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Review

Med1 Subunit of the Mediator Complex in Nuclear Receptor-Regulated Energy Metabolism, Liver Regeneration, and Hepatocarcinogenesis

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Several nuclear receptors regulate diverse metabolic functions that impact on critical biological processes, such as development, differentiation, cellular regeneration, and neoplastic conversion. In the liver, some members of the nuclear receptor family, such as peroxisome proliferator-activated receptors (PPARs), constitutive androstane receptor (CAR), farnesoid X receptor (FXR), liver X receptor (LXR), pregnane X receptor (PXR), glucocorticoid receptor (GR), and others, regulate energy homeostasis, the formation and excretion of bile acids, and detoxification of xenobiotics. Excess energy burning resulting from increases in fatty acid oxidation systems in liver generates reactive oxygen species, and the resulting oxidative damage influences liver regeneration and liver tumor development. These nuclear receptors are important sensors of exogenous activators as well as receptor-specific endogenous ligands. In this regard, gene knockout mouse models revealed that some lipid-metabolizing enzymes generate PPARα-activating ligands, while others such as ACOX1 (fatty acyl-CoA oxidase1) inactivate these endogenous PPARα activators. In the absence of ACOX1, the unmetabolized ACOX1 substrates cause sustained activation of PPARα, and the resulting increase in energy burning leads to hepatocarcinogenesis. Ligand-activated nuclear receptors recruit the multisubunit Mediator complex for RNA polymerase II-dependent gene transcription. Evidence indicates that the Med1 subunit of the Mediator is essential for PPARα, PPARγ, CAR, and GR signaling in liver. Med1 null hepatocytes fail to respond to PPARα activators in that these cells do not show induction of peroxisome proliferation and increases in fatty acid oxidation enzymes. Med1-deficient hepatocytes show no increase in cell proliferation and do not give rise to liver tumors. Identification of nuclear receptor-specific coactivators and Mediator subunits should further our understanding of the complexities of metabolic diseases associated with increased energy combustion in liver.

Key words: Liver regeneration; Mediator complex; Med1; Hepatocarcinogenesis

INTRODUCTION

Liver, a complex metabolic organ located strategically in the organism, orchestrates a variety of metabolic functions including the maintenance of fat and carbohydrate energy homeostasis, synthesis of serum proteins, formation and excretion of bile acids as products of cholesterol catabolism, and the detoxification of xenobiotics, including alcohol (1,2). Disturbances in these processes contribute invariably to acute or chronic liver injury. For example, the burgeoning pandemic of dietary obesity adversely impacts on hepatic energy homeostasis with a risk of developing nonalcoholic fatty liver disease (NAFLD), which leads to the development of cirrhosis of the liver and hepatocellular carcinoma (HCC), outcomes similar to those seen with alcoholic fatty liver disease (2,3). Liver is also the main target for the five most common hepatotropic viruses, namely, hepatitis A, hepatitis B, hepatitis C, hepatitis D, and hepatitis E (4). Of these, chronic hepatitis B virus and hepatitis C virus infections cause 75–80% of liver cancers diagnosed worldwide (5). Many of the chronic liver injury conditions create a microenvironment that is conducive to the confounding confluence of cell death, inflammation, fibrosis, and lingering hepatocyte regeneration, necessary for the development of end-stage liver disease of cirrhosis and HCC (3,6). The normal replicative capacity of hepatocytes, with an average life span

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of 200–300 days, and mitosis occurring at the rate of 1 in 20,000 cells, is low (7,8), but liver is evolutionarily endowed with enormous potential to regenerate, designed to restore the functional capacity of diminished liver (9). End-stage liver disease poses a major health challenge and points to the increasing importance of a deeper understanding of the molecular signaling mechanisms responsible for hepatocellular regeneration known to contribute to the carcinogenic process, although cell proliferation per se may not initiate cancer (10,11).

Liver regeneration is a predictable biological process triggered in response to many types of injury (7,9). Surgical removal of part of the liver, a process which emanates healing signals that instruct remaining mature hepatocytes to resume growth and division, has yielded fundamental clues governing liver regeneration (9,12). The predominant mode of liver regeneration entails normally functioning, quiescent, but conditionally dividing, differentiated hepatocytes that reenter cell cycle, multiply, and grow to replenish cells lost due to injury (9). Regeneration of differentiated hepatocytes is controlled by metabolic needs such that the process terminates once an appropriate liver to body weight ratio is achieved to prevent regenerating cells from running amok (13). A relatively minor mode of liver regeneration is the participation of putative liver stem cells that are activated only when mature hepatocytes can no longer engage in regeneration due to senescence or other constraints (9,12). Of interest is that during regeneration, liver cells, like all other proliferating cells, continue to perform critical metabolic functions, such as glucose and lipid homoeostasis and protein synthesis. A better understanding of how cell proliferation and metabolism are interconnected and coregulated may provide therapeutic leads (14).

Many of the important metabolic functions in liver are regulated by nuclear receptors (2). Activation of some nuclear receptors, such as peroxisome proliferator-activated receptor-α (PPARα) or constitutive androstane receptor (CAR), by their cognate ligands stimulates hepatocellular proliferation along with the characteristic metabolic effects. For example, Wy-14,643, ciprofibrate, and other peroxisome proliferators are potent hepatic mitogens as they activate PPARα (11,15,16). Likewise, 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP), a potent activator of CAR, is well recognized as a hepatocellular mitogen (17).

The biological processes regulated by nuclear receptors require coordinated assembly of transcription coactivator complexes on liganded nuclear receptors to facilitate chromatin remodeling and to recruit the Mediator complex for the expression of RNA polymerase II (Pol II)-transcribed genes (18). Recent evidence indicates a role for Med1, an important subunit of the Mediator complex in controlling the metabolic status and regeneration in liver (19,20). We focus this minireview on Med1 because it is an essential subunit of the multisubunit Mediator complex in regulating fatty acid oxidation and glucose metabolism (19). Furthermore, Med1 is also necessary for liver regeneration induced, in particular, by nuclear receptor PPARα and CAR activators (9,21). Recent evidence indicates that Med1 is also sufficient by itself in inducing hepatocellular proliferation (20).

NUCLEAR RECEPTORS IN LIVER REGENERATION

Nuclear receptor superfamily consists of 48 members in the human genome, and most of these receptors function as ligand-dependent transcription factors with a major role in development and metabolic homeostasis (2,22,23). In liver, several of these receptors serve as intracellular sensors of endogenous, naturally occurring, small molecules, such as lipid-soluble hormones and dietary lipid intermediates. Transcriptional activators that play a key role in the varied pathogenesis of NAFLD include three members of the PPAR subfamily of nuclear receptors (namely, PPARα, PPARδ/β, and PPARγ), CAR, farnesoid X receptor (FXR), liver X receptor-α (LXRα), pregnane X receptor (PXR), glucocorticoid receptor (GR), and others (2,22,23). These nuclear receptors and other transcriptional activators are associated with lipid sensing, lipid synthesis, and fatty acid oxidation (2). Nuclear receptors, such as PPARα and CAR, are also activated by exogenous molecules including several xenobiotics (2,11,22,24). They participate in the modulation of hepatocyte proliferation, and some of these receptors are implicated in hepatocarcinogenesis resulting from sustained activation by cognate exogenous or endogenous ligand(s) (11,25–28) (Table 1).

PPARα

PPARα is a lipid- and xenobiotic-sensing nuclear receptor expressed in tissues such as the liver, kidney, heart, and skeletal muscle with high capacity for fatty acid oxidation and energy burning (2,11,15,16). In liver, PPARα is responsible for the peroxisome proliferator-induced pleiotropic responses that include hepatocellular proliferation, increase in peroxisome population in hepatocytes, and enhanced fatty acid oxidation resulting in excess energy burning in liver (11,16,25). Accordingly, PPARα activation can modulate fatty acid oxidation and influence the progression of NAFLD (2,16). Synthetic PPARα agonists, called peroxisome proliferators, include structurally diverse compounds such as fibrates with hypolipidemic activity, phthalate ester plasticizers, industrial solvents, herbicides, food flavoring agents, and others (29,30). PPARα heterodimerizes with RXRα and transcriptionally regulates peroxisomal and mitochondrial β-oxidation and microsomal ω-oxidation of fatty acids and plays an essential role in burning energy, resulting in the generation

Table 1. Nuclear Receptors in Liver Regeneration

Nuclear Receptor	Nuclear Receptor Responsive Element	Natural Ligands	Synthetic Ligands	Function: Lipogenesis Versus Fatty Acid Oxidation	Function: Liver Regeneration
PPARα	5′-AGGTCA(N)AGGTCA-3′	Free fatty acids Fatty acid derivatives	Fibrates Wy-14,643 Ciprofibrates	Increases fatty acid oxidation	Increases
PPARγ	5′-AGGTCA(N)AGGTCA-3′	Free fatty acids Fatty acid derivatives	Glitazones	Increases lipid storage/ lipogenesis	Increases
LXRα,β	5′-(A/G)G(G/T)T(C/T) Annnn-(A/G)G(G/T)T (C/T)A-3′	Oxysterols	T0901317 GW3965	Increases lipogenesis	Decreases
FXR	5′-AGAGCA(N) AGGGGA-3′	Bile acids Oxysterols Polyunsaturated fatty acids	GW4064 6ECDCA MFA1 Obeticholic acid	Decreases lipogenesis	Increases
CAR	5′-(A/G)GTTCAnnnn -(A/G)GTTCA-3′	Xenobiotics Androstenol	Phenobarbital TCPOBOP Antimalarial drug artemisinin	Decreases lipogenesis/ increases fatty acid oxidation	Increases
PXR	5′-(A/G)GTTCAnnnn-(A/G)GTTCA-3′	Bile acids Cholesterol derived 5-cholestane-3, 7, 12-triol	Drugs Xenobiotics	Decreases lipogenesis/fatty acid oxidation	Increases
TR	5′-(A/G)GGTCAnnnn-(A/G)GGTCA-3′	T3 and T4	CO23,GC-1 KB-141 KB2115 MB07811	Increases fatty acid oxidation	Increases

of hydrogen peroxide and other reactive oxygen species (11,16,31). Sustained activation of PPARα, either by synthetic or endogenous ligands, results in the development of liver tumors, attributable in part to increased oxidative damage caused by fatty acid oxidation and to liver cell proliferation (11,26–28).

PPARα activators, in particular the peroxisome proliferators such as ciprofibrate and Wy-14,643, are powerful liver mitogens (29,32). Peroxisome proliferators were identified as primary mitogens and proposed that the effects are mediated by a receptor, which was subsequently identified as PPARα (33). The first indication for the formation and degradation of endogenous PPARα activators came from fatty acyl-CoA oxidase (ACOX1) null mice that display profound spontaneous peroxisome proliferation in hepatocytes with induction of genes that are activated by PPARα, implying that ACOX1 is essential for the inactivation of endogenous PPARα activators (16,28,34). PPARα agonists, depending on their potency, exert primary hepatomitogenic properties within the first week of treatment, as they are able to induce PPARα-mediated hepatocyte proliferation in the absence of liver injury (direct hyperplasia) (11,32,33). Chronic administration of these agents does not maintain a high level of sustained mitogenic response (31). Stimulation of PPARα represses the microRNA let-7, which degrades the c-myc oncogene and induces oncogenic mir-17 miRNA (35). Mice deficient in PPARα show a complete loss of cell proliferative response in liver following exposure to peroxisome proliferators, and these mice also show no induction of fatty acid oxidation systems and fail to develop liver tumors (36,37). These data contribute to the concept that energy burning-related increase in oxidative stress combined with increased liver cell proliferation contributes to liver cancer development by a PPARα-dependent mechanism (11,16,38). Species- and cell-specific differences in response to peroxisome proliferators, as reviewed recently (11), appear mostly quantitative but not qualitative in nature. Adenovirally directed expression of human PPARα in PPARα–/– mice induced liver cell proliferation similar to that in magnitude seen with mouse PPARα (39,40). The other two members of PPAR subfamily, PPARδ/β and PPARγ, are also involved in lipid metabolism and energy homeostasis in that PPARδ/β, which is ubiquitously expressed, participates in fatty acid oxidation and energy burning, and PPARγ is a major factor responsible for adipogenesis and energy conservation (16,22).

CAR

The constitutive androstane receptor (CAR) is most abundantly expressed in liver and regulates the transcription of drug-metabolizing enzymes and transporters to

minimize the toxicity of harmful chemicals (41–43). Like PPARs, CAR also plays a role in the regulation of energy homeostasis by influencing glucose and lipid metabolism. Activation of CAR results in the reduction of blood glucose by suppressing the expression of hepatic gluconeogenic enzymes phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6P) in mouse liver (44). Activation of CAR inhibits hepatic lipogenesis and induces fatty acid oxidation, and these appear beneficial in reducing hepatic steatosis (44). In liver, endogenous CAR resides in the cytoplasm of hepatocytes, and upon exposure to its agonist phenobarbital or 1,4-bis[2-(3,5-dichloropyridyloxy)] benzene (TCPOBOP), CAR translocates from the cytoplasm to the nucleus and triggers the transcription of target genes (43). Activation of CAR by TCPOBOP leads to profound hepatomegaly that involves both hypertrophic and hyperplastic responses (42,45). CAR agonists also translocate adenovirally expressed exogenous CAR from hepatocyte cytoplasm to the nucleus (46). Of interest is that PPARα ligands, which induce fatty acid oxidation enzymes, have been shown to drive CAR into the hepatocyte nucleus, but the functional significance of PPARα ligand-mediated translocation of CAR to the nucleus is unclear (47). Mice deficient in CAR show complete loss of hyperplastic response of hepatocytes to CAR activators (42). Of interest is that PPARα–/– mice show enhanced hepatocyte proliferation in response to CAR agonist TCPOBOP (48). CAR activation by phenobarbital is known to induce liver tumor promotion (49). Recently, it has been shown that the growth arrest and the DNA damage-inducible 45-β (Gadd45β) gene are most strongly induced by TCPOBOP during early liver regeneration (50).

PXR

PXR, also called steroid X receptor (SXR), like CAR, is a xenobiotic sensor. It is localized in the cytoplasm of hepatocytes and, when activated, it translocates to the nucleus (2,44,51). In the nucleus, PXR heterodimerizes with RXRα, and this heterodimer binds to xenobiotic-responsive enhancer module-bearing target genes to enhance their transcription (52). PXR is activated by several drugs and xenobiotics, which include pregnenolone-16-carbonitrile (PCN), taxol, rifampicin, and clotrimazole, among others (53). The major endogenous activators of PXR include bile acids and cholesterol-derived 5-cholestane-3,7,12-triol (triol) (54). PXR controls the expression of phase I and phase II drug-metabolizing enzymes as well as some members of the drug-transporter family (55). PXR reduces hepatic fatty acid oxidation by downregulating the expression of carnitine palmitoyltransferase 1α (CPT1α) and mitochondrial 3-hydroxy-3-methylglutarate-CoA synthase 2, the enzymes involved in mitochondrial β-oxidation of fatty acids (56). CPT1α is required for the carnitination of fatty acids, which is essential for entry of fatty acids into the mitochondria for β-oxidation (25).

PXR also plays a role in hepatocyte regeneration by virtue of its role in fatty acid metabolism as it increases de novo lipogenesis and inhibits fatty acid oxidation. Mice lacking PXR reveal a reduction of hepatocyte proliferation at 36 h following partial hepatectomy (57). PXR ligand PCN produced hepatomegaly in the wild-type mice but not in the PXR-KO mice (58). PCN also increased both the number of proliferating cell nuclear antigen immunopositive nuclei and apparent cell size in the wild-type mice but not in the PXR-KO mice (59).

FXR

Farnesoid X receptor (FXR) is the primary nuclear receptor for sensing bile acids (59,60). Bile acids are important products of cholesterol metabolism and are excreted in bile as byproducts of metabolism by liver. Bile acid levels are tightly regulated, as they serve as activators of FXR and also other xenobiotic nuclear receptors, CAR and PXR (22,58,61,62). FXR controls the synthesis and transport of bile acids in the liver and gut (60–65). Upon activation by bile acids, FXR positively regulates a number of genes that decrease cellular levels of toxic bile acids. FXR induces the small heterodimer partner (SHP) in liver that downregulates *Cyp7a1* and *Cyp8b1* genes encoding enzymes that synthesize bile acids from cholesterol. FXR is also known to inhibit hepatic lipogenesis by repressing SREBP-1c (2,22). FXR agonists decrease serum triglyceride levels. Activation of FXR by elevated bile acid levels accelerates liver regeneration, whereas decreased bile acid levels and absence of FXR inhibit liver growth (60,62). FXR appears to exert a dual functioning role, first by inhibiting cholesterol 7α-hydroxylase (CYP7a1) to reduce bile acid stress and then enhance hepatocellular proliferation by activating Foxm1b and FGF15 (62). Of note is that although the absence of FXR inhibits liver growth, FXR null mice spontaneously develop liver tumors as they age, asserting that bile acid-induced DNA damage in FXR null mice may be critical in liver tumor development even if FXR absence limits liver regeneration (63,64).

LXR

Liver X receptors α and β regulate cholesterol, glucose, and fatty acid homeostasis and are highly expressed in the liver (65–68). LXRs are endogenously activated by various oxygenated cholesterol derivatives or oxysterols, such as 22(R)-hydroxycholesterol, 24(S)-hydroxycholesterol, 24(S),25-epoxycholesterol, 27-hydroxycholesterol, and cholestenoic acid (65–68). LXRs function as important regulators of cholesterol catabolism by inducing the transcription of genes that participate in the conversion of

cholesterol into bile acids and enhancing reverse cholesterol transport from peripheral sources to the liver (69). Activation of LXRs by endogenous oxysterols and synthetic ligands, such as T0901317 and GW3965, mediates increased transcription of lipogenic genes and cholesterol export genes (2). Hepatic steatosis is promoted by LXRs in a CD36-dependent manner (70). In addition, LXR-regulated cholesterol metabolism has implications in liver cell proliferation. Proliferating cells require excess cholesterol, and LXR activation affects cell proliferation (71). Also, the specific synthetic ligands for LXRs are known to inhibit cell proliferation. Hepatocyte proliferation resulting from partial hepatectomy is accompanied by the suppression of LXR-driven pathways to ensure increased intracellular cholesterol levels required for dividing cells. Reactivation of LXR pathways by synthetic ligands hampers the liver regenerative capacity by decreasing the hepatic cholesterol content (72).

TR

Thyroid hormone (T3) influences metabolism, growth, and development, and these effects are mediated by thyroid hormone receptors (TRs). Like CAR and PPARα activators, T3 is a strong inducer of liver cell proliferation (73). T3-mediated hepatocyte mitogenic response is mediated by PKA-dependent β-catenin activation (73).

MEDIATOR COMPLEX AND NUCLEAR RECEPTOR FUNCTION

Mediator is a large multisubunit complex composed of up to at least 31 subunits in all eukaryotes (18,74). This complex acts as a molecular bridge between gene-specific transcription factors and the RNA polymerase II machinery (74). The Mediator complex was first isolated from *Saccharomyces cerevisiae* and was shown to be necessary for transcriptional activation (75,76). Mammalian Mediator was then isolated as thyroid hormone receptor-associated protein (TRAP) complex (18,74). Subsequently, Mediator complexes were isolated using specific nuclear receptors and designated as ARC (activator recruited cofactor), DRIP (vitamin D receptor-interacting protein), and others (77,78). The Mediator subunits are functionally conserved throughout the evolution from yeast to human, and the current nomenclature is based on the original yeast MED proteins (79). Yeast and human Mediator subunits are organized in a similar core structure comprised of a head, middle, and tail module (80,81). The middle and head modules interface with the pol II basal transcription machinery (82). The tail and middle modules are mainly targeted by gene-specific activators. The Mediator complex associates with the C-terminal domain of the largest subunit of RNA Pol II and is considered essential for basal and regulated expression of most of the RNA polymerase II-dependent genes (82). Depletion of human Mediator from nuclear extracts abolishes transcription by pol II (83). Some Mediator subunits govern the expression of many genes, whereas others appear necessary for a specific gene (84). In this review, we focus on the emerging role of Med1 subunit in liver function.

Med1 AS KEY SUBUNIT OF THE MEDIATOR COMPLEX

Transcription coactivator Med1 is regarded as a key subunit of the mammalian mediator complex. Med1, also called PBP/PPARBP/TRAP220/DRIP205/RB18A, contains two LXXLL motifs located at amino acids 589–593 and 630–634 (85). These signature motifs of Med1 are necessary for the binding of a variety of cofactors, including SRC family members, PGC-1 family members, p300/CBP, and RIP140 (86). Med1 is a pivotal component of the TRAPs, DRIP complex, and ARC complex (87–89). PPARα-interacting cofactor complex (PRIC) also contains Med1 (78). Med1 also interacts with several transcription factors and cofactors, such as PRIP, PIMT, C/EBPβ, SRC, GATA family members, CBP/P300, PGC-1, and tumor-suppressor P53 (90–94). These interactions signify that Med1 has a major role in nuclear receptor-regulated metabolic functions, cellular proliferation, and differentiation. Med1 is expressed in various tissues of adult mice, including brain, heart, liver, lung, kidney, adipose tissues, and testis (85).

Deletion of Med1 Results in Embryonic Lethality

Med1 null mutation in mice is embryonically lethal at midgestation (day 11.5 postcoitum; E11.5), illustrating that Med1 is an essential and nonredundant coactivator and is necessary for embryonic development (95). Embryonic lethality is attributed to impaired development of placental vasculature and defects in the heart, eye, vascular, and hematopoietic system (21,96). The phenotypic changes in Med1 null mice are somewhat similar to those observed in mice deficient in members of GATA transcription factor family (91). Since Med1 interacts with GATA factors, it is possible that these are involved in similar developmental pathways (91). Med1 null fibroblasts failed to show PPARγ-stimulated adipogenesis, as they do not express adipogenic genes in response to PPARγ stimulation. These observations establish that Med1 is required for PPARγ regulated transcription (97).

Med1 Is Required for Liver Regeneration After Partial Hepatectomy

The embryonic lethality of Med1 null mutants necessitated the generation of conditional null mice using the *Cre-loxP* strategy for elucidating the cell- and gene-specific roles of Med1. Med1 liver conditional null mice were used to determine the role of Med1 in mouse liver regeneration (98). Liver regeneration is regulated by a

complex network of signals involving cytokines, chemokines, growth factors, nuclear receptors, and cofactors. Disruption of Med1 in liver impairs liver regeneration with low survival after partial hepatectomy (98). In general, Med1 null hepatocytes are smaller in size compared with hepatocytes in littermate control (98). In wild-type mice, Med1 mRNA expression levels begin to increase 2 h after partial hepatectomy with maximal at 18 and 24 h (Fig. 1). The liver to body weight ratio increased progressively in wild-type mice, whereas similar increases were not observed in conditional Med1 liver null mice (98). In wild-type mice, DNA synthesis, as measured by BrdUrd incorporation, was prominent, with a peak labeling at 36 and 48 h after partial hepatectomy. In contrast, Med1 null liver demonstrated minimal labeling at all times after partial hepatectomy (98). Over 25 genes are upregulated more than sixfold 3 h after partial hepatectomy in wild-type mice compared to Med1 liver null mice (98). Most of these genes are related to cell cycle, cell growth, apoptosis, and signal transduction, such as insulin-like growth factor 1 (Igf1), IGF-binding protein 1 (IGFBP1), E2F transcription factor, growth arrest, and DNA damage-inducible 45γ (GADD45 γ) (98). The reduced expression of these cell cycle genes and cell growth regulatory factors in Med1 null liver indicates that Med1 null hepatocyte fails to respond to partial hepatectomy. Impaired liver regeneration in Med1 null livers suggests a defect in exit from quiescence and diminished entry into G_2/M phase (98). Med1 null hepatocytes also failed to respond to hepatocyte growth factor/scatter factor, implying that hepatic Med1 deficiency affects c-met signaling. Taken together, Med1 plays a critical role in stimulation of liver regeneration following partial hepatectomy.

Med1 Is Essential for PPARα-Regulated Gene Expression

PPARα is activated by a structurally diverse array of synthetic chemicals that are potent hepatic mitogens and carcinogens in mice and rats (24,99). In PPARα null mice, the pleiotropic responses induced by peroxisome proliferators such as Wy,14,643, ciprofibrate, and others fail to occur, and these mice also do not develop hepatocellular carcinomas in response to peroxisome proliferators (36). Thus, it is now well established that PPARα is necessary for peroxisome proliferator-induced liver cell proliferation. Recent evidence indicates that Med1 subunit of the Mediator complex is essential for PPARα signal transduction in that Med1 null hepatocytes are similar to PPARα null liver cells in their inability to respond to PPARα ligands (21). Med1 deficiency in liver parenchymal cells results in the abrogation of peroxisome proliferative response and PPARα target gene transcription mimicking the absence of PPARα (21). No DNA synthesis was noted in Med1 null hepatocytes in response to PPARα activators, whereas scattered residual Med1-positive hepatocytes that escaped Cre-mediated excision of floxed alleles in Med1 null livers showed DNA synthesis and were markedly hypertrophic with peroxisome proliferation in response to PPARα ligands (21). Med1 null hepatocytes fail to respond to PPARα ligand-induced peroxisome proliferation (Fig. 2). Moreover, in Med1 null mouse livers, the rare Med1-positive hepatocytes exhibit dramatic increases in peroxisome proliferation and clonal expansion when chronically treated with PPARα ligands such as Wy-14,643 (Fig. 2). Interestingly, the Med1 liver null mice develop liver tumors on long-term exposure to PPARα ligand, but all these tumors are derived from residual Med1-positive cells, but none developed from Med1 null hepatocytes (100). These results imply that Med1 plays a pivotal role in PPARα ligand-induced liver tumor development. In essence, the absence of Med1 in hepatocytes in vivo mimics the absence of PPARα (21,100), indicating that both PPARα and Med1 are essential for PPARα-regulated gene expression including hepatocyte proliferation and liver tumor development.

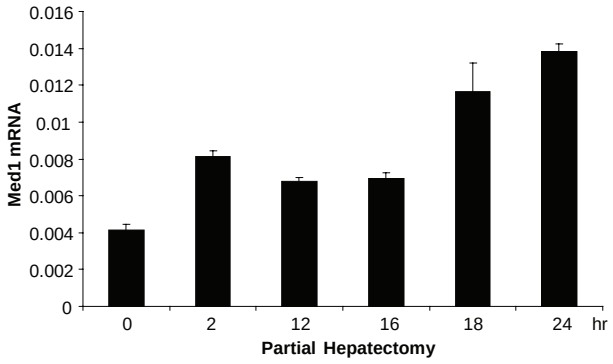

Figure 1. Increased Med1 expression following partial hepatectomy, as revealed by real-time RT-PCR. Livers were harvested at time points as indicated following hepatectomy.

Med1 Is Necessary for CAR-Regulated Gene Expression

Nuclear receptor CAR mediates the hypertrophic and hyperplastic effects in liver upon treatment with CAR activators, phenobarbital, and TCPOBOP. As a nuclear receptor, CAR interacts with the two nuclear receptor-interacting LXXLL motifs in Med1 in a ligand-dependent manner. The unliganded CAR is located in the cytoplasm in hepatic parenchymal cells. It is rapidly translocated to the nucleus in response to activation by CAR agonists (17). In Med1-deficient hepatocytes, CAR fails to translocate to the nucleus in response to activation by phenobarbital or TCPOBOP (21). Adenoviral reconstitution of Med1 in Med1 null mouse livers restores Med1-mediated nuclear translocation of CAR

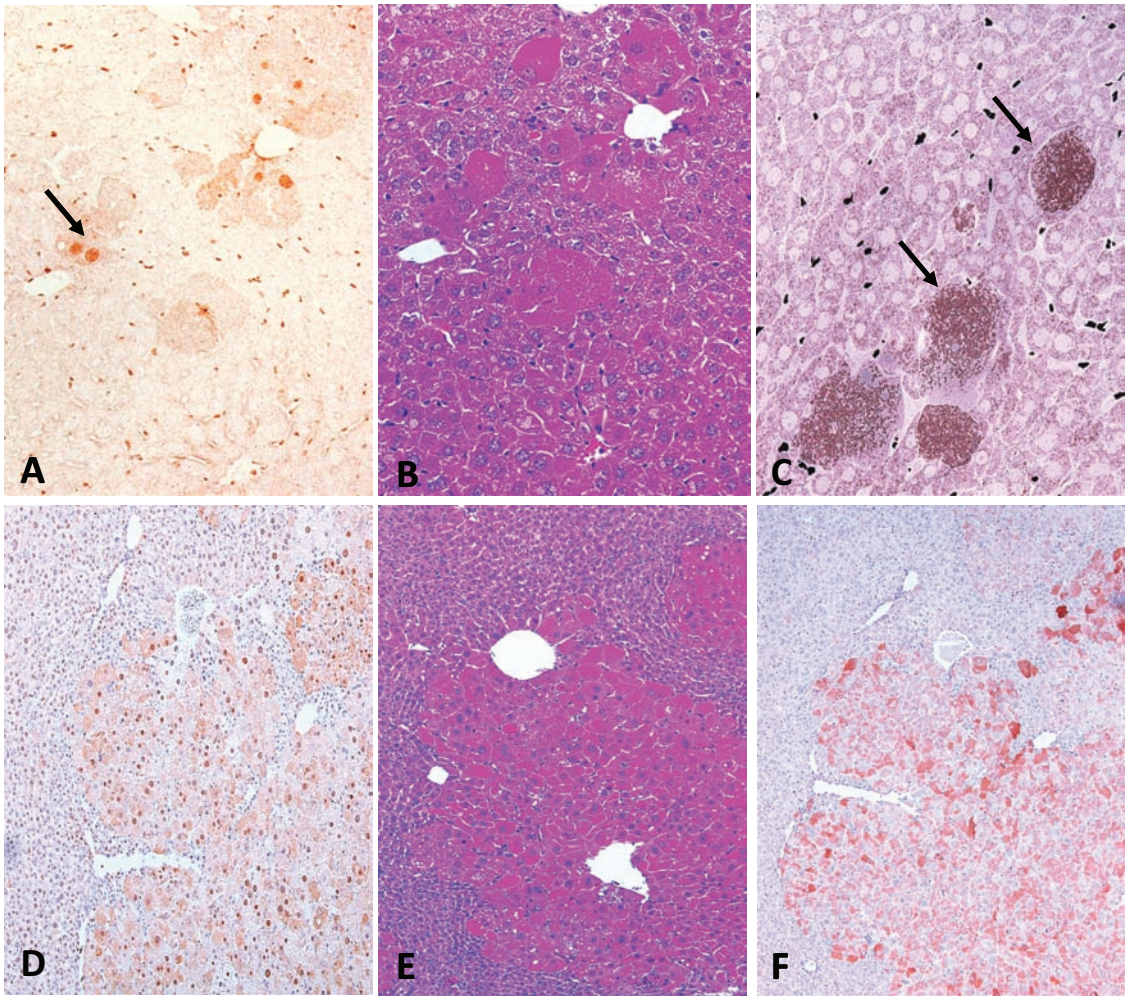

Figure 2. Effects of Med1 null on PPARα ligand induced responses in liver. Med1 liver null mice treated with Wy-14,643 (0.125% w/w) for 2 weeks (A, B, and C) and for 6 months (D, E, and F). Immunohistochemical localization of Med1 in Med1 liver conditional null mouse demonstrating absence of Med1 nuclear staining in hepatocytes except for positive staining for Med1 in few hepatocytes (A) and expanding colonies of escaped Cre deletion of large hepatocytes (D). These expanding colonies also reveal abundant cytoplasmic expression of L-PBE (F).

as well as the overexpression of CAR-regulated genes of CYP1A2, CYP2B10, CYP3A11, and CYP7A1 (21). Accordingly, Med1 is considered essential for the translocation, retention, and/or concentration of CAR in the nucleus (101,102). CAR-mediated induction of hepatic CYP3A11, CYP2B10, and CYP1A2 in Med1 null livers is reduced significantly when compared to wild-type mouse livers (21). In wild-type mice, cell cycle proteins cyclin A and cyclin D1 are upregulated in liver at 48 and 96 h after TCPOBOP injection, but not in Med1 null mice. Taken together, the Med1 is considered essential for the function of CAR (21,46). Consistent with these findings is that Med1 deficiency in liver abrogates acetaminophen hepatotoxicity (21). Of interest is striking abrogation of CCl_4-induced hepatocyte proliferation and hepatotoxicity in Med1 null livers (98). Furthermore, the diethylnitrosamine (DEN) initiation–phenobarbital promotion (CAR activation) experiment demonstrated that no tumors developed from Med1 null hepatocytes, and all tumors were Med1 positive (100). These observations suggested that all tumors were derived from residual hepatocytes, which escaped Cre-mediated deletion with intact Med1 gene, and hepatocytes deficient in Med1 were not susceptible to neoplastic transformation (100). Med1(fl/fl) HCC cell line generated from these tumors expressed Med1, and deletion of Med1(fl/fl) allele by adeno-Cre in situ injection into tumors led to necrosis of tumor cells. These data illustrate that Med1 is essential for the development of HCC in the mouse (100). In summary, Med1 is required for CAR-regulated gene expression, liver cell proliferation, and hepatocarcinogenesis. These effects may be due to the fact that CAR

translocation is Med1 dependent and that CAR target gene expression requires nuclear CAR.

Med1 Overexpression Induces Hepatocyte Proliferation

In earlier studies, Med1 was shown to function as estrogen receptor (ER) coactivator, and Med1 gene was found to be amplified in breast cancers (101). Med1 is also overexpressed in prostate cancer cells, implying that Med1 may play a pivotal role in neoplastic and non-neoplastic cell proliferation (102). Since Med1 null hepatocytes failed to respond to PPARα and CAR activators, and Med1 null hepatocytes did not give rise to tumors, it was hypothesized that Med1 is essential for nuclear receptor signal transduction in liver or that this coactivator per se might be a hepatomitogen. Liver cell proliferative response, if any, that is directly related to Med1 expression was assessed by using adenovirally driven expression of Med1 in mouse liver (20). Overexpression of Med1 in liver cells led to the induction of a broad spectrum of genes as well as hepatocyte proliferation (20). Microarray analysis revealed that Med1 upregulates many genes, including those belonging to initiation and elongation of DNA replication and cell cycle progression, those related to cell growth and mitosis (20). Induction of genes regulated by nuclear receptors PPARα, FXR, CAR, HNF4, and LXR as well as Wnt signaling pathways and genes related to NF-κB regulation was also noted in with Med1 overexpression (20). Interestingly, among these genes is the Foxm1, a key transcription factor for liver regeneration regulated by FXR (103). Foxm1 is upregulated during early cancer development and is involved in tumorigenesis due to its role in cell cycle progression and proliferation (103). Gene expression profiling data revealed that most genes involved in liver regeneration are induced significantly during Med1 overexpression (20). These include early response genes Egr1, JunB, Fos, and C-myc-binding protein (Mycbp). Med1 overexpression also dramatically induced several genes, including cyclins (B1, D1, and E1), Cdks (Cdk1, 2, 4), Cdca8, Cdc16, Cdc20, polo-like kinase 1 (PLK1), centromere proteins (CENPs), E2F family members (E2F1, 4, 6), and survivin. Almost all of these induced genes are involved in G_2/M transition. Some genes related to cell growth and mitosis are also induced significantly (greater than fourfold) and include IGFBP1, IGF2, FGF21, GADD45, Gab1 (growth factor receptor bound protein 2-associated protein 1), Mapk 14/p38, Mapk6, AKT1s1 (AKT1 substrate 1), and Gsk3α. It is suggested that Med1 is a key regulator for G_1/S and G_2/M transition and M phase progression, indicating that Med1 has a critical role in cell cycle progression and cell proliferation. Some important genes involved in a variety of liver functions include Foxm1, FoxO1, nuclear protein 1, interleukin-6-dependent-binding protein, GATA-binding protein 6 (Gata6), transcriptional enhancer factor 1(Tef1), chREBP, and C/EBP. These were induced greater than fourfold, indicating that Med1 has a pivotal role in regulating several transcriptional pathways. Interestingly, due to Med1 overexpression, ~15 subunits of the Mediator complex were also induced greater than twofold (Med25) (20), implying that Mediator complex formed in these cells may have been changed with respect to their capacity to activate transcription. Med1 overexpression induced PPARα greater than twofold along with the upregulation of approximately eight peroxisomal proteins. Our microarray data also showed induction of the DNA repair and DNA damage response-related genes as well as several apoptosis-related genes, indicating that some of the Med1-overexpressing liver cells may undergo apoptosis.

As discussed above, Med1 null hepatocytes fail to respond to peroxisome proliferator and abrogated PPARα function (19). Of interest is that Med1 overexpression-induced liver cell proliferation does not depend on PPARα. It is well recognized that Med1 is needed for PPARα-regulated gene expression, and available data also points the essential role of Med1 in the activation of several other nuclear receptors, such as CAR, FXR, TR, and GR (21,103–105). These receptors also induce liver cell proliferation. According to our microarray data, Med1 could induce hepatocyte proliferation by amplifying the signaling of various nuclear receptors and transcription factors.

Med1 Is Required for GR Function

Glucocorticoid receptor (GR) agonist dexamethasone (Dex) induces hepatic steatosis and also increases CAR receptor expression (105). Med1 is needed for GR- and CAR-mediated transcriptional activation; it suggests that Med1 deficiency would result in the attenuation of Dex-induced hepatic steatosis. Med1 null livers exhibited reduced levels of GR- and CAR-regulated mRNA compared to wild-type mouse livers (21,105). Administration of glucocorticoids resulted in diminished liver regeneration, in part attributable to GR-induced hepatic steatosis.

PHOSPHORYLATION OF Med1

Phosphorylation augments functional diversity of nuclear receptors and cofactors and provides the basis for a combinatorial code required for specific gene activation. Phosphorylation is the most common post-translational modification that dynamically regulates the molecular properties of coactivators and endows complexity to nuclear receptor-dependent gene expression and associated physiological processes. Evolutionarily, cells have developed several means to respond to internal and external stimuli that signal imbalances in metabolic processes and energy utilization. These include

rapid responses, such as phosphorylation events, as well as relatively latent effects on gene transcription (20). PPAR and other nuclear receptors are phosphoproteins, and their transcriptional activity is affected by cross-talk with kinases and phosphatases. Phosphorylation by various kinases such as ERK-/p38-MAPK, PKA/PKC, AMPK, and glycogen synthase kinase-3 (GSK3) that are activated by growth factors and cellular ATP levels regulate PPAR transcriptional activity in a context-dependent manner (106). The activity and specificity of coactivators is subject to regulation by phosphorylation. Coactivators such as SRCs, CBP/p300, PGC-1, and Med1 are phosphorylated by kinases that are involved in diverse cellular signaling (107,108).

Med1 protein has several motifs containing potential serine and threonine residues that are well conserved across the species, suggesting that Med1 is a phosphoprotein. Using in vitro phosphorylation assays, mouse Med1/PBP/TRAP220 by MAPK (ERK1 and ERK2), PKA, and PKC (109) was investigated. These studies revealed an exclusive protein kinase A (PKA) phosphorylation site at serine 656, two protein kinase C (PKC) sites at serine 796 and serine 1345, a common PKA/PKC site at serine 756, and two extracellular signal-regulated kinase 2 sites of the mitogen-activated protein kinase (MAPK) family at threonine 1017 and threonine 1444 (109). Later, it was demonstrated that ERK phosphorylates human TRAP220/Med1 in vivo (HeLa cells) at two specific sites: threonine 1032 and threonine 1457 (110). It is important to note that the motif containing threonine 1017 and 1444 in mouse Med1 is the same as human motif-containing threonine 1032 and 1444. The difference is due to the number of amino acids in mouse (1560) and human (1581) Med1 protein. Phosphorylation at these two sites by ERK stabilizes and increases the intrinsic activity of Med1/TRAP220 (109). The external stimulus for the activation of MAPK–ERK for the phosphorylation of Med1 was shown to be thyroid and gonadal hormone (dihydrotestosterone). This phosphorylation in Hela cells is required for the nuclear receptor MED1 association with the Mediator (109). Functional significance of Med1 phosphorylation at threonine 1032 was addressed in the castration-resistance prostate cancer cell growth (102). UBEC2 overexpress in many types of solid tumors, including androgen receptor (AR)-negative castration-resistant prostate cancer (CRPC) cells. PI3K/AKT-mediated Med1 phosphorylation causes interaction with proteins bound to the promoter element (FoxA1, RNA polymerase II, and TATA-binding protein) of the UBE2C oncogene with the far upstream enhancer making UBE2C locus chromatin looping to stimulate transcription (111). Phosphorylation of Med1 by ERK and/or AKT in prostate cancer cells augments androgen receptor transcriptional activity, which in turn enhances Med1 overexpression and upregulation of genes involved in inflammation, cell cycle progression, and survival (102). Persistent activation of ERK/MAPK leads to Med1 overexpression, resulting in ER-positive breast cancer cells resistant to tamoxifen (112). Phosphorylated Med1 exhibits nuclear accumulation, increased recruitment of ER–Med1 complex on the promoter of ER-responsive upon tamoxifen treatment (112). Recently, phosphorylation of Med1 by energy-sensing kinase AMP-activated protein kinase (AMPK) was demonstrated (20).

AMPK interacts with and directly phosphorylates Med1 in vitro at serine 656, serine 756, and serine 796. AMPK also phosphorylates Med1 in vivo in mouse liver and in cell lines. Of interest is that PPARα activators such as fenofibrate and Wy-14,643 increase AMPK-mediated Med1 phosphorylation in vivo (20). Furthermore, inhibition of AMPK by compound C decreases hepatocyte proliferation induced by Med1 and by PPARα activators implying a link between energy sensing, AMPK phosphorylation of Med1, and hepatocyte proliferation induced by nuclear receptors in concert with the metabolic perturbations.

PERSPECTIVE

Nuclear receptors and coactivators modulate the expression of many RNA polymerase II (Pol II)-transcribed genes involved in metabolic homeostasis and cell proliferation in liver. Perturbations of metabolic functions and changes in cell proliferation appear tightly regulated by nuclear receptors such as PPARα, CAR, FXR, and others. Of the many transcription coactivators identified to date, the Med1 subunit of the Mediator complex appears essential for the PPARα, CAR, and GR-regulated gene expression. Med1 null hepatocytes do not respond to the hepatocyte proliferative effects of PPARα activators. Med1-deficient hepatocytes do not respond to CAR activators, and this is because, in the absence of Med1, CAR remains in the cytoplasm and fails to translocate to the nucleus in response to CAR agonists. Since these two receptors also regulate fat metabolism by elevating fatty acid oxidation systems, absence of Med1 also affects energy metabolism. Mediator contains nearly 30 proteins, but as of now, there is insufficient knowledge as to the role of individual subunits of the Mediator complex in the nuclear receptor-regulated metabolic functions (113–115). Although there is interest in Med1 subunit regulated functions, it is unclear as to the role of other subunits of Mediator complex in relaying the transcriptional signal, for example, from the PPARα and Med1 relay. It is possible that other coactivators and other components of the Mediator may be required for Med1 to transmit the signal to transcriptional machinery. These issues can be explored as mice lacking other subunits of the Mediator become available.

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