ/δ signals to PPARα and activates FAO in muscle via the lipid molecule PC (18:0/18:1), whose production in the liver is PPARβ/δ-dependent [40].

Different roles for PPARα and PPARβ/δ in mitochondri-ogenesis are also beginning to emerge. A transitory upregula-tion of PPARα, and consequent induction of PGC-1α, is nec-essary to promote mitochondriogenesis in the early steps of differentiating embryonic stem cells. A robust upregulation of PPARβ/δ is instead needed to promote mitochondriogenesis at later stages of cells differentiation and correlates with the expression of mature hepatocytic markers [41].

Functional peroxisome proliferator response elements have been identified in the distal promoter of PGC-1α, pro-viding the mechanistic basis for PPAR-induced mitochon-drial biogenesis. However, the contribution of the diverse PPAR isotypes to PGC-1α induction appears to be cell context-dependent. PGC-1α is activated by PPARα in brown adipose tissue [42] and by PPARγ in both white and brown adipose tissue [42]. In skeletal muscle, PPARβ/δ but not PPARα induce PGC-1α expression [43, 44].

In liver, PCG-1α is induced by fasting, paralleling PPARα activation, and promotes gluconeogenesis, a process medi-ated by PPARβ/δ [45].

2.3. PPARγ. PPARγ is the main PPAR isotype expressed in white and brown adipose tissue. It is the master inducer of adipogenesis and promotes glucose uptake and utilization in the novo lipogenic pathway, therefore regulating whole body lipid metabolism and insulin sensitivity. Natural PPARγ ligands are lipid molecules derivates, such as unsaturated FA, PGJ$_2$, and oxidized LDL [14, 46, 47] while potent synthetic ligands include the insulin sensitizer class of drug TZD [48].

PPARγ induces the expression of genes regulating glucose sensitivity (GLUT-4, IRS-1, IRS-2, and PI3K), as well as genes involved in FA uptake and mobilization (FAT/CD36, fatty acids binding proteins aP2, and lipoprotein lipase) and triglyceride synthesis (acyl-CoA synthetase, glycerol kinase, and PEPCK) [46, 49] (Figure 1). In the liver, PPARγ is expressed in macrophages, endothelial cells, quiescent (nonactivated) stellate cells, and hepatocytes. Its complex actions on liver physiology are mostly mediated by its anti-inflammatory functions on macrophages and endothe-lial cells, antifibrotic function in hepatic stellate cells, and metabolic cross-talk between hepatocytes and adipocytes via FGF family members (FGF21, FGF-1). Mice with selective deletion of PPARγ in hepatocytes developed relative fat intolerance, increased adiposity, hyperlipidemia, and insulin resistance. Loss of hepatic PPARγ increased TG blood level and redistribution to other tissues, aggravating insulin resistance in muscle and adipose tissue [50, 51]. These models highlighted the role of liver PPARγ in maintaining lipid/glucose homeostasis and insulin sensitivity.

PPARγ also induces the expression of mitochondrial pro-teins, common to the other PPARs, such as CPT-1 and UCPs, suggesting a possible degree of overlap in mitochondrial metabolism regulation with other PPAR members. Probably the most relevant function of PPARγ in mitochondria biology comes with its interaction with PGC-1 family members. PGC-1α was initially identified as a nuclear PPARγ coactivator in mitochondrial rich brown adipose tissue-tissue [52]. Since then, it has become clear that PGC-1α and β control virtually any aspect of mitochondria function and biogenesis [53], by thoroughly coordinating a plethora of nuclear receptors (including all three PPARs, EERα) and nonnuclear receptor protein [54]. Indeed, PPARγ can promote the expression of PGC-1α, which in turn potentiates PPARγ activity [55]. Recently, steatogenic FA were shown to induce PPARγ via PGC-1α, suggesting a link between mitochondria biogenesis and triglyceride accumulation [56].

3. Mechanisms of Mitochondrial Oxidative Stress Damage

Reactive oxygen species (ROS) are small reactive molecules generated by the normal cell metabolism, involved in homeo-stasis and signaling. ROS such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical ($HO^\bullet$) consist of radical and nonradical oxygen species formed by the partial reduction of oxygen. Cellular ROS levels are controlled by antioxidant systems such as reduced/oxidized glutathione (GSH/GSSG), reduced/oxidized cysteine

(Cys/CySS), tioredoxin (Trx), peroxiredoxin (Prx), superoxide dismutase (SOD), and catalase.

An imbalance of the generation/neutralization of ROS, driven by an overproduction of ROS or a depletion of the antioxidant defenses, leads to a prooxidant state defined as "oxidative stress." Oxidative stress can directly damage proteins, lipids, and DNA, leading to damaged macromolecules and organelles, but also deranges the redox circuits that regulate many signaling pathways [57]. In fact, while excessively high levels of oxidative stress lead the cell to apoptosis, a controlled increase of ROS serves as critical signaling molecules in cell proliferation and survival [58]. ROS can be generated by growth factor signaling through activation of the NADPH oxidase NOX1 or through the mitochondria. In turn, they can induce cellular signaling cascades by oxidation of phosphatases such as PTEN or PTP or kinases such as Src. This leads to the activation of several pathways such as a Src/PKD1-dependent NF-κB activation mechanism, MAPK (Erk1/2, p38, and JNK), and the PI3K/Akt signaling. Aberrant levels of ROS induce a deregulation of these pathways, which are involved in several pathological conditions, such as NAFLD [59], diabetes [60], and cancer [58, 61].

Several different sources of ROS exist in mitochondria. ETC complex I and complex II, as well as other mitochondrial enzymes such as α-ketoglutarate dehydrogenase, pyruvate phosphate dehydrogenase, fatty acyl-CoA dehydrogenase, and glycerol 3-phosphate dehydrogenase, can produce $O_2^{\bullet-}$ as byproduct, releasing it within the mitochondrial matrix. Moreover, H_2O_2 is produced by the monoamine oxidases (MAOs) located in the outer mitochondrial membrane [62, 63]. Therefore, mitochondria can produce a significant amount of ROS during OXPHOS and FAO, especially in the context of reduced antioxidant defense such as depletion of the mitochondrial glutathione pool [64].

Four main alterations are the direct result of ROS formation: lipid, protein and DNA oxidation, and depletion of antioxidant molecules.

Mitochondrial DNA (mtDNA) is particularly susceptible to oxidative damage due to the absence of protective histones, incomplete DNA repair mechanisms, and the close proximity to ROS production site, which increase the risk of double-strand breaks and somatic mutations with increased ROS production [65]. Since the ETC proteins are encoded exclusively in mtDNA, oxidative damage leads to defective mitochondrial respiration and to a second burst of ROS production that damages mitochondrial membrane and eventually results in loss of mitochondrial membrane potential and activation of proapoptotic pathways due to the ROS induced-ROS-release avalanche [64, 65]. Indeed, depletion and mutation of mtDNA have been described in several type of liver injury, including NASH [66].

Lipid peroxidation is the process under which lipids, mainly polyunsaturated fatty acids, are attacked by oxidants such as ROS. These reactions can form a variety of primary and secondary products, among which malondialdehyde (MDA) appears to be the most mutagenic and 4-hydroxynonenal (4-HNE) the most toxic. MDA induced mutations are involved in cancer and other genetic diseases. 4-HNE can also act as a signaling molecule modulating many pathways and inducing the expression of proteins, such as NF-κB, Akt, MAPK, JNK, and PPARs. Lipid peroxidation occurs through a radical reaction; it is therefore extremely harmful to biological membranes where the damage can rapidly spread.

The depletion of mitochondrial ROS scavenger is a key step in the pathogenesis of ROS-related liver disease.

In NASH animal model, depletion of mGSH occurs due to cholesterol accumulation in the mitochondrial membrane [67] that disrupts the functionality of GSH transport from cytosol to mitochondria. Depletion of mGSH and other antioxidant systems are documented in NASH patients [68].

ROS can also act as second messengers in cellular signaling oxidizing proteins on cysteine residues, resulting in protein activation or inhibition. High levels of ROS can therefore activate pathways in a signal-independent manner and self-sustain many proproliferative pathways highly involved in cancer and liver diseases such as NASH/NAFLD.

For example, it has been demonstrated that ROS can directly oxidize and activate complexes such as inflammasomes: protein platforms that assemble in the presence of exogenous or endogenous danger signals such as pathogen associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) to activate and amplify inflammatory pathways [69]. Typically, inflammasomes consist of a sensor (NLRs, ALRs, and TLRs), an adaptor (ASC), and the effector molecule caspase-1 [70]. Once caspase-1 is recruited and activated through autocatalytic cleavage by the inflammasome, it can proteolytically process the inflammatory cytokines IL-1β and IL-18 that lead to a specialized form of cell death called pyroptosis. Pyroptosis causes the release of IL-1β and amplify the inflammatory response downstream of inflammasome activation [70]. In the liver, inflammasomes are expressed in hepatocytes as well as in immune cells and can also be activated by fatty acids through a mechanism involving mitochondrial ROS, decreased autophagy, and IL-1β secretion. Inflammasomes are found overexpressed in NAFLD and NASH and their silencing reduced hepatic injury, steatosis, and fibrosis [69]. Interestingly, agonists of PPARβ/δ were shown to reduce fatty acid induced inflammation and steatosis by inhibiting inflammasomes [69, 71].

Lipid overload in NAFLD and NASH leads to mitochondrial dysfunction and increased oxidative stress, which results from both increased electron flux through the ETC and depletion of the mitochondrial antioxidant defense systems [64].

Reduced levels of GSH, SOD, and catalase as well as increased protein oxidation, a hallmark of increased oxidative stress, are found in NASH patients [68]. Consistently, the mitochondria of NASH patients have altered morphology [72, 73], reduced or mutated mDNA content [66], and reduced oxidative phosphorylation capacity [74]. Oxidative stress constitutes one of the key factors driving NAFLD progression to NASH [75]. Indeed, histological markers of oxidative stress, such as oxidized phosphatidylcholine, localize into steatotic/apoptotic hepatocytes and macrophages and correlate with the degree of steatosis [76].

Depletion of mtGSH and mitochondria oxidative damage are recapitulated also in several animal models of NASH. Interestingly, Llacuna and colleagues highlighted that mitochondrial damage in diverse animal models of NASH seemed to be dependent more on mitochondria cholesterol accumulation (ob/ob mice or HFD administration), rather than only fatty acid/triglyceride overload (choline deficiency model) [67]. Consistently, statins reduced mitochondrial damage in ob/ob mice and HFD models.

This line of view is confirmed by a recent report highlighting the crucial role of dietary cholesterol in delivering the "second hit" for NASH onset, in context of moderate dietary fat administration (45% of total calories from fat) [77]. In this study, addition of a moderate level of cholesterol in HF elicits the onset of hepatocellular damage and inflammation through activation of the inflammasomes response, while neither dietary cholesterol nor HF alone produced the NASH phenotype. Importantly, addition of cholesterol to HF resulted in blunted adaptation of mitochondrial metabolism to HF and markedly reduced mitochondrial biogenesis, effects paralleled by a decrease in PGC-1α and TFAM expression levels [77]. Moreover, while hepatic inflammation recovered after removal of excess dietary cholesterol, mitochondrial functions remained hampered alongside elevated NRLP3 inflammasome protein levels, indicating slow recovery dynamics from mitochondrial damage.

Excess accumulation of free cholesterol in mitochondrial membranes emerges as a hallmark of cellular transformation, potentially fueling the metabolic derangement required for cancer cell growth and resistance to apoptosis [78].

4. PPARs and Mitochondrial Dysfunction, from NAFLD to HCC

4.1. PPARα. A role for PPARα in NASH pathogenesis in animal models has long been established.

PPAR$\alpha^{-/-}$ mice fed a MCD diet developed more severe NASH than WT mice, and Wy-14,643 administration completely prevented the development of NASH in WT mice, but not in PPAR$\alpha^{-/-}$ mice [79]. The protective effect of the PPARα agonist Wy-14,643 was unexpected, since the authors had foreseen a detrimental effect of the oxidative stress produced by peroxysomal ω-oxidation after PPARα activation. However, PPARα activation also resulted in increased hepatic lipid turnover through the β-oxidative pathway, preventing accumulation of lipoperoxides despite peroxysomal induction [79]. The beneficial effects of PPARα activation by Wy-14,643 were also confirmed in a severe NASH model with established fibrosis [80].

PPARα deletion in mice results in mild, age and sex-dependent, lipid accumulation in the liver [81]. Moreover, overnight fasting results in severe hypoglycemia, hypoketonemia, and increased plasma free FA levels, impaired β-oxidation, and ketogenesis in PPAR$\alpha^{-/-}$ mice [19]. As a result, HFD feeding worsens NAFLD in PPAR$\alpha^{-/-}$ mice [19, 82]. More recently, the use of a hepatocytic specific PPAR$\alpha^{-/-}$ mice model confirmed the protective role of PPARα in

NAFLD induced by MCD and short-term HFD. Interestingly, PPAR$\alpha^{hep-/-}$ mice developed steatosis and hypercholesterolemia with aging similarly to whole body PPAR$\alpha^{-/-}$ mice but did not become obese nor hyperglycaemic [20], confirming that hepatocytic PPARα deletion by itself is a primary cause of liver steatosis.

On the other hand, in leptin deficient (ob/ob) and leptin resistant (db/db) mouse models, PPARα expression was found reduced, unchanged, or increased [83]. Rate of FAO also varies greatly depending on the study. While these discrepancies could be generated by different study protocols, they may be interpreted also in the light of different PPARα pools that can be differentially activated in the metabolism of dietary, versus adipose tissue-derived fatty acids.

Since FA can bind and activate PPARα, thus promoting mitochondrial and peroxysomal FAO, downregulation of PPARα in NASH mice models and patients may be counterintuitive. Moreover, high FAO can increase oxidative stress; therefore stimulating PPARα activity and FAO is somewhat expected to worsen the oxidative damage in hepatocytes. However, it should be recalled that although mitochondria are potentially a major source of ROS, they are also very well equipped with antioxidant defense systems. In fact, whether significant ROS production occurs in mitochondria in vivo is highly debated, and the endoplasmic reticulum is currently emerging as the major source or toxic ROS within the cell [64]. The current view is that liver triglycerides accumulation per se does not result in inflammation [84, 85]. Rather, accumulation of free fatty acids, in particular saturated fatty acids (SFA), results in marked lipotoxicity, hepatocellular damage, and inflammation [86, 87]. The onset of inflammation drives the progression from NAFLD to NASH and causes PPARα downregulation by TNFα [88]. Moreover, TNFα also reduces adiponectin levels. Adiponectin promotes FAO and blunts liver gluconeogenesis signaling through AdipoR2 receptor, which promotes PPARα activity [89] and depends upon PPARα induction. Thus, inflammation-mediated disruption of the metabolic cross-talk between the adipose tissue and the liver may account for reduced PPARα activity, mitochondrial dysfunction, and NASH development (Figure 2). A recent report by Ande and coworkers highlights the importance of the inflammatory cross-talk between adipose tissue and liver, in a sex-dependent manner, in the induction of hepatocytes mitochondrial dysfunction, NASH, and HCC development [90].

This line of view is consistent with the emerging role of PPARα in the control of inflammation [12] and provides additional rationale for pharmacological induction of PPARα in NASH treatment.

Reports on PPARα in human NAFLD are scarce. Very recently a thorough investigation of PPARs expression in NAFLD patients was assessed by Staels' group. The expression of PPARα, PPARβ/δ, and PPARγ was evaluated on mRNA extracted from paired liver biopsies collected 1 year apart in 85 patients. They found a significant association between decreased PPARα expression and histological severity of NASH. No correlation was found with PPARβ/δ or PPARγ expression [91].
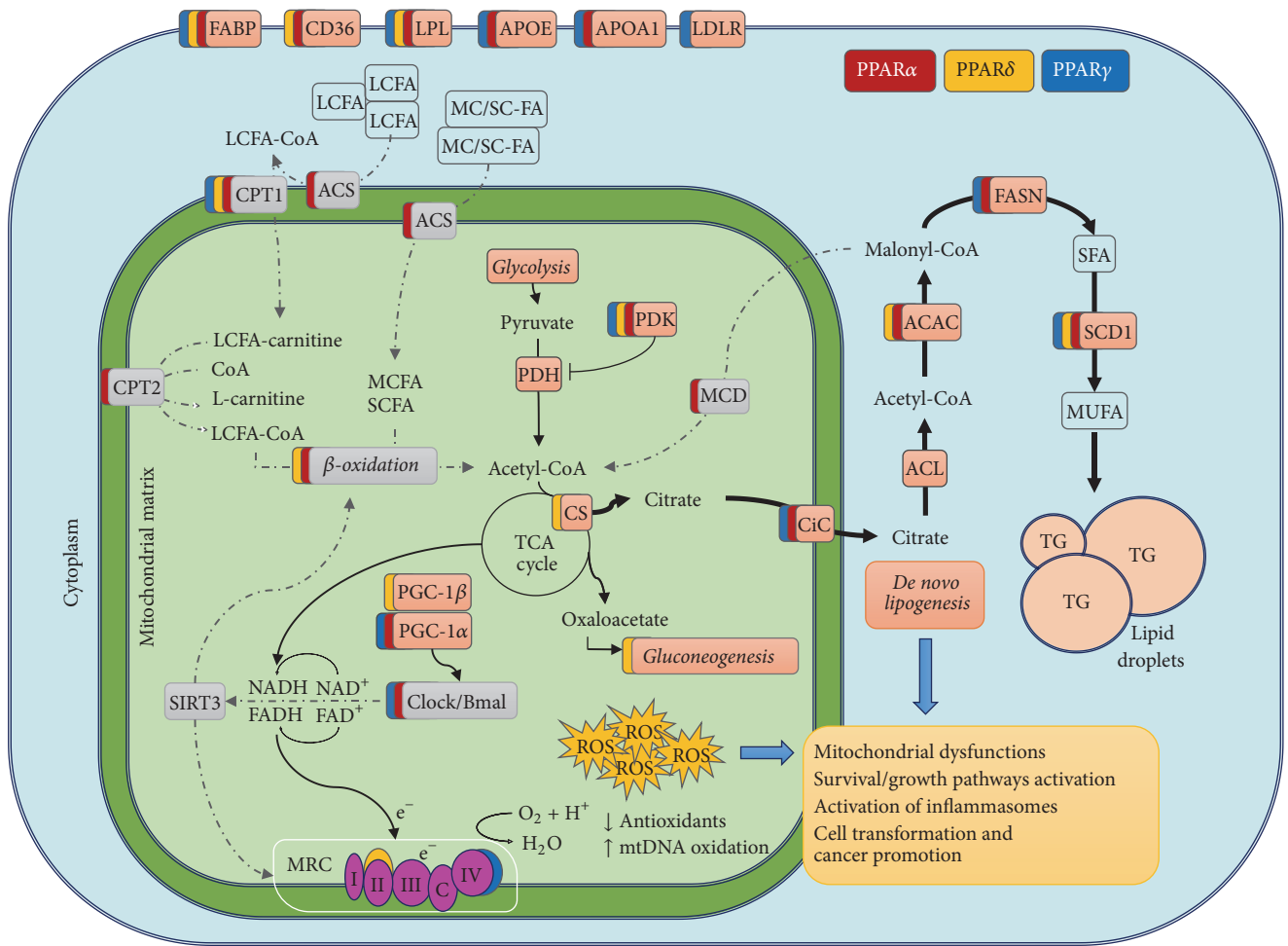
FIGURE 2: Altered mitochondrial metabolism in NASH and HCC: role of PPARs. Altered PPARs expression drives metabolic dysfunctions in the mitochondria leading to suppression of FAO, disruption of circadian rhythms, increased ROS levels, and upregulation of de novo lipogenesis. Color-coding depicts PPAR isotypes-dependency of target genes.

The PPARα agonists peroxisome proliferators exhibit liver cancerogenic activity when chronically administered in mice. The tumor promoting activity has been related to massive proliferation of peroxisomes, with consequent oxidative stress, and to inhibition of let-7c, a microRNA that represses c-myc expression [92]. Long-term HCC development was also found to be dependent with sustained PPARα activation in a transgenic model overexpressing the HCV core protein [93]. However, humans are resistant to peroxisome proliferation and indeed no association between fibrates and increased risk of any cancer has ever been found [94, 95].

Recently, PPARα$^{-/-}$ mice were found to be more susceptible to DEN-induced HCC, and PPARα anticancer activity was shown to be mediated by NF-kB inhibition [96].

Interestingly, PPARα regulation of mitochondrial metabolism may be exploited for cancer treatment. Many cancer types exhibit highly glycolytic metabolism, and cancer cell's mitochondria have a strong commitment toward anabolism and cataplerosis. Since TCA intermediates are used mainly in biosynthetic reactions, mitochondria of cancer cells often have scarce OXPHOS and rely mainly on glycolysis for ATP production. Activation of PPARα induces pyruvate dehydrogenase kinase 4 (PDK4) [97], which inhibits the pyruvate dehydrogenase complex, thus preventing pyruvate from glycolysis to enter mitochondria for acetyl-CoA synthesis and anaplerosis. The net result is the blockage of TCA and fatty acid synthesis, which requires acetyl-CoA, and the slowing-down of glycolytic rate [98].

Activation of PPARα suppresses anaplerosis from glutamine, by repressing the expression of glutaminase and glutamate dehydrogenase, thus potentially counteracting c-myc-dependent activation of glutaminolysis in tumor [97].

Therefore, the transrepression activity of PPARα on lipid biosynthesis and anaplerosis is just as relevant as its transactivation activity on FAO genes. The transrepression activity of PPARα indeed impacts on mitochondria metabolism through SIRT1, by competing with ERR transcriptional pathway [99]. Interestingly, Pawlak and colleagues recently showed that the transrepression activity of PPARα also regulates the inflammatory response in liver, preventing transition from NAFLD to NASH and fibrosis, and occurs

independently on PPARα DNA binding activity and its lipid handling properties [100].

A very recent report established a direct connection between PPARα-driven FAO and hepatocyte proliferation. CyclinD1, expressed in proliferating cells and a typical protooncogene, was found to inhibit PPARα expression, thereby reducing β-oxidation, both in normal hepatocytes and in HCC cells lines. This link was confirmed also in liver after partial hepatectomy, where induction of CyclinD1 timed with a reduction of PPARα and its target genes [101].

4.2. PPARβ/δ.

As summarized above, PPARβ/δ functions significantly overlap with PPARα in peripheral tissues, while in the liver its functions are more closely related to PPARγ regulated processes.

In genetic mice model of NAFLD (ob/ob), adenoviral overexpression of PPARβ/δ reduced the lipogenic program activated by SREBP-1c, via downregulation of the SREBP-1c activator insig-1, thus ameliorating hepatic steatosis [102]. Conversely, increased activation of SREBP-1c was found in PPARβ/δ$^{-/-}$ versus WT mice, fed either a control or ethanol liquid diet [103], suggesting that PPARβ/δ may play a role in suppressing the lipogenic pathway trough SREBP-1c.

In another study, adenoviral-mediated overexpression of PPARβ/δ in hepatocytes improved glucose utilization and hepatic insulin sensitivity. After overnight fasting, PPARβ/δ overexpressing livers had higher triglyceride and glycogen content than wild-type mice, while fatty acids and cholesterol level were similar [38]. Moreover, adenoviral-mediated overexpression in C57/BL6 mice induced SREBP-1c and PGC-1β expression. PPARβ/δ overexpression protected mice liver from fatty acid overload by promoting (i) FA conversion into nontoxic MUFA and (ii) FA storage into lipid droplets as triglycerides (Figure 2). As a result, activation of inflammatory pathways by FA overload was reduced in PPARβ/δ overexpressing mice fed HFD although steatosis was increased [38]. Treatment of db/db mice with the high affinity PPARβ/δ ligand GW501516 resulted in marked increase of genes involved in fatty acids synthesis and pentose phosphate pathways, promoting FA synthesis in the liver (in parallel with FA oxidation in muscle) [104].

These discrepancies are difficult to reconcile and might be related to the different mice model used, although in both genetic and dietary models PPARβ/δ has been shown to either promote or inhibit liver lipogenesis. Moreover, PPARβ/δ inhibits hepatic FGF21 expression [105], while PPARα is a potent activator of FGF21 [20]. Since FGF21 is known to inhibit SREBP-1c and several other lipogenic genes in the liver [106, 107], the potential cross-talk of different PPAR isotypes on FGF21 may contribute to eliciting context-dependent effects.

Despite these striking differences, activation of PPARβ/δ consistently resulted in a beneficial effect on liver damage.

Pharmacological activation of PPARβ/δ has been explored in several rodents and human studies. Administration of PPARβ/δ agonists improved hepatic steatosis and reduced insulin resistance and hepatic inflammation [71, 108–111].

Consistently, PPARβ/δ$^{-/-}$ mice were prone to inflammation derived liver damage.

In humans, PPARβ/δ agonists for NASH treatment are currently under investigation in clinical trials. The first evidence in men was obtained with GW501516, which proved to be equal to the PPARα agonist GW590735 in reducing plasma triglycerides levels and superior to the PPARα agonist in reducing cholesterol LDL, apolipoprotein B, liver fat content, and urinary isoprostane [112]. More recently, the PPARβ/δ agonist MBX-8025 was tested in 181 dyslipidemic patients in combination with atorvastatin or alone. MBX-8025 proved effective in reducing apolipoprotein B levels, non-HDL-cholesterol, triglycerides, free fatty acids, and high-sensitive C-reactive protein [113].

PPARβ/δ-driven mitochondriogenesis has been implicated in the differentiation of hepatic-like tissue from mouse of ES cells [41]. At the early phase of differentiation, a transitory upregulation of PPARα was observed, which resulted in induction of PGC-1α and mitochondriogenesis. Instead, the late phase of differentiation required a robust and sustained expression of PPARβ/δ, which was timely associated with albumin expression and acquisition of high mitochondrial membrane potential. PPARβ/δ agonists L165041 promoted differentiation into hepatic-like tissue that was abolished by PPARβ/δ inhibitor GSK0660 [41]. Therefore, PPARβ/δ may promote terminal hepatocyte differentiation associated with acquisition of mature mitochondria metabolism and function.

Indeed, PPARβ/δ$^{-/-}$ mice show a delay in liver regeneration after partial hepatectomy, associated with lack of Akt activation, lack of induction of glycolytic and lipogenic genes, and suppression of E2F transcription factors activation [114].

Interestingly, PPARβ/δ was associated with nonproliferating hepatocytes in a gene signature analysis of nuclear receptor in proliferating livers and HCC [115]. The authors analyzed the expression of all 49 members of the nuclear receptor superfamily in regenerating mouse liver and PPARβ/δ (together with TRα and FXRβ) was found consistently downregulated throughout the process. PPARβ/δ was found significantly reduced in a small series of HCC with respect to the surrounding nontumoral tissue and the PPARβ/δ agonist GW501516 suppressed CyclinD1 expression and cell proliferation in Hepa1-6 cells [115]. However, whether PPARβ/δ agonists suppress HCC cells growth is still controversial [116, 117]. Both PPARβ/δ and PPARγ have been implicated in mediating beta-catenin-Tcf/lef signaling [118].

Recently, PPARβ/δ was identified as a target gene of FHL2, a tumor suppressor gene also involved in hepatocellular carcinoma [119, 120].

4.3. PPARγ.

The effectiveness of the insulin sensitizers TZD in ameliorating the lipidemic profile, inflammation, and steatosis in T2DM patients is well established. Several clinical trials have explored the potential of TZDs in the treatment of NASH and have recently been reviewed [121, 122].

A recent meta-analysis of RCT on TZD and NASH (3 with pioglitazone, 1 with rosiglitazone) confirmed the effectiveness of TZD in improving steatosis, necroinflammation, and

hepatocyte ballooning [123]. A significant improvement in fibrosis was obtained only when the analysis was restricted to the pioglitazone studies only. Rosiglitazone failed to improve necroinflammation, ballooning, and fibrosis in the 1-year FLIRT trial [124] and even when treatment was extended for additionally 2 years [125]. Combinatory treatment of rosiglitazone with metformin or losartan did not improve the histological endpoint versus rosiglitazone alone [126]. A very recent report suggests that rosiglitazone administration may exert opposite outcome on liver steatosis depending on liver PPARγ expression levels: RGZ worsen steatosis in PPARγ overexpressing mice fed a HFD and protected mice with low PPARγ expression level [121, 127].

PPARγ is indeed markedly overexpressed in the liver of obese patients with NAFLD and NASH, and its expression positively correlates with plasma insulin, HOMA-IR, and SREBP1-c mRNA levels and inversely correlates with adiponectin [128]. High PPARγ levels, in particular of PPARγ2, promotes de novo lipogenesis and liver steatosis and is associated with HFD feeding in mice [129–131]. However as recalled above, induction of PPARγ by TZD, in particular pioglitazone, ameliorates steatosis and NASH. This discrepancy may be interpreted in the light of the double nature of PPARγ target genes, which comprises both genes of de novo lipid synthesis and mitochondrial genes promoting FAO [132]. Moreover, pioglitazone also binds and activates PPARα with low potency [133], which could explain its better performance than rosiglitazone in ameliorating steatosis. Mechanistically, induction of PPARγ in steatotic hepatocytes may serve as a protective mechanism to reduce liver FFA levels by storing them as less toxic triglycerides [134, 135]. Therefore, the prosteatotic action of PPARγ [136] may not be entirely detrimental. However, excess triglyceride accumulation eventually results in hepatocyte ballooning and necroinflammation, promoting transition to NASH.

The role of PPARγ in hepatocellular carcinoma is still debated. A large body of literature on PPARγ and cancer was produced using TZD, which eventually were proved to have several anticancer pleiotropic effects also independently of PPARγ [137–140].

We and others have investigated the role of PPARγ on hepatocarcinogenesis in mice harboring a hepatocyte specific deletion of PPARγ gene (PPAR$\gamma^{hep-/-}$ mice). Yu and colleagues found increased DEN-induced HCC in mice lacking one PPARγ allele, thus suggesting a tumor-suppression function for PPARγ [141]. Moreover, RGZ reduced HCC development in DEN-treated WT mice but not in PPAR$\gamma^{+/-}$ mice [141]. Using a transgenic model of HBV-related HCC, we found that RGZ or PGZ effectively reduced HCC onset [142]. Strikingly, TZD treatment resulted more effective in PPAR$\gamma^{hep-/-}$ mice than in WT mice [142], highlighting that (i) TZD antitumor activity is independent of PPARγ; (ii) PPARγ expression reduced TZD activity; therefore in this model PPARγ may support, rather than inhibiting, tumor growth.

As the master regulator of adipogenic differentiation, PPARγ has been described to promote differentiation programs in a variety of tumor cell types [143, 144],

inducing cell-cycle arrest [145], apoptosis/anoikis [146–148], and inhibiting EMT [149, 150], angiogenesis [151], and metastasis [152].

However, several lines of evidence also support the notion that this nuclear receptor may support the growth in several cancer types. Conflicting results have been reported in breast cancer model. Recently, Avena et al. showed that breast cancer growth was inhibited by PPARγ overexpression epithelial cancer cells but promoted by PPARγ overexpression in cancer associated stroma [153]. The authors identify the tumor promoting role of PPARγ in the metabolic symbiosis between stoma and epithelial cancer cells, where cancer associated fibroblasts provided intermediates for mitochondrial metabolism to cancer cells [153]. Moreover, increased de novo lipogenesis, that is promoted by PPARγ, is now recognized as a metabolic hallmark of cancer cell [154], including HCC [155–159] (Figure 2). Indeed, de novo lipogenesis is activated downstream of the Akt/mTOR pathway, one of the most common signaling pathways altered in cancer. Forced activation of Akt/mTOR induces liver cancer [160, 161], a process mediated at least in part by activation of FASN [155, 156]. Consistently, inactivation of FASN was recently shown to completely inhibit Akt-driven HCC in mice [158]. Importantly, FASN is not oncogenic per se. However, when the PI3K/Akt/mTOR pathway becomes hyperactive, the induction of the de novo lipogenesis is a requisite for supporting cancer cell growth. Importantly, PPARγ is a direct transcriptional target of mTORC1 [162]. Moreover, in PTEN null mice PPARγ was found to directly induce the expression of key glycolytic gene HK and oncogenic PKM2, inducing hepatocyte steatosis, hypertrophy, and hyperplasia [163].

Therefore, PPARγ may inhibit or promote HCC development depending on the metabolic context, the cell type expressing it, the oncogenic signaling pathways involved, and dietary or pharmacological treatment. It is however conceptually very attractive to explore the therapeutic potential interference with the cancer cell lipid handling capacity, through modulation of mitochondrial FA, ketogenesis, and lipogenesis, as an integrated anticancer approach.

5. PPARs and Circadian Regulation of Mitochondria Metabolism

Many processes of our metabolism and physiology are regulated by circadian clocks, endogenous time-tracking systems that coordinate daily rhythms of rest, activity, feeding behavior, energy utilization, and storage. Although circadian rhythms are endogenous they respond to external stimuli, which include light, temperature, and redox cycles [164]. Circadian regulation is coordinated by the suprachiasmatic nucleus in the brain, but most peripheral organs contain their own independent pacemakers [165]. At a cellular level these oscillations are driven by transcriptional feedback loops associated with changes in chromatin remodeling, mRNA processing, protein turnover, and activity [166–169]. Main factors that control circadian rhythmicity in the cells include BMAL1 and CLOCK ("activators") and CRYs and PERs ("inhibitors"). Their effects are tissue-specific and in the liver

they control approximately 10% of the transcriptome [170], influencing metabolic pathways by modifying the expression or activity of key enzymes and transporters involved in lipid, glucose, and mitochondrial oxidative metabolism. Reciprocally, intracellular metabolites and transcriptional factors modulate CLOCK activity in response to the energy status.

Circadian dysregulation of lipid metabolism, ROS production, and cell-cycle control is linked to various pathological conditions including metabolic syndrome, diabetes, chronic liver diseases, and cancer [171–173].

5.1. Clock and Lipid Metabolism: Regulation of PPARs and Mitochondrial Functions.

The redox state of the cell also seems to play an important part in the rhythmicity of metabolism, especially in the mitochondria. NAD+ levels oscillate and are under direct control of clock transcription factors that upregulate the rate-limiting enzyme in NAD^+ biosynthesis, NAMPT (nicotinamide phosphoribosyl transferase). In mitochondria NAD+ activates SIRT3, an important regulator of intrinsic mitochondrial function including FAO. In the cytoplasm NAD+ activates SIRT1 that operates a small feedback regulating Clock and Bmal. Disruption of circadian rhythms in mice leads to defects in mtFAO and decreased OCR mainly through deregulation of NAD+ dependent SIRT3 activity [174, 175] (Figure 2).

Several genes involved in lipid metabolism (such as SREBP, HMGCoAR, and FAS) are modulated by PPARα and display circadian fluctuations that are lost in PPARα-KO mice [176, 177].

PPARα is a direct transcriptional target of BMAL1 and CLOCK [178–180] and in the rodent liver operates a feedback loop binding BMAL1 and REV-ERBα gene promoters. BMAL1-KO and CLOCK-mutant mice display abolished PPARα oscillation and decreased expression in the liver, whereas PPARα-KO mice display altered oscillation of PER3 and BMAL1 [181]. Moreover, administration of PPARα agonists fenofibrates upregulates the expression of *Bmal1* in mouse liver [180].

Fatty acids are known to be PPARα activators, binding directly to the transcriptional factor. Interestingly, hepatic fatty acids are also produced in a circadian manner by acyl-CoA thioesterases (ACOTs) and lipoprotein lipases (LPLs).

The expression of both enzyme families displays circadian rhythmicity; it is regulated by PPARα and can in fact be induced by WY14643. Moreover, silencing members of ACOTs lead to a downregulation of Cyp4a10 and Cyp4a14, PPARa targets [182–186].

Another clock controlled gene, Nocturnin, binds to PPARγ modulating its transcriptional activity [187], and PPARγ systemic inactivation in mice leads to impaired rhythmicity of the canonical clock genes in liver and adipose tissues [188]. PGC-1α is also rhythmically expressed in mouse liver and muscle, upregulates circadian factors BMAL1, CLOCK, and REV-ERBα [189], and modulates the length of circadian oscillations by controlling Bmal1 transcription in a REV-ERB-dependent manner. Mice lacking PGC-1α show abnormal circadian rhythms and altered expression of metabolic genes [189]. Interestingly, circadian regulation was lost also in

mice lacking PGC-1β, but this resulted in markedly decreased activity during the dark cycle, as opposed to the hyperactive PGC-1α KO mice [190] (Figure 2).

The liver-specific deletion of PPARδ in mice showed that it is involved in the temporal regulation of several lipogenic genes, such as fatty acid synthase (FAS) and acetyl-CoA carboxylase 1 and acetyl-CoA carboxylase 2 [40]. BMAL1 also induces the expression of REV-ERBα, a nuclear receptor that downregulates BMAL1 itself, operating a negative feedback, and upregulates the expression of a liver-specific microRNA: miR-122 [191]. miR-122 is also involved in lipid metabolism in mouse liver [192] and PPARδ was proven to be one of its targets, suggesting that PPARδ plays a role in hepatic circadian regulation [193].

The circadian regulation of mitochondrial metabolism is still in its early days. Using a MS-based proteomic approach, the expression of rate-limiting enzymes and metabolites in mitochondria was quantitatively evaluated throughout the day [194]. Many key mitochondrial enzymes involved in carbohydrates and lipid metabolism were found to peak in the early morning period and to be regulated by PER2/3 proteins. Mitochondrial respiration displayed an oscillatory behavior, peaking several times of the day. In mice KO for Per2/3, as well as in those fed a HFD, period protein oscillation was lost, together with OXPHOS oscillation [194].

5.2. Circadian Disturbances in Liver Disease.

It is now clear that circadian rhythms are fundamental in liver physiology and their disruption is observed in many hepatic pathologic conditions, such as NASH, NAFLD, ALD, and HCC [110, 172, 195–198].

In a mouse model of NASH it was found that HFD induces the susceptibility to develop NASH through desynchronized Clock gene expression and altered cellular redox status, accompanied by reduced sirtuin abundance [197]. HFD in mice is sufficient to induce the loss of circadian fluctuations of insulin secretion [199]. Conversely, BMAL1 whole body-KO mice and Clock-mutant mice display hepatic steatosis, obesity, hypoinsulinemia, and increased glucose intolerance [200].

The molecular alterations found in the liver of HFD-fed mice include loss of oscillation or phase advance of rhythmicity of many genes involved in lipid and mitochondrial metabolism (such as NAMPT, acetyl-coenzyme A synthetase, and ornithine decarboxylase 1) and gain of oscillation of other genes such as PPARγ and its targets [201]. This transcriptional reprogramming relies on changes in the oscillation and chromatin recruitment of PPARγ that also induces the oscillation of *Cidec* (cell death activator CIDE-3) [201], a protein that is substantially elevated in the livers of the obese ob/ob mice [202]. Administration of GW9662, a specific PPARγ antagonist, into HFD-fed animals produced a decrease in PPARγ-induced Cidec expression [201]. The expression of another known PPARγ target, pyruvate carboxylase (Pcx), an important regulator of hepatic gluconeogenesis, was significantly elevated and rhythmic in livers of HFD-fed mice [201]. In Nocturnin-KO mice fed with HFD, liver PPARγ oscillation was abolished, accompanied by a reduced expression of many

genes related to lipid metabolism and resistance to hepatic steatosis [203].

Accumulating evidence supports the importance of the disruption of circadian rhythms in various types in cancer. Specifically, in HCC patients, low expression of clock genes was observed in the cancerous tissue, but not in the non-cancerous liver tissue, and correlated with tumor size and tumor grade [204]. A number of mechanisms may explain the circadian control on HCC. For example, it was found that DEN exposure in mice is associated with circadian disturbance, suggesting that liver clocks are involved in the carcinogenesis [196]. Mutations and polymorphisms of the clock proteins are being screened to assess their association with HCC. Interestingly, a functional polymorphism of PER3 was recently associated with a lower risk of death in HCC patients treated with TACE [205].

6. Perspectives and Conclusions

It is now clear that expression or activation of nuclear receptors, including PPARs, is not sufficient to predict their biological output. The net effect of a nuclear receptor activation in a given cell actually depends on the context of coactivators, corepressors, dimerization events, availability of endogenous/synthetic ligands, posttranslational modifications, competition, and interactions with other NRs. This led to the development of partial agonist selective PPAR modulators (SPPARMs), a second generation of PPAR agonists able to selectively activate a subset of target genes downstream a specific PPAR isotype.

K-877 is a SPPARαM currently being tested in dyslipidemic patients that exhibits higher lipid lowering activity than fibrates and has a favorable risk profile [206, 207]. INT-131, SPPARγM, has potent glucose lowering effects not associated with TZD side-effects [208].

A different approach to PPAR modulation is to simultaneously activate, with different potency, more than one isotype: dual PPAR agonist or pan-agonists are currently under investigation. The dual PPARα/δ agonist GFT-505 is proving effective in reducing plasma triglyceride levels, improving insulin sensitivity, and increasing HDL-cholesterol in obese patients [209, 210] and showed promising results in mice model of NASH [211]. Very recently a phase 2 multicenter randomized controlled trial, enrolling 274 subjects with histologically proven NASH, showed that GFT505 produces a dose-dependent improvement in histology of patients with NASH [212].

As we gain knowledge of the metabolic circadian regulation and of its disruption in disease, an entire new area of intervention begins to emerge. Modulation of amplitude and phase of PPARs circadian regulation could be exploited to drive complex metabolic remodeling of mitochondrial metabolism in NASH and cancer models. Finally, the integration of the above-mentioned approaches with the metabolic and genetic profiling of cancers holds the promise for new therapeutic approaches that can selectively target the fuel requirements of HCC.

Abbreviations

ACAA2:	Acetyl-CoA acyltransferase 2
ACADM:	Medium-chain specific acyl-CoA dehydrogenase
ACADs:	Acyl-CoA dehydrogenases
ACADVL:	Very long-chain specific acyl-CoA dehydrogenase
ACC1 and ACC2:	Acetyl-CoA carboxylase 1 and acetyl-CoA carboxylase 2
ACS:	Acetyl-coenzyme A synthetase
AdipoR2:	Adiponectin receptor 2
MAL1:	Aryl hydrocarbon receptor nuclear translocator-like protein 1
CPT-1 and CPT-2:	Carnitine palmitoyl transferase 1 and carnitine palmitoyl transferase 2
DAMPs:	Damage-associated molecular pattern
ERRalpha:	Estrogen related receptor alpha
ETC:	Electron transport chain
FAO:	Fatty acid oxidation
FAS:	Fatty acid synthase
FAT/CD36:	Fatty acid translocase
FHL2:	Four and a half LIM domains protein 2
FXR:	Farnesoid X receptor
GK:	Glycerol kinase
GLUT2:	Glucose transporter 2
GLUT-4:	Glut transporter 4
HADHA and HADHB:	Trifunctional enzyme subunit alpha and beta
H-FABP:	Heart-type fatty acid-binding protein
HMGCoAR:	3-hydroxy-3-methylglutaryl-CoA reductase
HNF-4:	Hepatocyte nuclear factor 4-alpha
IRS-1, IRS-2:	Insulin receptor substrate 1 and insulin receptor substrate 2
JNK:	Jun N-terminal kinase
LC-FA:	Long-chain fatty acids
LPL:	Lipoprotein lipase
LXR:	Liver X receptor
M/S-FA:	Medium/short chain fatty acids
MAPK:	Mitogen-activated protein kinase 1
MCD:	Methionine and choline deficient diet
MDA:	Malondialdehyde

MTP:	Mitochondrial trifunctional protein
MUFA:	Monounsaturated fatty acids
NAD+:	Nicotinamide adenine dinucleotide
NAMPT:	Nicotinamide phosphoribosyltransferase
NCOR:	Nuclear receptor corepressor 1
NF-κB:	Nuclear factor NF-kappa-B
PAMPs:	Pathogen associated molecular patterns
PDK4:	Pyruvate dehydrogenase kinase 4
PEPCK:	Phosphoenolpyruvate carboxykinase
PGC-1α/β:	Peroxisome proliferator activated receptor gamma coactivator 1 α/β
PI3K:	Phosphatidylinositol 3-kinase
PKM2:	Pyruvate kinase M2
PTEN:	Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase
REV-ERBa:	Nuclear receptor subfamily 1 group D member 1
SCD1:	Acyl-CoA desaturase 1
SFA:	Saturated fatty acids
SIRT-1 andSIRT-3:	NAD-dependent protein deacetylase sirtuin-1 andNAD-dependent protein deacetylase sirtuin-3
SOD:	Superoxide dismutase
TRα:	Thyroid hormone receptor alpha (TR-alpha)
UCP-1, UCP-2, and UCP-3:	Mitochondrial uncoupling protein 3
4-HNE:	4-hydroxynonenal.

Competing Interests

The authors declare that they have no competing interests.

References

[1] J. Ferlay, I. Soerjomataram, M. Ervik et al., *GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide*, IARC CancerBase no. 11, International Agency for Research on Cancer, 2013, http://globocan.iarc.fr.

[2] H. B. El-Serag, "Hepatocellular carcinoma," *New England Journal of Medicine*, vol. 365, no. 12, pp. 1118–1127, 2011.

[3] Z. M. Younossi, A. B. Koenig, D. Abdelatif, Y. Fazel, L. Henry, and M. Wymer, "Global epidemiology of nonalcoholic fatty liver disease-meta-analytic assessment of prevalence, incidence, and outcomes," *Hepatology*, vol. 64, no. 1, pp. 73–84, 2016.

[4] B. Q. Starley, C. J. Calcagno, and S. A. Harrison, "Nonalcoholic fatty liver disease and hepatocellular carcinoma: a weighty connection," *Hepatology*, vol. 51, no. 5, pp. 1820–1832, 2010.

[5] H. B. El-Serag, T. Tran, and J. E. Everhart, "Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma," *Gastroenterology*, vol. 126, no. 2, pp. 460–468, 2004.

[6] N. Chalasani, Z. Younossi, J. E. Lavine et al., "The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association," *Hepatology*, vol. 55, no. 6, pp. 2005–2023, 2012.

[7] M. Masarone, A. Federico, L. Abenavoli, C. Loguercio, and M. Persico, "Non alcoholic fatty liver: epidemiology and natural history," *Reviews on Recent Clinical Trials*, vol. 9, no. 3, pp. 126–133, 2014.

[8] N. Kawada, K. Imanaka, T. Kawaguchi et al., "Hepatocellular carcinoma arising from non-cirrhotic nonalcoholic steatohepatitis," *Journal of Gastroenterology*, vol. 44, no. 12, pp. 1190–1194, 2009.

[9] O. Warburg, "On the origin of cancer cells," *Science*, vol. 123, no. 3191, pp. 309–314, 1956.

[10] O. warburg, "On respiratory impairment in cancer cells," *Science*, vol. 124, no. 3215, pp. 269–270, 1956.

[11] C. S. Ahn and C. M. Metallo, "Mitochondria as biosynthetic factories for cancer proliferation," *Cancer & Metabolism*, vol. 3, no. 1, article 1, 2015.

[12] A. W. F. Janssen, B. Betzel, G. Stoopen et al., "The impact of PPARα activation on whole genome gene expression in human precision cut liver slices," *BMC Genomics*, vol. 16, no. 1, article 760, 2015.

[13] M. Rakhshandehroo, G. Hooiveld, M. Müller, and S. Kersten, "Comparative analysis of gene regulation by the transcription factor PPARα between mouse and human," *PLoS ONE*, vol. 4, no. 8, Article ID e6796, 2009.

[14] W. Wahli and L. Michalik, "PPARs at the crossroads of lipid signaling and inflammation," *Trends in Endocrinology and Metabolism*, vol. 23, no. 7, pp. 351–363, 2012.

[15] M. V. Chakravarthy, Z. Pan, Y. Zhu et al., "'New' hepatic fat activates PPARα to maintain glucose, lipid, and cholesterol homeostasis," *Cell Metabolism*, vol. 1, no. 5, pp. 309–322, 2005.

[16] D. Patsouris, J. K. Reddy, M. Müller, and S. Kersten, "Peroxisome proliferator-activated receptor α mediates the effects of high-fat diet on hepatic gene expression," *Endocrinology*, vol. 147, no. 3, pp. 1508–1516, 2006.

[17] P. G. P. Martin, H. Guillou, F. Lasserre et al., "Novel aspects of PPARα-mediated regulation of lipid and xenobiotic metabolism revealed through a nutrigenomic study," *Hepatology*, vol. 45, no. 3, pp. 767–777, 2007.

[18] L. M. Sanderson, P. J. de Groot, G. J. E. J. Hooiveld et al., "Effect of synthetic dietary triglycerides: a novel research paradigm for nutrigenomics," *PLoS ONE*, vol. 3, no. 2, Article ID e1681, 2008.

[19] S. Kersten, J. Seydoux, J. M. Peters, F. J. Gonzalez, B. Desvergne, and W. Wahli, "Peroxisome proliferator-activated receptor α mediates the adaptive response to fasting," *Journal of Clinical Investigation*, vol. 103, no. 11, pp. 1489–1498, 1999.

[20] A. Montagner, A. Polizzi, E. Fouche et al., "Liver PPARalpha is crucial for whole-body fatty acid homeostasis and is protective against NAFLD," *Gut*, vol. 65, no. 7, pp. 1202–1214, 2016.

[21] M. V. Chakravarthy, I. J. Lodhi, L. Yin et al., "Identification of a Physiologically Relevant Endogenous Ligand for PPARα in Liver," *Cell*, vol. 138, no. 3, pp. 476–488, 2009.

[22] T. Yamauchi, J. Kamon, Y. Ito et al., "Cloning of adiponectin receptors that mediate antidiabetic metabolic effects," *Nature*, vol. 423, no. 6941, pp. 762–769, 2003.

[23] P. Iglesias, R. Selgas, S. Romero, and J. J. Díez, "Biological role, clinical significance, and therapeutic possibilities of the recently discovered metabolic hormone fibroblastic growth factor 21," *European Journal of Endocrinology*, vol. 167, no. 3, pp. 301–309, 2012.

[24] F. M. Fisher, P. C. Chui, I. A. Nasser et al., "Fibroblast growth factor 21 limits lipotoxicity by promoting hepatic fatty acid activation in mice on methionine and choline-deficient diets," *Gastroenterology*, vol. 147, no. 5, pp. 1073.e6–1083.e6, 2014.

[25] E. Szalowska, H. A. Tesfay, S. A. van Hijum, and S. Kersten, "Transcriptomic signatures of peroxisome proliferator-activated receptor α (PPARα) in different mouse liver models identify novel aspects of its biology," *BMC Genomics*, vol. 15, no. 1, article 1106, 2014.

[26] S. Kersten, M. Rakhshandehroo, B. Knoch, and M. Müller, "Peroxisome proliferator-activated receptor alpha target genes," *PPAR Research*, Article ID 612089, 2010.

[27] D. G. Cotter, B. Ercal, X. Huang et al., "Ketogenesis prevents diet-induced fatty liver injury and hyperglycemia," *The Journal of Clinical Investigation*, vol. 124, no. 12, pp. 5175–5190, 2014.

[28] Y. Wang, A.-W. Mohsen, S. J. Mihalik, E. S. Goetzman, and J. Vockley, "Evidence for physical association of mitochondrial fatty acid oxidation and oxidative phosphorylation complexes," *Journal of Biological Chemistry*, vol. 285, no. 39, pp. 29834–29841, 2010.

[29] F. Villarroya, R. Iglesias, and M. Giralt, "PPARs in the control of uncoupling proteins gene expression," *PPAR Research*, vol. 2007, Article ID 74364, 12 pages, 2007.

[30] L. J. Kelly, P. P. Vicario, G. M. Thompson et al., "Peroxisome proliferator-activated receptors γ and α mediate in vivo regulation of uncoupling protein (UCP-1, UCP-2, UCP-3) gene expression," *Endocrinology*, vol. 139, no. 12, pp. 4920–4927, 1998.

[31] G. Y. Lee, N. H. Kim, Z.-S. Zhao, B. S. Cha, and Y. S. Kim, "Peroxisomal-proliferator-activated receptor α activates transcription of the rat hepatic malonyl-CoA decarboxylase gene: a key regulation of malonyl-CoA level," *Biochemical Journal*, vol. 378, no. 3, pp. 983–990, 2004.

[32] L. Cheng, G. Ding, Q. Qin et al., "Cardiomyocyte-restricted peroxisome proliferator-activated receptor-δ deletion perturbs myocardial fatty acid oxidation and leads to cardiomyopathy," *Nature Medicine*, vol. 10, no. 11, pp. 1245–1250, 2004.

[33] D. M. Muoio, P. S. MacLean, D. B. Lang et al., "Fatty acid homeostasis and induction of lipid regulatory genes in skeletal muscles of peroxisome proliferator-activated receptor (PPAR) α knock-out mice. Evidence for compensatory regulation by PPARδ," *Journal of Biological Chemistry*, vol. 277, no. 29, pp. 26089–26097, 2002.

[34] U. Dressel, T. L. Allen, J. B. Pippal, P. R. Rohde, P. Lau, and G. E. O. Muscat, "The peroxisome proliferator-activated receptor β/δ agonist, GW501516, regulates the expression of genes involved in lipid catabolism and energy uncoupling in skeletal muscle cells," *Molecular Endocrinology*, vol. 17, no. 12, pp. 2477–2493, 2003.

[35] B. Brunmair, K. Staniek, J. Dörig et al., "Activation of PPAR-delta in isolated rat skeletal muscle switches fuel preference from glucose to fatty acids," *Diabetologia*, vol. 49, no. 11, pp. 2713–2722, 2006.

[36] L. Jiang, J. Wan, L.-Q. Ke, Q.-G. Lü, and N.-W. Tong, "Activation of PPARδ promotes mitochondrial energy metabolism and decreases basal insulin secretion in palmitate-treated β-cells," *Molecular and Cellular Biochemistry*, vol. 343, no. 1-2, pp. 249–256, 2010.

[37] M. C. Manio, K. Inoue, M. Fujitani, S. Matsumura, and T. Fushiki, "Combined pharmacological activation of AMPK and PPARδ potentiates the effects of exercise in trained mice," *Physiological Reports*, vol. 4, no. 5, Article ID e12625, 2016.

[38] 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.

[39] 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.

[40] S. Liu, J. D. Brown, K. J. Stanya et al., "A diurnal serum lipid integrates hepatic lipogenesis and peripheral fatty acid use," *Nature*, vol. 502, no. 7472, pp. 550–554, 2013.

[41] D.-Y. Zhu, J.-Y. Wu, H. Li et al., "PPAR-β facilitating maturation of hepatic-like tissue derived from mouse embryonic stem cells accompanied by mitochondriogenesis and membrane potential retention," *Journal of Cellular Biochemistry*, vol. 109, no. 3, pp. 498–508, 2010.

[42] E. Hondares, M. Rosell, J. Díaz-Delfín et al., "Peroxisome proliferator-activated receptor α (PPARα) induces PPARγ coactivator 1α (PGC-1α) gene expression and contributes to thermogenic activation of brown fat: involvement of PRDM16," *Journal of Biological Chemistry*, vol. 286, no. 50, pp. 43112–43122, 2011.

[43] M. Schuler, F. Ali, C. Chambon et al., "PGC1α expression is controlled in skeletal muscles by PPARβ, whose ablation results in fiber-type switching, obesity, and type 2 diabetes," *Cell Metabolism*, vol. 4, no. 5, pp. 407–414, 2006.

[44] E. Hondares, I. Pineda-Torra, R. Iglesias, B. Staels, F. Villarroya, and M. Giralt, "PPARδ, but not PPARα, activates PGC-1α gene transcription in muscle," *Biochemical and Biophysical Research Communications*, vol. 354, no. 4, pp. 1021–1027, 2007.

[45] S. Herzig, F. Long, U. S. Jhala et al., "CREB regulates hepatic gluconeogenesis through the coactivator PGC-1," *Nature*, vol. 413, no. 6852, pp. 179–183, 2001.

[46] M. T. Nakamura, B. E. Yudell, and J. J. Loor, "Regulation of energy metabolism by long-chain fatty acids," *Progress in Lipid Research*, vol. 53, no. 1, pp. 124–144, 2014.

[47] 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.

[48] R. E. Soccio, E. R. Chen, and M. A. Lazar, "Thiazolidinediones and the promise of insulin sensitization in type 2 diabetes," *Cell Metabolism*, vol. 20, no. 4, pp. 573–591, 2014.

[49] M. Ahmadian, J. M. Suh, N. Hah et al., "PPARγ signaling and metabolism: the good, the bad and the future," *Nature Medicine*, vol. 19, no. 5, pp. 557–566, 2013.

[50] 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.

[51] K. Matsusue, M. Haluzik, G. Lambert et al., "Liver-specific disruption of PPARγ in leptin-deficient mice improves fatty liver but aggravates diabetic phenotypes," *The Journal of Clinical Investigation*, vol. 111, no. 5, pp. 737–747, 2003.

[52] P. Puigserver, Z. Wu, C. W. Park, R. Graves, M. Wright, and B. M. Spiegelman, "A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis," *Cell*, vol. 92, no. 6, pp. 829–839, 1998.

[53] J. St-Pierre, J. Lin, S. Krauss et al., "Bioenergetic analysis of peroxisome proliferator-activated receptor γ coactivators 1α

and 1β (PGC-1α and PGC-1β) in muscle cells," *The Journal of Biological Chemistry*, vol. 278, no. 29, pp. 26597–26603, 2003.

[54] W. Fan and R. Evans, "PPARs and ERRs: molecular mediators of mitochondrial metabolism," *Current Opinion in Cell Biology*, vol. 33, pp. 49–54, 2015.

[55] E. Hondares, O. Mora, P. Yubero et al., "Thiazolidinediones and rexinoids induce peroxisome proliferator-activated receptor-coactivator (PGC)-1α gene transcription: an autoregulatory loop controls PGC-1α expression in adipocytes via peroxisome proliferator-activated receptor-γ coactivation," *Endocrinology*, vol. 147, no. 6, pp. 2829–2838, 2006.

[56] H. Maruyama, S. Kiyono, T. Kondo, T. Sekimoto, and O. Yokosuka, "Palmitate-induced regulation of PPARγ via PGC1α: a mechanism for lipid accumulation in the liver in nonalcoholic fatty liver disease," *International Journal of Medical Sciences*, vol. 13, no. 3, pp. 169–178, 2016.

[57] P. D. Ray, B.-W. Huang, and Y. Tsuji, "Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling," *Cellular Signalling*, vol. 24, no. 5, pp. 981–990, 2012.

[58] M. Schieber and N. S. Chandel, "ROS function in redox signaling and oxidative stress," *Current Biology*, vol. 24, no. 10, pp. R453–R462, 2014.

[59] L. Zeng, W. J. Tang, J. J. Yin, and B. J. Zhou, "Signal transductions and nonalcoholic fatty liver: a mini-review," *International Journal of Clinical and Experimental Medicine*, vol. 7, no. 7, pp. 1624–1631, 2014.

[60] I. Afanas'ev, "Signaling of reactive oxygen and nitrogen species in diabetes mellitus," *Oxidative Medicine and Cellular Longevity*, vol. 3, no. 6, pp. 361–373, 2010.

[61] I. Afanas'ev, "Reactive oxygen species signaling in cancer: comparison with aging," *Aging and Disease*, vol. 2, no. 3, pp. 219–230, 2011.

[62] J. St-Pierre, J. A. Buckingham, S. J. Roebuck, and M. D. Brand, "Topology of superoxide production from different sites in the mitochondrial electron transport chain," *The Journal of Biological Chemistry*, vol. 277, no. 47, pp. 44784–44790, 2002.

[63] E. B. Tahara, F. D. T. Navarete, and A. J. Kowaltowski, "Tissue-, substrate-, and site-specific characteristics of mitochondrial reactive oxygen species generation," *Free Radical Biology and Medicine*, vol. 46, no. 9, pp. 1283–1297, 2009.

[64] T. Mello, F. Zanieri, E. Ceni, and A. Galli, "Oxidative stress in the healthy and wounded hepatocyte: a cellular organelles perspective," *Oxidative Medicine and Cellular Longevity*, vol. 2016, Article ID 8327410, 15 pages, 2016.

[65] C. Ricci, V. Pastukh, J. Leonard et al., "Mitochondrial DNA damage triggers mitochondrial-superoxide generation and apoptosis," *American Journal of Physiology—Cell Physiology*, vol. 294, no. 2, pp. C413–C422, 2008.

[66] H. Kawahara, M. Fukura, M. Tsuchishima, and S. Takase, "Mutation of mitochondrial DNA in livers from patients with alcoholic hepatitis and nonalcoholic steatohepatitis," *Alcoholism: Clinical and Experimental Research*, vol. 31, supplement 1, pp. S54–S60, 2007.

[67] L. Llacuna, A. Fernández, C. V. Montfort et al., "Targeting cholesterol at different levels in the mevalonate pathway protects fatty liver against ischemia-reperfusion injury," *Journal of Hepatology*, vol. 54, no. 5, pp. 1002–1010, 2011.

[68] L. A. Videla, R. Rodrigo, M. Orellana et al., "Oxidative stress-related parameters in the liver of non-alcoholic fatty liver disease patients," *Clinical Science*, vol. 106, no. 3, pp. 261–268, 2004.

[69] J. Xiao and G. L. Tipoe, "Inflammasomes in non-alcoholic fatty liver disease," *Frontiers in Bioscience*, vol. 21, pp. 683–695, 2016.

[70] D. Sharma and T. Kanneganti, "The cell biology of inflammasomes: mechanisms of inflammasome activation and regulation," *The Journal of Cell Biology*, vol. 213, no. 6, pp. 617–629, 2016.

[71] M. Y. Lee, R. Choi, H. M. Kim et al., "Peroxisome proliferator-activated receptor delta agonist attenuates hepatic steatosis by anti-inflammatory mechanism," *Experimental & Molecular Medicine*, vol. 44, no. 10, pp. 578–585, 2012.

[72] S. Seki, T. Kitada, T. Yamada, H. Sakaguchi, K. Nakatani, and K. Wakasa, "In situ detection of lipid peroxidation and oxidative DNA damage in non-alcoholic fatty liver diseases," *Journal of Hepatology*, vol. 37, no. 1, pp. 56–62, 2002.

[73] S. H. Caldwell, R. H. Swerdlow, E. M. Khan et al., "Mitochondrial abnormalities in non-alcoholic steatohepatitis," *Journal of Hepatology*, vol. 31, no. 3, pp. 430–434, 1999.

[74] M. Pérez-Carreras, P. Del Hoyo, M. A. Martín et al., "Defective hepatic mitochondrial respiratory chain in patients with nonalcoholic steatohepatitis," *Hepatology*, vol. 38, no. 4, pp. 999–1007, 2003.

[75] R. Gambino, G. Musso, and M. Cassader, "Redox balance in the pathogenesis of nonalcoholic fatty liver disease: mechanisms and therapeutic opportunities," *Antioxidants and Redox Signaling*, vol. 15, no. 5, pp. 1325–1365, 2011.

[76] Y. Ikura, M. Ohsawa, T. Suekane et al., "Localization of oxidized phosphatidylcholine in nonalcoholic fatty liver disease: impact on disease progression," *Hepatology*, vol. 43, no. 3, pp. 506–514, 2006.

[77] S. Li, X.-Y. Zeng, X. Zhou et al., "Dietary cholesterol induces hepatic inflammation and blunts mitochondrial function in the liver of high-fat-fed mice," *Journal of Nutritional Biochemistry*, vol. 27, pp. 96–103, 2016.

[78] V. Ribas, C. García-Ruiz, and J. C. Fernández-Checa, "Mitochondria, cholesterol and cancer cell metabolism," *Clinical and Translational Medicine*, vol. 5, article 22, 2016.

[79] 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.

[80] E. Ip, G. Farrell, P. Hall, G. Robertson, and I. Leclercq, "Administration of the potent PPARα agonist, Wy-14,643, reverses nutritional fibrosis and steatohepatitis in mice," *Hepatology*, vol. 39, no. 5, pp. 1286–1296, 2004.

[81] P. Costet, C. Legendre, J. Moré, A. Edgar, P. Galtier, and T. Pineau, "Peroxisome proliferator-activated receptor α-isoform deficiency leads to progressive dyslipidemia with sexually dimorphic obesity and steatosis," *The Journal of Biological Chemistry*, vol. 273, no. 45, pp. 29577–29585, 1998.

[82] 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," *The Journal of Nutrition*, vol. 141, no. 4, pp. 603–610, 2011.

[83] K. Begriche, J. Massart, M.-A. Robin, F. Bonnet, and B. Fromenty, "Mitochondrial adaptations and dysfunctions in nonalcoholic fatty liver disease," *Hepatology*, vol. 58, no. 4, pp. 1497–1507, 2013.

[84] M. Monetti, M. C. Levin, M. J. Watt et al., "Dissociation of hepatic steatosis and insulin resistance in mice overexpressing DGAT in the liver," *Cell Metabolism*, vol. 6, no. 1, pp. 69–78, 2007.

[85] W. Liao, T. Y. Hui, S. G. Young, and R. A. Davis, "Blocking microsomal triglyceride transfer protein interferes with apoB secretion without causing retention or stress in the ER," *Journal of Lipid Research*, vol. 44, no. 5, pp. 978–985, 2003.

[86] Z. Z. Li, M. Berk, T. M. McIntyre, and A. E. Feldstein, "Hepatic lipid partitioning and liver damage in nonalcoholic fatty liver disease: role of stearoyl-Coa desaturase," *Journal of Biological Chemistry*, vol. 284, no. 9, pp. 5637–5644, 2009.

[87] M. Sharma, S. Mitnala, R. K. Vishnubhotla, R. Mukherjee, D. N. Reddy, and P. N. Rao, "The riddle of nonalcoholic fatty liver disease: progression from nonalcoholic fatty liver to non-alcoholic steatohepatitis," *Journal of Clinical and Experimental Hepatology*, vol. 5, no. 2, pp. 147–158, 2015.

[88] K. Beier, A. Völkl, and D. Fahimi, "TNF-α downregulates the peroxisome proliferator activated receptor-α and the mRNAs encoding peroxisomal proteins in rat liver," *FEBS Letters*, vol. 412, no. 2, pp. 385–387, 1997.

[89] V. G. Giby and T. A. Ajith, "Role of adipokines and peroxisome proliferator-activated receptors in nonalcoholic fatty liver disease," *World Journal of Hepatology*, vol. 6, no. 8, pp. 570–579, 2014.

[90] S. R. Ande, K. H. Nguyen, B. L. Grégoire Nyomba, and S. Mishra, "Prohibitin-induced, obesity-associated insulin resistance and accompanying low-grade inflammation causes NASH and HCC," *Scientific Reports*, vol. 6, Article ID 23608, 2016.

[91] S. Francque, A. Verrijken, S. Caron et al., "PPARα gene expression correlates with severity and histological treatment response in patients with non-alcoholic steatohepatitis," *Journal of Hepatology*, vol. 63, no. 1, pp. 164–173, 2015.

[92] 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.

[93] 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," *The Journal of Clinical Investigation*, vol. 118, no. 2, pp. 683–694, 2008.

[94] 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.

[95] S. Bonovas, G. K. Nikolopoulos, and P. G. Bagos, "Use of fibrates and cancer risk: a systematic review and meta-analysis of 17 long-term randomized placebo-controlled trials," *PLoS ONE*, vol. 7, no. 9, Article ID 0045259, 2012.

[96] N. Zhang, E. S. H. Chu, J. Zhang et al., "Peroxisome proliferator activated receptor alpha inhibits hepatocarcinogenesis through mediating NF-κB signaling pathway," *Oncotarget*, vol. 5, no. 18, pp. 8330–8340, 2014.

[97] M. C. Sugden and M. J. Holness, "Mechanisms underlying regulation of the expression and activities of the mammalian pyruvate dehydrogenase kinases," *Archives of Physiology and Biochemistry*, vol. 112, no. 3, pp. 139–149, 2006.

[98] M. Grabacka, M. Pierzchalska, and K. Reiss, "Peroxisome proliferator activated receptor α ligands as anticancer drugs targeting mitochondrial metabolism," *Current Pharmaceutical Biotechnology*, vol. 14, no. 3, pp. 342–356, 2013.

[99] S. Oka, R. Alcendor, P. Zhai et al., "PPARα-Sirt1 complex mediates cardiac hypertrophy and failure through suppression of the ERR transcriptional pathway," *Cell Metabolism*, vol. 14, no. 5, pp. 598–611, 2011.

[100] M. Pawlak, E. Baugé, W. Bourguet et al., "The transrepressive activity of peroxisome proliferator-activated receptor alpha is necessary and sufficient to prevent liver fibrosis in mice," *Hepatology*, vol. 60, no. 5, pp. 1593–1606, 2014.

[101] S. Kamarajugadda, J. R. Becker, E. A. Hanse et al., "Cyclin D1 represses peroxisome proliferator-activated receptor alpha and inhibits fatty acid oxidation," *Oncotarget*, vol. 7, no. 30, pp. 47674–47686, 2016.

[102] X. Qin, X. Xie, Y. Fan et al., "Peroxisome proliferator-activated receptor-δ induces insulin-induced gene-1 and suppresses hepatic lipogenesis in obese diabetic mice," *Hepatology*, vol. 48, no. 2, pp. 432–441, 2008.

[103] M. Goudarzi, T. Koga, C. Khozoie et al., "PPARβ/δ modulates ethanol-induced hepatic effects by decreasing pyridoxal kinase activity," *Toxicology*, vol. 311, no. 3, pp. 87–98, 2013.

[104] 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.

[105] M. Zarei, E. Barroso, R. Leiva et al., "Heme-regulated eIF2α kinase modulates hepatic FGF21 and is activated by PPARβ/δ deficiency," *Diabetes*, vol. 65, no. 10, pp. 3185–3199, 2016.

[106] Y. Zhang, T. Lei, J. F. Huang et al., "The link between fibroblast growth factor 21 and sterol regulatory element binding protein 1c during lipogenesis in hepatocytes," *Molecular and Cellular Endocrinology*, vol. 342, no. 1-2, pp. 41–47, 2011.

[107] J. Xu, D. J. Lloyd, C. Hale et al., "Fibroblast growth factor 21 reverses hepatic steatosis, increases energy expenditure, and improves insulin sensitivity in diet-induced obese mice," *Diabetes*, vol. 58, no. 1, pp. 250–259, 2009.

[108] L. Serrano-Marco, R. Rodríguez-Calvo, I. El Kochairi et al., "Activation of peroxisome proliferator—activated receptor-β/δ (PPAR-β/δ) ameliorates insulin signaling and reduces SOCS3 levels by inhibiting STAT3 in interleukin-6-stimulated adipocytes," *Diabetes*, vol. 60, no. 7, pp. 1990–1999, 2011.

[109] X. Li, J. Li, X. Lu et al., "Treatment with PPARδ agonist alleviates non-alcoholic fatty liver disease by modulating glucose and fatty acid metabolic enzymes in a rat model," *International Journal of Molecular Medicine*, vol. 36, no. 3, pp. 767–775, 2015.

[110] H. J. Lee, J. E. Yeon, E. J. Ko et al., "Peroxisome proliferator-activated receptor-delta agonist ameliorated inflammasome activation in nonalcoholic fatty liver disease," *World Journal of Gastroenterology*, vol. 21, no. 45, pp. 12787–12799, 2015.

[111] L. A. Bojic, D. E. Telford, M. D. Fullerton et al., "PPARδ activation attenuates hepatic steatosis in *Ldlr*$^{-/-}$ mice by enhanced fat oxidation, reduced lipogenesis, and improved insulin sensitivity," *Journal of Lipid Research*, vol. 55, no. 7, pp. 1254–1266, 2014.

[112] 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.

[113] H. E. Bays, S. Schwartz, T. Littlejohn III et al., "MBX-8025, a novel peroxisome proliferator receptor-δ 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.

[114] H.-X. Liu, Y. Fang, Y. Hu, F. J. Gonzalez, J. Fang, and Y.-J. Y. Wan, "PPARβ regulates liver regeneration by modulating Akt and E2f signaling," *PLoS ONE*, vol. 8, no. 6, Article ID e65644, 2013.

[115] M. Vacca, S. D'Amore, G. Graziano et al., "Clustering nuclear receptors in liver regeneration identifies candidate modulators of hepatocyte proliferation and hepatocarcinoma," *PLoS ONE*, vol. 9, no. 8, Article ID e104449, 2014.

[116] H. E. Hollingshead, R. L. Killins, M. G. Borland et al., "Peroxisome proliferator-activated receptor-β/δ (PPARβ/δ) ligands do not potentiate growth of human cancer cell lines," *Carcinogenesis*, vol. 28, no. 12, pp. 2641–2649, 2007.

[117] L. Xu, C. Han, K. Lim, and T. Wu, "Cross-talk between peroxisome proliferator-activated receptor δ and cytosolic phospholipase $A_2\alpha$/cyclooxygenase-2/prostaglandin E_2 signaling pathways in human hepatocellular carcinoma cells," *Cancer Research*, vol. 66, no. 24, pp. 11859–11868, 2006.

[118] S. Handeli and J. A. Simon, "A small-molecule inhibitor of Tcf/β-catenin signaling down-regulates PPARγ and PPARδ activities," *Molecular Cancer Therapeutics*, vol. 7, no. 3, pp. 521–529, 2008.

[119] C. Y. Cao, S. W.-F. Mok, V. W.-S. Cheng, and S. K.-W. Tsui, "The FHL2 regulation in the transcriptional circuitry of human cancers," *Gene*, vol. 572, no. 1, pp. 1–7, 2015.

[120] C.-F. Ng, P. K.-S. Ng, V. W.-Y. Lui et al., "FHL2 exhibits antiproliferative and anti-apoptotic activities in liver cancer cells," *Cancer Letters*, vol. 304, no. 2, pp. 97–106, 2011.

[121] H. Yau, K. Rivera, R. Lomonaco, and K. Cusi, "The future of thiazolidinedione therapy in the management of type 2 diabetes mellitus," *Current Diabetes Reports*, vol. 13, no. 3, pp. 329–341, 2013.

[122] D. M. Torres, C. D. Williams, and S. A. Harrison, "Features, diagnosis, and treatment of nonalcoholic fatty liver disease," *Clinical Gastroenterology and Hepatology*, vol. 10, no. 8, pp. 837–858, 2012.

[123] E. Boettcher, G. Csako, F. Pucino, R. Wesley, and R. Loomba, "Meta-analysis: pioglitazone improves liver histology and fibrosis in patients with non-alcoholic steatohepatitis," *Alimentary Pharmacology and Therapeutics*, vol. 35, no. 1, pp. 66–75, 2012.

[124] V. Ratziu, P. Giral, S. Jacqueminet et al., "Rosiglitazone for non-alcoholic 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.

[125] V. Ratziu, F. Charlotte, C. Bernhardt et al., "Long-term efficacy of rosiglitazone in nonalcoholic steatohepatitis: results of the Fatty Liver Improvement by Rosiglitazone Therapy (FLIRT 2) extension trial," *Hepatology*, vol. 51, no. 2, pp. 445–453, 2010.

[126] D. M. Torres, F. J. Jones, J. C. Shaw, C. D. Williams, J. A. Ward, and S. A. Harrison, "Rosiglitazone versus rosiglitazone and metformin versus rosiglitazone and losartan in the treatment of nonalcoholic steatohepatitis in humans: a 12-month randomized, prospective, open-label trial," *Hepatology*, vol. 54, no. 5, pp. 1631–1639, 2011.

[127] M. Gao, Y. Ma, M. Alsaggar, and D. Liu, "Dual outcomes of rosiglitazone treatment on fatty liver," *The AAPS Journal*, vol. 18, no. 4, pp. 1023–1031, 2016.

[128] 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.

[129] 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.

[130] 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.

[131] A. Vidal-Puig, M. Jimenez-Liñan, B. B. Lowell et al., "Regulation of PPAR γ gene expression by nutrition and obesity in rodents," *The Journal of Clinical Investigation*, vol. 97, no. 11, pp. 2553–2561, 1996.

[132] G. P. Ables, "Update on Pparγ and nonalcoholic fatty liver disease," *PPAR Research*, vol. 2012, Article ID 912351, 5 pages, 2012.

[133] J. Sakamoto, H. Kimura, S. Moriyama et al., "Activation of human peroxisome proliferator-activated receptor (PPAR) subtypes by pioglitazone," *Biochemical and Biophysical Research Communications*, vol. 278, no. 3, pp. 704–711, 2000.

[134] E. Xu, M.-P. Forest, M. Schwab et al., "Hepatocyte-specific Ptpn6 deletion promotes hepatic lipid accretion, but reduces NAFLD in diet-induced obesity: potential role of PPARγ," *Hepatology*, vol. 59, no. 5, pp. 1803–1815, 2014.

[135] C. W. Wu, E. S. H. Chu, C. N. Y. Lam et al., "PPARγ is essential for protection against nonalcoholic steatohepatitis," *Gene Therapy*, vol. 17, no. 6, pp. 790–798, 2010.

[136] 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," *FASEB Journal*, vol. 25, no. 8, pp. 2538–2550, 2011.

[137] S. Kuntz, S. Mazerbourg, M. Boisbrun et al., "Energy restriction mimetic agents to target cancer cells: comparison between 2-deoxyglucose and thiazolidinediones," *Biochemical Pharmacology*, vol. 92, no. 1, pp. 102–111, 2014.

[138] 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.

[139] C.-W. Wu, G. C. Farrell, and J. Yu, "Functional role of peroxisome-proliferator-activated receptor γ in hepatocellular carcinoma," *Journal of Gastroenterology and Hepatology*, vol. 27, no. 11, pp. 1665–1669, 2012.

[140] A. Laganà, S. Vitale, A. Nigro et al., "Pleiotropic Actions of Peroxisome Proliferator-Activated Receptors (PPARs) in dysregulated metabolic homeostasis, inflammation and cancer: current evidence and future perspectives," *International Journal of Molecular Sciences*, vol. 17, no. 7, p. 999, 2016.

[141] 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.

[142] 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.

[143] 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.

[144] X. Ren, D. Zheng, F. Guo et al., "PPARγ suppressed Wnt/β-catenin signaling pathway and its downstream effector SOX9 expression in gastric cancer cells," *Medical Oncology*, vol. 32, no. 4, 2015.

[145] K. Wu, Y. Yang, D. Liu et al., "Activation of PPARγ suppresses proliferation and induces apoptosis of esophageal cancer cells by inhibiting TLR4-dependent MAPK pathway," *Oncotarget*, 2016.

[146] S. Dionne, E. Levy, D. Levesque, and E. G. Seidman, "PPARγ ligand 15-deoxy-delta 12,14-prostaglandin J2 sensitizes human colon carcinoma cells to TWEAK-induced apoptosis," *Anticancer Research*, vol. 30, no. 1, pp. 157–166, 2010.

[147] O. Pellerito, A. Notaro, S. Sabella et al., "WIN induces apoptotic cell death in human colon cancer cells through a block of autophagic flux dependent on PPARγ down-regulation," *Apoptosis*, vol. 19, no. 6, pp. 1029–1042, 2014.

[148] D. M. Ray, S. H. Bernstein, and R. P. Phipps, "Human multiple myeloma cells express peroxisome proliferator-activated receptor γ and undergo apoptosis upon exposure to PPARγ ligands," *Clinical Immunology*, vol. 113, no. 2, pp. 203–213, 2004.

[149] 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.

[150] L.-Q. Cao, Z.-L. Shao, H.-H. Liang et al., "Activation of peroxisome proliferator-activated receptor-γ (PPARγ) inhibits hepatoma cell growth via downregulation of SEPT2 expression," *Cancer Letters*, vol. 359, no. 1, pp. 127–135, 2015.

[151] I. Cellai, G. Petrangolini, M. Tortoreto et al., "*In vivo* effects of rosiglitazone in a human neuroblastoma xenograft," *British Journal of Cancer*, vol. 102, no. 4, pp. 685–692, 2010.

[152] B. Shen, E. S. H. Chu, G. Zhao et al., "PPARgamma inhibits hepatocellular carcinoma metastases in vitro and in mice," *British Journal of Cancer*, vol. 106, no. 9, pp. 1486–1494, 2012.

[153] P. Avena, W. Anselmo, D. Whitaker-Menezes et al., "Compartment-specific activation of PPARγ governs breast cancer tumor growth, via metabolic reprogramming and symbiosis," *Cell Cycle*, vol. 12, no. 9, pp. 1360–1370, 2013.

[154] J. A. Menendez, "Fine-tuning the lipogenic/lipolytic balance to optimize the metabolic requirements of cancer cell growth: molecular mechanisms and therapeutic perspectives," *Biochimica et Biophysica Acta—Molecular and Cell Biology of Lipids*, vol. 1801, no. 3, pp. 381–391, 2010.

[155] D. F. Calvisi, C. Wang, C. Ho et al., "Increased lipogenesis, induced by AKT-mTORC1-RPS6 signaling, promotes development of human hepatocellular carcinoma," *Gastroenterology*, vol. 140, no. 3, pp. 1071–1083, 2011.

[156] J. Hu, L. Che, L. Li et al., "Co-activation of AKT and c-Met triggers rapid hepatocellular carcinoma development via the mTORC1/FASN pathway in mice," *Scientific Reports*, vol. 6, Article ID 20484, 2016.

[157] L. Li, L. Che, K. M. Tharp et al., "Differential requirement for *de novo* lipogenesis in cholangiocarcinoma and hepatocellular carcinoma of mice and humans," *Hepatology*, vol. 63, no. 6, pp. 1900–1913, 2016.

[158] L. Li, G. M. Pilo, X. Li et al., "Inactivation of fatty acid synthase impairs hepatocarcinogenesis driven by AKT in mice and humans," *Journal of Hepatology*, vol. 64, no. 2, pp. 333–341, 2016.

[159] D. Cao, X. Song, L. Che et al., "Both *de novo* synthetized and exogenous fatty acids support the growth of hepatocellular carcinoma cells," *Liver International*, 2016.

[160] J. Samarin, V. Laketa, M. Malz et al., "PI3K/AKT/mTOR-dependent stabilization of oncogenic far-upstream element binding proteins in hepatocellular carcinoma cells," *Hepatology*, vol. 63, no. 3, pp. 813–826, 2016.

[161] C. Wang, L. Che, J. Hu et al., "Activated mutant forms of PIK3CA cooperate with RasV12 or c-Met to induce liver tumour formation in mice via AKT2/mTORC1 cascade," *Liver International*, vol. 36, no. 8, pp. 1176–1186, 2016.

[162] M. Laplante and D. M. Sabatini, "Regulation of mTORC1 and its impact on gene expression at a glance," *Journal of Cell Science*, vol. 126, pp. 1713–1719, 2013.

[163] G. Panasyuk, C. Espeillac, C. Chauvin et al., "PPARγ contributes to PKM2 and HK2 expression in fatty liver," *Nature Communications*, vol. 3, article 672, 2012.

[164] D. P. King and J. S. Takahashi, "Molecular genetics of circadian rhythms in mammals," *Annual Review of Neuroscience*, vol. 23, pp. 713–742, 2000.

[165] U. Schibler and P. Sassone-Corsi, "A web of circadian pacemakers," *Cell*, vol. 111, no. 7, pp. 919–922, 2002.

[166] D. Feng and M. A. Lazar, "Clocks, metabolism, and the epigenome," *Molecular Cell*, vol. 47, no. 2, pp. 158–167, 2012.

[167] N. Koike, S.-H. Yoo, H.-C. Huang et al., "Transcriptional architecture and chromatin landscape of the core circadian clock in mammals," *Science*, vol. 338, no. 6105, pp. 349–354, 2012.

[168] J. Morf, G. Rey, K. Schneider et al., "Cold-inducible RNA-binding protein modulates circadian gene expression posttranscriptionally," *Science*, vol. 338, no. 6105, pp. 379–383, 2012.

[169] G. Rey, F. Cesbron, J. Rougemont, H. Reinke, M. Brunner, and F. Naef, "Genome-wide and phase-specific DNA-binding rhythms of BMAL1 control circadian output functions in mouse liver," *PLoS Biology*, vol. 9, no. 2, Article ID e1000595, 2011.

[170] H. Yoshitane, H. Ozaki, H. Terajima et al., "CLOCK-controlled polyphonic regulation of circadian rhythms through canonical and noncanonical E-boxes," *Molecular and Cellular Biology*, vol. 34, no. 10, pp. 1776–1787, 2014.

[171] Y. Tahara and S. Shibata, "Circadian rhythms of liver physiology and disease: experimental and clinical evidence," *Nature Reviews Gastroenterology and Hepatology*, vol. 13, no. 4, pp. 217–226, 2016.

[172] X. Tong and L. Yin, "Circadian rhythms in liver physiology and liver diseases," *Comprehensive Physiology*, vol. 3, no. 2, pp. 917–940, 2013.

[173] S. Sahar and P. Sassone-Corsi, "Metabolism and cancer: the circadian clock connection," *Nature Reviews Cancer*, vol. 9, no. 12, pp. 886–896, 2009.

[174] C. B. Peek, A. H. Affinati, K. M. Ramsey et al., "Circadian clock NAD$^+$ cycle drives mitochondrial oxidative metabolism in mice," *Science*, vol. 342, no. 6158, Article ID 1243417, 2013.

[175] M. D. Hirschey, T. Shimazu, J.-Y. Huang, B. Schwer, and E. Verdin, "SIRT3 regulates mitochondrial protein acetylation and intermediary metabolism," *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 76, pp. 267–277, 2011.

[176] G. F. Gibbons, D. Patel, D. Wiggins, and B. L. Knight, "The functional efficiency of lipogenic and cholesterogenic gene expression in normal mice and in mice lacking the peroxisomal proliferator-activated receptor-alpha (PPAR-α)," *Advances in Enzyme Regulation*, vol. 42, pp. 227–247, 2002.

[177] D. D. Patel, B. L. Knight, D. Wiggins, S. M. Humphreys, and G. F. Gibbons, "Disturbances in the normal regulation of SREBP-sensitive genes in PPARα-deficient mice," *Journal of Lipid Research*, vol. 42, no. 3, pp. 328–337, 2001.

[178] K. Oishi, H. Shirai, and N. Ishida, "CLOCK is involved in the circadian transactivation of peroxisome-proliferator-activated receptor α (PPARα) in mice," *Biochemical Journal*, vol. 386, no. 3, pp. 575–581, 2005.

[179] P. Gervois, S. Chopin-Delannoy, A. Fadel et al., "Fibrates increase human REV-ERBα expression in liver via a novel peroxisome proliferator-activated receptor response element," *Molecular Endocrinology*, vol. 13, no. 3, pp. 400–409, 1999.

[180] L. Canaple, J. Rambaud, O. Dkhissi-Benyahya et al., "Reciprocal regulation of brain and muscle Arnt-like protein 1 and peroxisome proliferator-activated receptor α defines a novel positive feedback loop in the rodent liver circadian clock," *Molecular Endocrinology*, vol. 20, no. 8, pp. 1715–1727, 2006.

[181] L. Chen and G. Yang, "PPARs integrate the mammalian clock and energy metabolism," *PPAR Research*, vol. 2014, Article ID 653017, 6 pages, 2014.

[182] F. Gachon, N. Leuenberger, T. Claudel et al., "Proline- and acidic amino acid-rich basic leucine zipper proteins modulate peroxisome proliferator-activated receptor α (PPARα) activity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 12, pp. 4794–4799, 2011.

[183] A. Benavides, M. Siches, and M. Llobera, "Circadian rhythms of lipoprotein lipase and hepatic lipase activities in intermediate metabolism of adult rat," *American Journal of Physiology—Regulatory Integrative and Comparative Physiology*, vol. 275, no. 3, part 2, pp. R811–R817, 1998.

[184] M. C. Hunt, P. J. G. Lindquist, J. M. Peters, F. J. Gonzalez, U. Diczfalusy, and S. E. H. Alexson, "Involvement of the peroxisome proliferator-activated receptor α in regulating long-chain acyl-CoA thioesterases," *Journal of Lipid Research*, vol. 41, no. 5, pp. 814–823, 2000.

[185] M. C. Hunt, K. Solaas, B. Frode Kase, and S. E. H. Alexson, "Characterization of an acyl-CoA thioesterase that functions as a major regulator of peroxisomal lipid metabolism," *Journal of Biological Chemistry*, vol. 277, no. 2, pp. 1128–1138, 2002.

[186] K. Schoonjans, J. Peinado-Onsurbe, A.-M. Lefebvre et al., "PPARα and PPARγ activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene," *The EMBO Journal*, vol. 15, no. 19, pp. 5336–5348, 1996.

[187] M. Kawai, C. B. Green, B. Lecka-Czernik et al., "A circadian-regulated gene, *Nocturnin*, promotes adipogenesis by stimulating PPAR-γ nuclear translocation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 23, pp. 10508–10513, 2010.

[188] G. Yang, Z. Jia, T. Aoyagi, D. McClain, R. M. Mortensen, and T. Yang, "Systemic pparγ deletion impairs circadian rhythms of behavior and metabolism," *PLoS ONE*, vol. 7, no. 8, Article ID e38117, 2012.

[189] C. Liu, S. Li, T. Liu, J. Borjigin, and J. D. Lin, "Transcriptional coactivator PGC-1α integrates the mammalian clock and energy metabolism," *Nature*, vol. 447, no. 7143, pp. 477–481, 2007.

[190] J. Sonoda, I. R. Mehl, L.-W. Chong, R. R. Nofsinger, and R. M. Evans, "PGC-1β controls mitochondrial metabolism to modulate circadian activity, adaptive thermogenesis, and hepatic steatosis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 12, pp. 5223–5228, 2007.

[191] H. R. Ueda, W. Chen, A. Adachi et al., "A transcription factor response element for gene expression during circadian night," *Nature*, vol. 418, no. 6897, pp. 534–539, 2002.

[192] C. Esau, S. Davis, S. F. Murray et al., "miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting," *Cell Metabolism*, vol. 3, no. 2, pp. 87–98, 2006.

[193] 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.

[194] A. Neufeld-Cohen, M. S. Robles, R. Aviram et al., "Circadian control of oscillations in mitochondrial rate-limiting enzymes and nutrient utilization by PERIOD proteins," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 113, no. 12, pp. E1673–E1682, 2016.

[195] E. Filipski, P. F. Innominato, M. W. Wu et al., "Effects of light and food schedules on liver and tumor molecular clocks in mice," *Journal of the National Cancer Institute*, vol. 97, no. 7, pp. 507–517, 2005.

[196] E. Filipski, P. Subramanian, J. Carrière, C. Guettier, H. Barbason, and F. Lévi, "Circadian disruption accelerates liver carcinogenesis in mice," *Mutation Research—Genetic Toxicology and Environmental Mutagenesis*, vol. 680, no. 1-2, pp. 95–105, 2009.

[197] K. D. Bruce, D. Szczepankiewicz, K. K. Sihota et al., "Altered cellular redox status, sirtuin abundance and clock gene expression in a mouse model of developmentally primed NASH," *Biochimica et Biophysica Acta (BBA)—Molecular and Cell Biology of Lipids*, vol. 1861, no. 7, pp. 584–593, 2016.

[198] U. S. Udoh, J. A. Valcin, K. L. Gamble, and S. M. Bailey, "The molecular circadian clock and alcohol-induced liver injury," *Biomolecules*, vol. 5, no. 4, pp. 2504–2537, 2015.

[199] K. Honma, M. Hikosaka, K. Mochizuki, and T. Goda, "Loss of circadian rhythm of circulating insulin concentration induced by high-fat diet intake is associated with disrupted rhythmic expression of circadian clock genes in the liver," *Metabolism*, vol. 65, no. 4, pp. 482–491, 2016.

[200] F. W. Turek, C. Joshu, A. Kohsaka et al., "Obesity and metabolic syndrome in circadian Clock mutant mice," *Science*, vol. 308, no. 5724, pp. 1043–1045, 2005.

[201] K. L. Eckel-Mahan, V. R. Patel, S. de Mateo et al., "Reprogramming of the circadian clock by nutritional challenge," *Cell*, vol. 155, no. 7, pp. 1464–1478, 2013.

[202] 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.

[203] C. B. Green, N. Douris, S. Kojima et al., "Loss of Nocturnin, a circadian deadenylase, confers resistance to hepatic steatosis and diet-induced obesity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 23, pp. 9888–9893, 2007.

[204] Y.-M. Lin, J. H. Chang, K.-T. Yeh et al., "Disturbance of circadian gene expression in hepatocellular carcinoma," *Molecular Carcinogenesis*, vol. 47, no. 12, pp. 925–933, 2008.

[205] B. Zhao, J. Lu, J. Yin et al., "A functional polymorphism in *PER3* gene is associated with prognosis in hepatocellular carcinoma," *Liver International*, vol. 32, no. 9, pp. 1451–1459, 2012.

[206] S. Ishibashi, S. Yamashita, H. Arai et al., "Effects of K-877, a novel selective PPARα modulator (SPPARMα), in dyslipidaemic patients: a randomized, double blind, active- and placebo-controlled, phase 2 trial," *Atherosclerosis*, vol. 249, pp. 36–43, 2016.

[207] S. Raza-Iqbal, T. Tanaka, M. Anai et al., "Transcriptome analysis of K-877 (A novel selective PPARα modulator (SPPARMα))-regulated genes in primary human hepatocytes and the mouse liver," *Journal of Atherosclerosis and Thrombosis*, vol. 22, no. 8, pp. 754–772, 2015.

[208] J. P. Taygerly, L. R. McGee, S. M. Rubenstein et al., "Discovery of INT131: a selective PPARγ modulator that enhances insulin sensitivity," *Bioorganic and Medicinal Chemistry*, vol. 21, no. 4, pp. 979–992, 2013.

[209] B. Cariou, R. Hanf, S. Lambert-Porcheron et al., "Dual peroxisome proliferator-activated receptor α/δ agonist gft505 improves hepatic and peripheral insulin sensitivity in abdominally obese subjects," *Diabetes Care*, vol. 36, no. 10, pp. 2923–2930, 2013.

[210] B. Cariou, Y. Zaïr, B. Staels, and E. Bruckert, "Effects of the new dual PPARα/δ agonist GFT505 on lipid and glucose homeostasis in abdominally obese patients with combined dyslipidemia or impaired glucose metabolism," *Diabetes Care*, vol. 34, no. 9, pp. 2008–2014, 2011.

[211] B. Staels, A. Rubenstrunk, B. Noel et al., "Hepatoprotective effects of the dual peroxisome proliferator-activated receptor alpha/delta agonist, GFT505, in rodent models of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis," *Hepatology*, vol. 58, no. 6, pp. 1941–1952, 2013.

[212] V. Ratziu, S. A. Harrison, S. Francque et al., "Elafibranor, an agonist of the peroxisome proliferator–activated receptor–α and $-\delta$, induces resolution of nonalcoholic steatohepatitis without fibrosis worsening," *Gastroenterology*, vol. 150, no. 5, pp. 1147.e5–1159.e5, 2016.