Review

Peroxisome proliferator-activated receptor-γ cross-regulation of signaling events implicated in liver fibrogenesis

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ABSTRACT

Peroxisome proliferator-activated receptor-γ (PPARγ) is a nuclear receptor with transcriptional activity controlling multiple physical and pathological processes. Recently, PPARγ has been implicated in the pathogenesis of liver fibrosis. Its depleted expression has strong associations with the activation and transdifferentiation of hepatic stellate cells, the central event in liver fibrogenesis. Studies over the past decade demonstrate that PPARγ cross-regulates a number of signaling pathways mediated by growth factors and adiponectins, and cellular events including apoptosis and senescence. These signaling and cellular events and their molecular interactions with PPARγ system are profoundly involved in liver fibrogenesis. We critically summarize these mechanistic insights into the PPARγ regulation in liver fibrogenesis based on the updated findings in this area. We conclude with a discussion of the impacts of these discoveries on the interpretation of liver fibrogenesis and their potential therapeutic implications. PPARγ activation could be a promising strategy for antifibrotic therapy.

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Abbreviations: adiponectin receptor; AMPK, 5′-AMP-activated protein kinase; AP1, activator protein 1; C/EBP, CCAAT/enhancer binding protein; CHB, chronic hepatitis B; Cidea, mitochondrial cell death-inducing DNA fragmentation factor α-like effector A; CTGF, connective tissue growth factor; DR, death receptor; ECM, extracellular matrix; Egr-1, early growth response-1; ERK, extracellular signal-regulated kinase; HGF, hepatocyte growth factor; HSC, hepatic stellate cell; JAK2, Janus kinase 2; JNK, c-Jun N-terminal kinase; LXR-α, liver X receptor-α; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-κB; NIK, NF-κB-inducing kinase; OB1R, obese gene product receptor; PDK1, platelet-derived growth factor; PI3K, phosphatidylinositol-3-kinase; PPAR, peroxisome proliferator-activated receptor; PPRE, peroxisome proliferator response elements; RXR-α, retinoid X receptor-α; SA-β-gal, senescence-associated β-galactosidase; SIRT1, silent information regulator type 1; SMP30, senescence marker protein 30; SREBP-1c, sterol regulatory element-binding proteins-1c; STAT3, signal transducer and activator of transcription 3; TLR, Toll-like receptor; Tnfr, tumor necrosis factor; TRAIL-R, TNF-related apoptosis-inducing ligand receptor.

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1. Introduction

Peroxisome-proliferator activated receptors (PPARs) belong to the superfamily of nuclear hormone receptors. To date, four isoforms of PPARs have been identified, namely PPARα, β, γ and δ, which regulate transcription of a number of target genes controlling many important physiological processes [1]. There are two major forms of PPARγ, namely γ1 and γ2, resulting from alternate promoter usage and splicing [2]. PPARγ structurally comprises a central DNA-binding domain, a carboxy terminal ligand-binding domain and two transcription activation function motifs termed AF-1 and AF-2 [3]. The core of PPARγ ligand-binding site is a large Y-shaped cavity composed of 12 α-helices located at the C-terminal, which functions as a molecular switch and ligand binding to which is required for full agonist activity toward PPARγ [4]. In the absence of ligands, PPARγ interacts with the co-repressor proteins and inhibits gene expression. Upon binding to cognate ligands, PPARγ dissociates the co-repressor and translocates from cytoplasm to the nucleus where it recruits co-activator proteins and forms a heterodimer with retinoid X receptor-α (RXR-α). The multiprotein complex then binds to peroxisome proliferator response elements (PPRE), the specific DNA sequences in target gene promoter, leading to transcriptional activation [5]. Posttranslational modifications such as phosphorylation [6], sumoylation [7] or ubiquitination [8] may also modulate the transcription activity of PPARγ.

PPARγ is expressed in various tissues and cell types, mainly in adipose tissue, where it regulates adipocyte differentiation and glucose homestasis [9]. PPARγ activation by its endogenous ligands such as fatty acids initiates transcription of adipogenesis-associated target genes, such as FABP4 (which encodes fatty acid-binding protein 4) and CD36 (which encodes fatty acid translocase) [10]. Moreover, PPARγ plays a pivotal role in metabolic syndrome through genetic correlation with insulin resistance and type 2 diabetes mellitus, because it is a key regulator of insulin sensitivity and the drug target of synthetic thiazolidinedione agents against type 2 diabetes. PPARγ activation ameliorates hyperglycemia and dyslipidemia in diabetes due to its insulin sensitization capability [11].

Increasing understanding toward PPARγ biology demonstrates that this transcription factor has pleiotropic functions in various fundamental pathways with more wide-ranging pathophysiological implications. PPARγ is shown to be involved in circadian regulatory system [12] and bone marrow adipogenesis [13]. PPARγ is also implicated in cell-fate determination in a variety of cell types, for instance, ligand activation of PPARγ can result in growth inhibition or apoptosis in fibroblasts [14] and several kinds of cancer cells [15, 16].

Recently, much attention has been paid to the anti-inflammatory and protective effects of PPARγ during tissue repair program. PPARγ regulates gene transcriptions involved in the wound-healing process of many organs, and thereby ameliorates inflammation, oxidative stress, and matrix remodeling in the injured tissue [17]. Mode for PPARγ-mediated gene transcription and the biological consequences are illustrated in Fig. 1. Furthermore, perhaps a breakthrough in understanding PPARγ pathophysiology over the past decade could be the recognition that PPARγ acts as a pivotal molecular in liver fibrogenesis. Its transcriptional dysfunction and interplay with a series of signaling events that are involved in fibrogenesis underlie the molecular pathogenesis of liver fibrosis [18].

Accumulating knowledge in this facet provides novel insights into the signal transduction-based pharmacological intervention of liver fibrosis, a widespread disorder for which effective therapies are still lack in current clinical context. In this review, we will first briefly describe the basic principles of liver fibrogenesis and the newly uncovered role of PPARγ in this pathology, and then critically discuss the updated findings on how PPARγ interacts with the primary signaling events implicated in liver fibrogenesis.

2. Role of PPARγ in liver fibrogenesis

Liver fibrogenesis is defined as a dynamic wound-healing process characterized by excessive production and deposition of extracellular matrix (ECM) mainly type I collagen in the liver. The ECM deposition distorts hepatic sinusoids and compromises hepatocyte function. It is well-established that activation of hepatic stellate cells (HSCs), a type of nonparenchymal cell resident in the space of Disse, is the central...
event in liver fibrogenesis [19]. Normally, HSCs are quiescent and stores up to 85% of the body’s total vitamin A content in their lipid droplets. Upon injury, they are activated and undergo transdifferentiation from non-proliferating cells to myofibroblasts responsible for the overproduction of collagen-rich ECM. Meanwhile, the lipid droplets and vitamin A contents are diminished rapidly, exhibiting a deletion of adipogenic phenotype [20].

Advances in understanding the molecular mechanisms underlying HSC activation and liver fibrogenesis suggest the involvement of complex signaling modulations performed by a number of cytokines and chemokines within HSCs or between HSCs and other cell types [21]. Transcriptional factor PPARγ has been identified to be the central regulator of these signaling events and functions as a switch molecule for HSC activation and phenotype alteration [22]. There has been clear evidence that HSC activation was accompanied by significantly decreased PPARγ expression, whereas ectopic PPARγ expression or PPARγ ligands reversed the biochemical features of HSC activation and reduced collagen secretion both in vitro and in vivo [23–25]. Mechanistic investigations showed that PPARγ maintained HSC quiescence through regulating a panel of adipogenic transcription factors, including CCAAT/enhancer binding protein (C/EBP), liver X receptor-α (LXRα), and sterol regulatory element-binding proteins-1c (SREBP-1c), which were rapidly depleted during HSC transdifferentiation [26]. Moreover, adipogenic transcriptional regulation in HSCs by PPARγ also occurred at the level of chromatin packaging, because DNA methylation inhibitor blocked HSC myofibroblastic transdifferentiation and prevented the diminished PPARγ expression in HSCs [27]. Collectively, these discoveries preliminarily address the adipogenic mechanisms of PPARγ in HSC activation and suggest the antifibrotic role of PPARγ activation. Indeed, ectopic PPARγ expression and PPARγ agonists have exhibited therapeutic benefits for experimental liver fibrosis in various animal models [28–30]. And thiazolidinedione PPARγ ligands have been undergoing clinical evaluation for their therapeutic efficacy and safety as therapies against chronic liver diseases including fibrosis in humans [31].

Although the precise molecular mechanisms underlying the antifibrotic potential of PPARγ system remain largely unknown, it is highly possible that the PPARγ interactions with a series of signaling processes that regulate HSC expression of fibrogenic molecules and the dynamic balance between HSC proliferative and apoptotic status could account for the pivotal role of PPARγ in liver fibrogenesis. The past decade has indeed witnessed tremendous advances in this research area. In the following sections, we will survey the current past decade has indeed witnessed tremendous advances in this research area. In the following sections, we will survey the current understanding of PPARγ cross-regulation of the key fibrosis-related signaling events. Of note is that some findings discussed below are not restricted to the pathology of liver fibrosis due to limited literature data, but we hold that they are relevant information of significance and can illuminate the interpretation of liver fibrosis and guide the development of antifibrotics.

3. PPARγ crosstalk with growth factors

3.1. Transforming growth factor-β signaling

A variety of cell types produce transforming growth factor-β (TGF-β) that consists of three major isoforms, namely β1, β2 and β3. TGF-β1 is the principal isoform implicated in liver fibrosis. TGF-β1 is stored as an inactivated form bound to a latency-associated protein. Following activation in liver injury, TGF-β1 initiates its fibrogenic signaling cascade by binding to the type I and type II surface receptors (TβRI and TβRII), which belong to the family of receptor serine/threonine kinase, leading to phosphorylation of Smad2 and Smad3, and allowing Smad2/3 to associate with the common mediator Smad4. Subsequently, the Smad heterodimers translocate into the nucleus and trigger target gene transcription, including collagen I and III. The inhibitory Smad6/7 can disrupt the binding of Smad2/3 to the receptor, constituting an endogenous suppression of the transduction [32]. Activated HSCs express TGF-β1 and Smads 2, 3, 4, and 7, and the levels of TGF-β1 and Smads 2 and 4 increase as fibrosis progresses, but Smad7 expression is in silence, leaving Smads 2, 3 and 4 to promote the fibrotic responses [33]. Moreover, Smad3 knockout resulted in decreased collagen I production and attenuated HSC proliferation [34]; whereas forced expression of Smad7 blocked HSC transdifferentiation due to inhibited Smad2/3 phosphorylation [35]. There are also reports showing the involvement of TβRII/Smad1/ERK pathway [36], the TGF-β-induced collagen stability via p38 activity [37], and the profibrogenic role of Nogo-B via facilitating TGF-β/Smad2 signaling in liver fibrogenesis [38].

A number of literature data support a direct role for PPARγ in modulation of TGF-β1/Smads signaling. PPARγ could abrogate TGF-β1/Smad stimulation of transcriptional responses and collagen gene expression in the profibrotic myofibroblasts [39]. PPARγ activation also abrogated TGF-β1-induced promoter activity of connective tissue growth factor (CTGF), a key molecular amplifying the profibrogenic responses in the fibrotic liver [40, 41], but this suppression could be completely rescued by Smad3 overexpression but not Smad4 [42]. This was the first evidence that CTGF is a PPARγ-regulated gene via TGF-β1/Smad3 signaling pathway. Ligand activation of PPARγ also potently inhibited CTGF expression in rat HSCs by disrupting TGF-β1 signaling [43], and inhibited TβRII/Smad3-mediated induction of ECM gene expression in cultured human HSCs [44]. However, the PPARγ ligands probably affected neither the Smad2/3 protein level nor Smad2/3 phosphorylation under these conditions. This may give rise to a hypothesis that activated PPARγ triggers its association with certain nuclear receptor co-activators, then becoming repressive for Smad-mediated transcription. Indeed, there has been preliminary proof that transcriptional co-activator p300, also a histone acetyltransferase, participated in PPARγ-mediated inhibition of TGF-β/Smad signaling [45]. Consistent with this, Ghosh et al. demonstrated that PPARγ disrupted Smad-mediated transcriptional pathway by blocking p300 recruitment and histone H4 hyperacetylation, leading to suppression of TGF-β1-induced collagen gene expression in human fibroblasts [46]. These results suggest PPARγ-mediated inhibition of p300/Smad2/3 association as a novel mechanism underlying PPARγ modulation of TGF-β1/Smad profibrotic responses. Moreover, PPARγ was shown to disrupt the binding of TGF-β1-activated Smads to their response elements [47], indicating that PPARγ transrepression toward TGF-β signaling may also occur at the level of specific mediators of the pathway, such as Smads. On the other hand, some details of how TGF-β affects PPARγ system have also been elucidated. Fu et al. proposed that TGF-β1 initially stimulated PPARγ expression via ERK/Egr-1 (early growth response-1) signaling, whereas late inhibited PPARγ expression via activator protein 1 (AP1) and Smad3, and possibly NAB2 [48]. Collectively, these findings provide important information on the cross-talk between PPARγ and TGF-β signaling. It would be interesting to envision that these molecular linkings are potential pharmacological targets for fibrosis therapy, but one should consider how to repress the fibrogenic activities of TGF-β1 in HSCs while retaining the other beneficial effects of TGF-β1 in the injured liver such as the anti-inflammatory property and its function as an important regulator of hepatocyte proliferation [49].

We recently demonstrated that natural polyphenolic compound curcumin significantly reduced the expression of TβRI and TβRII in a PPARγ activation-dependent manner, leading to TGF-β3 signaling disruption in HSCs and potent antifibrotic effects. In addition, curcumin activation of PPARγ abrogated TGF-β1-induced CTGF expression [50–52]. These data are consistent with the aforementioned discoveries on the PPARγ crosstalk with TGF-β signaling and imply that pharmacological intervention via PPARγ activation can indeed block the profibrogenic TGF-β1/Smad pathway, and thereby inhibit HSC activation and treat hepatic fibrosis. Actually, the safety of curcumin for human use has been demonstrated by clinical evidence in some other pathological
contexts [53]. More basic and clinical studies are needed to recommend this natural PPARγ ligand for human liver disease therapy.

3.2. Platelet-derived growth factor signaling

Platelet-derived growth factor (PDGF) is another key fibrogenic stimulus to HSC activation and hepatic fibrogenesis. PDGF is structurally identified as a disulfide-linked dimeric protein composed of varying combinations of four polypeptide chains (A, B, C, and D). PDGF transmits its signal via the tyrosine kinase receptors PDGFR-α or -β and the downstream mitogen-activated protein kinase (MAPK) cascades including extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 [54]. In liver, quiescent HSCs are negative for PDGF-βR, whose expression, however, is significantly upregulated in activated HSCs both in vitro and in vivo, rendering HSCs highly responsive to PDGF-B [54]. Recently, PDGF-C and -D have also been linked to liver fibrogenesis in two in vivo studies, where PDGF-C overexpression led to fibrosis and hepatocellular carcinoma [55], and PDGF-D significantly facilitated HSC proliferation and ECM expression [56].

Although numerous studies have described how PPARγ activity is regulated by receptor tyrosine kinases [57], information on PPARγ regulation of PDGF/MAPK pathway is newly emerging. It was found that PDGF signaling led to progressive reduction in PPRE reporter activity; whereas natural and synthetic PPARγ ligands or transfection with PPARγ repressed PDGF-induced human HSC proliferation in culture [24, 58]. Activation of PPARγ by ligands also inhibited ERK activity and PDGF-induced c-Fos promoter activity, suggesting that PPARγ affected PDGFR/MAPK pathway [59]. Moreover, some other studies covering a variety of pathological situations demonstrated the repression of PPARγ toward JNK signaling [60], and p38 MAPK pathway [61]. However, there is also evidence that PPARγ ligands could activate MAPK pathway independently of PPARγ [62]. Thus, it could be a little bit hard to give a clear overview on the molecular basis for PPARγ ligand regulation of PDGF/MAPK signaling implicated in liver fibrotic responses, but this issue should be addressed with much importance, because it implies potential therapeutic interventions. Indeed, PDGFR-kinase inhibitor imatinib was shown to be promising for treating liver fibrosis [63]. Additionally, the natural antifibrotic curcumin dependent on PPARγ activation interrupted PDGF signaling in activated HSCs by reducing PDGFR-β phosphorylation and gene expression, and suppressing its downstream cascade including ERK1/2 and JNK1/2 [64]. These results not only further elucidate the mechanisms of curcumin for inhibition of HSC activation and blockade of hepatic fibrogenesis, but also provide new insights into the implications of PPARγ interaction with PDGF/MAPK pathway in liver fibrosis.

3.3. Hepatocyte growth factor signaling

Hepatocyte growth factor (HGF) is originally identified as a powerful mitogen for hepatocytes. HGF stimulates cellular growth and motility, and morphogenesis through activating its specific tyrosine kinase receptor known as c-Met (an oncogene product) at cell surface [65]. HGF/c-Met system plays an essential role in liver development and regeneration, and its disruption affects hepatocyte survival and tissue remodeling [66]. Unlike TGF-β and PDGF discussed above, HGF has emerged as a cytokine that potently prevents liver fibrogenesis. There has been in vivo evidence that HGF suppressed liver fibrosis by reducing HSC activation and collagen synthesis, and promoted liver recovery from chronic injuries in distinct animal models [67-69]. HGF-mediated antifibrosis is probably attributed to disruption of profibrogenic TGF-β signaling including decreased expression of TGF-β1 and its receptor and inhibited Smad2/3 nuclear translocation. In addition, HGF was found to enhance the interaction of phosphorylated Smad3 with galectin-7 that inhibited TGF-β-stimulated COL1A2 and plasminogen activator inhibitor-1 transcription in activated HSCs [70].

Increasing studies have suggested PPARγ regulation of HGF/c-Met signaling system. Although it is known that regulation of HGF gene expression involves a number of transcription factors such as COUP-TF, C/EBP, estrogen receptor, nuclear factor-1, and AP2 [71-73], a PPARγ responsive element was also found to localize upstream from the transcription start site of HGF gene promoter and played a crucial role. Ligand activation of PPARγ strongly stimulated HGF promoter activity, and induced HGF mRNA and protein expression in fibroblasts [74]. This provided clear evidence that HGF is a target gene of PPARγ. HGF expression induced by PPARγ agonists was also observed in other pathological contexts of tissue injury [75]. Furthermore, PPARγ played a role in HGF-mediated disruption of TGF-β signaling implicated in liver fibrogenesis, because PPARγ activation promoted c-Met receptor phosphorylation, upregulated expression of TG-interacting factor, a Smad transcriptional co-repressor, and suppressed Smad-mediated gene transcription, suggesting novel mechanisms that couple PPARγ, HGF, and TGF-β/Smad signaling [76].

Although to address how PPARγ mediates the antifibrotic potential of HGF signaling requires more illuminating evidence in vivo, HGF gene therapy has indeed shown therapeutic benefits in a series of animal studies through various delivery methods such as transfection of human HGF gene into skeletal muscles [77], infusion of plasmid DNA via portal vein followed by electroporation [78], transfection with HGF-expressing adenovirus vectors [79], or transfection with human HGF naked plasmid DNA [80]. Fibrogenic makers and collagen synthesis were all significantly suppressed under these conditions. Since PPARγ activation can directly stimulate HGF/c-Met system, it would be attractive to suggest a therapeutic approach combining HGF gene therapy and small molecule PPARγ agonists for synergistic effects. This may hold promise in manipulation of liver fibrosis worthy of further investigations.

4. PPARγ crosstalk with adipokines

4.1. Leptin signaling

Adipokines are polypeptide cytokines predominantly secreted in adipose tissue by a regulated pattern. The obese gene product leptin is a best-investigated adipokine actively involved in liver fibrogenesis. Leptin transmits its biological activity through leptin receptors (ObRs), which consist of six isoforms, and downstream Janus kinase 2 (JAK2) signal transducer and activator of transcription 3 (STAT3) pathway [81]. A number of investigations in vitro [82, 83] and in vivo [84, 85] demonstrated that leptin is a potent stimulator for HSC profibrogenic behaviors, including increased proliferation, prevented apoptosis, collagen overproduction, and upregulated proinflammatory and proangiogenic cytokines. Leptin also induces HSC phagocytosis, during which Rho- guanosine triphosphatase-ases mediate the cytokskeleton regulation, suggesting a novel role for leptin responsible for fibrogenic process [86]. In addition, leptin also activates kupffer cells and macrophages, and stimulates hepatic sinusoid endothelial cells to secrete TGF-β [87].

The critical role of PPARγ in lipometabolism makes it relevant to adipokine signal transductions. Indeed, several in vivo studies demonstrated PPARγ involvement in leptin signaling in various physiopathological conditions. Leptin stimulated expression of PPARγ co-activator-1 [88], and enhanced hepatic triglyceride content associated with fat-specific protein 27 that is a PPARγ target gene [89]. Recently, evidence in vitro showed that leptin downregulated PPARγ gene expression by activating phosphatidylinositol-3-kinase (PI3K)/Akt or ERK pathway in rat HSCs [90] and Egr-1 was involved in suppressed PPARγ expression by leptin [91]. Leptin also suppressed PPARγ protein expression in HSCs potentially through leptin-induced ERK1/2 activation in vivo [92].
Compelling evidence has also highlighted the PPARγ modulation of leptin signaling. Activation of PPARγ by thiazolidinediones inhibited leptin expression in cell culture system [93] and rodent models [94]. Thiazolidinedione-induced leptin suppression was shown to be associated with PPARγ antagonism to C/EBP-α on at least two separate promoters of leptin at the transcriptional level [95]. Furthermore, PPARγ ligand ciglitazone diminished ObR expression in leptin-activated human HSCs potentially associated with suppression of ERK activation, but it failed to reverse leptin-induced inhibition of SREBP-1c expression presumably because mere PPARγ ligand might not sufficiently activate PPARγ to the extent of changing SREBP-1c mRNA level [96]. Although the in vivo evidence for PPARγ interaction with leptin signaling in the context of liver fibrogenesis is eagerly awaited so far, clinical data has indicated the correlation between PPARγ gene expression (both γ1 and γ2 isoform) and leptin secretion in humans [97]. These insights provide rationale for developing novel therapeutics targeting PPARγ/leptin system for liver fibrosis therapy. Indeed, the natural PPARγ agonist curcumin has recently been shown to protect HSCs against leptin-induced activation, making it a more promising therapeutic candidate for the management of hepatic fibrosis in obese patients [98].

4.2. Adiponectin signaling

Adiponectin and its receptor (AdipoR) represent a new adipokine system involved in liver fibrogenesis. Adiponectin structurally contains a collagen-like domain connecting to a globular moiety that retains the functional significance after cleavage. Adiponectin bioactivity is mostly attributed to the high-molecular weight form rather than the full-length adiponectin [99]. Two specific receptors, namely AdipoR1 and AdipoR2, serve as transducers for globular and full-length adiponectin, respectively, but which isoform is more susceptible to adiponectin remains to be defined [100]. Contrary to its adipokine counterpart leptin, adiponectin is a beneficial molecule for hepatic inflammation and fibrosis. Adiponectin knockout led to severe liver fibrogenesis with elevated TGF-β1 and CTGF expression in animals [101]. The mechanisms underlining the antiobrogenic effects of adiponectin include induction of caspase-dependent apoptosis in activated HSCs [100], upregulation of 5′-AMP-activated protein kinase (AMPK) and subsequent inhibition of AKT pathway [102], activation of mitochondrial respiratory chain complexes involving uncoupling protein 2 [103], and recruitment and phenotype polarization of kuffer cells [104].

PPARγ also plays a role in regulation of adiponectin expression and secretion. PPRE was identified to be present in human adiponectin promoter [105], and PPARγ agonists could induce transactivation of adiponectin promoter [106]. Adiponectin expression induced by PPARγ ligands required PPARγ2 isoform rather than C/EBPα in vivo [107], and involved activation of AMPK pathway in vivo [108]. In the context of metabolic syndrome, PPARγ agonist rosiglitazone was found to result in significantly increased adiponectin gene expression in rodents [109], PPARγ agonists MK-0767 [110] and pioglitazone [111] could also improve adiponectin secretion and sensitivity in humans. Population genetic analysis further indicated that the Pro12Ala polymorphism of the PPARγ gene might influence the serum level of adiponectin in humans [112, 113]. In the context of liver pathology, it was recently reported that ligand activation of PPARγ was unable to halt HSC activation in HSCs genetically lacking adiponectin, suggesting adiponectin as a major downstream effector for PPARγ actions; however, adiponectin-induced inhibition of HSC activation could be PPARγ-independent, because adiponectin overexpression in PPARγ-null HSCs suppressed collagen expression in the absence of PPARγ [114]. These findings highlight a complicated relationship between PPARγ and adiponectin in HSC activation. Further investigations are needed to elucidate the mechanisms responsible for these observations.

How endogenous PPARγ associates with adiponectin is increasingly understood. PPARγ could be negatively regulated by poly(ADP-ribose) polymerase 1, which in turn contributed to the inhibited expression of adiponectin and Adipor1 in cardiac fibroblasts [115]. In addition, PPARγ suppressed the transcriptional activity of endoplasmic reticulum p44, which increased adiponectin secretion in human embryonic kidney cells [116]. Intriguingly, PPARγ together with its endogenous ligands was recently found to mediate the vitamin E-induced adiponectin expression in 3T3-L1 cells [117]. What implications of these findings in the pathology of liver fibrosis are largely unknown, but should be further investigated as they may be instrumental for therapeutic interventions of liver fibrosis given the antifibrotic potential of both PPARγ and adiponectin. Surprisingly, adiponectin and PPARγ agonist rosiglitazone were recently indicated to promote HBV replication in vitro, suggesting that control of adiponectin might be a therapeutic modality for treatment of chronic hepatitis B (CHB) [118]. This probably creates a dilemma for adiponectin use in chronic liver disease therapy considering the higher risk of CHB progressing to fibrosis.

5. PPARγ modulation of apoptosis

Apoptosis is highly regulated cell death biochemically characterized by nuclear fragmentation, chromatin condensation, membrane alterations, and cell shrinkage. Apoptosis is triggered primarily through the extrinsic and intrinsic pathways, two mutually inclusive signalings. The two pathways share common downstream effectors called caspases, a group of cysteine proteases acting as ultimate executors of apoptosis [119]. Caspase-independent apoptosis also exists [120]. We here do not describe the general mechanisms of apoptosis (for review [121]), but focus on the apoptosis in the pathogenesis of liver fibrosis. Fibrogenesis is concomitant with significant hepatocyte apoptosis [122], whereas HSC apoptosis contributes to fibrosis regression [123], and thus HSC apoptosis-inducing strategy has high potential to reverse liver fibrosis [124]. PPARγ-regulated apoptosis is a critical cellular event implicated in liver fibrosis.

5.1. The extrinsic pathway

The extrinsic cascade of apoptosis is initiated by membrane-bound death receptors (DRs), including Fas, tumor necrosis factor receptor 1 (TNF-R1), and TNF-related apoptosis-inducing ligand receptor 1 and 2 (TRAIL-R1 and -R2), binding to their ligands FasL, TNF-α, and TRAIL. Then the adaptor Fas-associated protein with death domain is recruited to form the death-induced signaling complex, which subsequently activates caspase-8 and -3 in sequence [125]. Death receptors and their ligands are ubiquitously expressed in liver, thus the apoptotic machinery in liver fibrosis is commonly triggered through the extrinsic pathway [126]. Activation of Fas and TRAIL-R1 is particularly associated with hepatocyte apoptosis in chronic liver injury [122]. TNF-α binding to TNF-R1 also activates nuclear factor-κB (NF-κB), a transcription factor for production of many pro-inflammatory cytokines, and further amplifies liver injury [127]. TRAIL-R2 is not expressed on hepatocytes, but is preferentially upregulated in activated HSCs, rendering them susceptible to TRAIL-mediated apoptosis [128]. This indicates that induction of apoptosis using TRAIL-R2 agonistic antibody may selectively kill HSCs. Fas/Fas-ligand-dependent mechanism was also found in HSC apoptosis [129].

PPARγ system has molecular associations with the signalings mediated by death ligands. TNF-α suppressed the PPARγ transactivation induced by ligands via NF-κB that was activated by the TAK1/TAB1/NIK (NF-κB-inducing kinase) cascade in osteoblasts [130]. TNF-α also induced protein cleavage of PPARγ in a caspase-dependent manner in adipocytes, leading to disrupted nuclear localization of PPARγ [131]. Conversely, ligand activation of PPARγ was found to inhibit TNF-α expression and the downstream cascade
such as NF-κB transcriptional activity in a variety of culture system [132–134].

Two studies have described the direct linking between PPARγ and the death receptor signaling in the pathology of liver fibrosis. Sung et al. reported that TNF-α inhibited PPARγ activity at post-translational level in HSCs by disrupting PPARγ-PPRE binding and ERK1/2-mediated phosphorylation of PPARγ at Ser82 but not via the NF-κB pathway [135]. These results may help account at least in part for the dramatically diminished PPARγ expression in liver fibrogenesis, where inflammation is persistent with excessive production of TNF-α [136]. The death ligands are likely to be external factors promoting PPARγ depletion in activated HSCs in addition to the intracellular genetic alterations. Furthermore, Wang et al. recently demonstrated that PPARγ interaction with death receptor (especially DR5) pathway was predominant in the apoptosis triggered by C/EBP-α in HSCs, underlying the in vivo observation that transfection of C/EBP-α gene ameliorated CCL4-induced hepatic fibrosis [137]. The data in this study provided clear evidence that PPARγ modulates the extrinsic pathway of apoptosis in HSCs. It will also be important to perform more studies with in vivo models to define the importance of these molecular associations in liver fibrogenesis.

5.2. The intrinsic pathway

The intrinsic pathway of apoptosis, known as mitochondrial pathway, is initiated by the intracellular stimuli and primarily mediated by the Bcl-2 family proteins that act as sensors to integrate death and survival signals at the level of mitochondria keeping the balance between proapoptotic (i.e., Bid, Bad, Bax) and antiapoptotic (Bcl-2 and Bcl-XL) members of the family. The intracellular apoptotic stress leads to mitochondrial outer membrane permeabilization and subsequently cytochrome C release, a nearly universal event in apoptosis, and triggers a final caspase-dependent apoptosis cascade (mainly involving caspase-9, -3 and -7 in sequence) culminating in cellular fragmentation. Meanwhile, endogenous cellular inhibitors of apoptosis proteins normally inhibiting accidentally activated caspases are neutralized [138]. In the context of liver fibrosis, rat HSCs can undergo apoptosis via Bcl-2 and caspase signaling pathway, and microRNA-15b and -16 are identified to be essentially involved by gene ontology analysis [139]. However, activated human HSCs in their myofibroblast-like phenotype to undergo caspase-dependent apoptosis are markedly resistant to most proapoptotic stimuli of potential pathophysiological or therapeutic relevance, possibly due to Bcl-2 overexpression [140]. These findings not only highlight the mechanistic disparities between rodents and humans, but of clinical significance, imply that the fibrosis reversal observed in humans may not be entirely dependent on HSC apoptosis and needs long-duration therapy.

Molecular interactions of PPARγ with the intrinsic pathway of apoptosis have been delineated. PPARγ was found to mediate mitochondrial apoptotic pathway in liver associated with transcriptional regulation of proapoptotic factor Cidea (mitochondrial cell death-inducing DNA fragmentation factor α-like effector A), a novel target gene of PPARγ [141]. PPARγ also induced apoptosis via upregulating cathepsin L expression in human monocyte-derived macrophages, which further promoted Bcl-2 degradation without affecting Bax protein level [142]. Activation of PPARγ by its natural or synthetic ligands has also been shown to induce apoptosis in many types of cells including HSCs. Our data demonstrated that the natural PPARγ ligand curcumin was a potent apoptosis-inducing agent in activated HSCs in vitro through increasing Bax abundance and reducing Bcl-2 level, and stimulating caspase-3 activity [50]. Synthetic ligand troglitazone induced apoptosis of liver cancer cells in a caspase-3-dependent manner via inhibiting expression of survivin, an apoptosis-inhibiting factor, and driving it to translocate from nucleus to cytoplasm, whereas Bax expression was not affected [143]. The proapoptotic effects of PPARγ agonists via mitochondrial pathway were also recapitulated in rat primary adipocytes, involving the inhibition of AKT-1 and Bcl-2, which decreased Bad phosphorylation and induced cytochrome C release and caspase-3 activation [144]. These findings suggest that activation of the intrinsic pathway may be an important event in apoptosis induced by ligand activation of PPARγ. However, PPARγ-independent mechanism was also observed in Bcl-2-mediated apoptosis induced by PPARγ ligands in prostate cancer cells [145].

Although it seems that PPARγ and its ligands are proapoptotic via stimulating mitochondrial apoptotic pathway, the converted effects also existed in some other cell types in various pathological contexts, such as hepatocytes in hemorrhagic shock [146], hippocampal neurons in oxidative stress [147], and cardiomyocytes in ischemic cardiac disease [148]. Under these situations, PPARγ was shown to upregulate the Bcl-2 anti-apoptotic protein and thereby protect the cells against apoptosis. Taken together, the molecular interpretations for PPARγ interaction with the intrinsic pathway of apoptosis have no exclusive conclusions yet, but they indeed provide novel insights into the apoptotic mechanisms in liver fibrogenesis. Their implications for targeted apoptosis in HSCs and for hepatocyte apoptosis prevention in fibrosis therapy remain to be defined.

6. PPARγ modulation of cellular senescence

Cellular senescence is defined as a process in which cells lose their ability to proliferate. Senescent cells are irreversibly arrested at the G1 phase of cell cycle without responses to external stimuli but maintain their metabolic activities [149]. Senescence is associated with a set of specific alterations in gene expression pattern, exhibiting several characteristics that distinguish senescent cells from quiescent ones, including shortened telomeres, upregulated activity of senescence-associated β-galactosidase (SA-β-gal), and elevated expression of p16INK4a and p21WAF1/CIP [150], of which p16INK4a is a key biomarker of aging in vivo [151], and its accumulation can trigger the onset of cellular senescence [152].

Cellular senescence is involved in pathology of various liver diseases. Hepatocyte-specific senescence due to telomere shortening correlated with liver cirrhosis in both experimental [153] and human contexts [154]. Telomere shortening also induced hepatocyte senescence in the HCV-infected liver via elevated cell-cycle turnover, and oxidative stress accelerated this process especially in the advanced stage of fibrosis [155]. The tumor suppressor p53 is another critical performer in cellular senescence program. Reactivation of endogenous p53 in p53-deficient hepatocellular carcinoma led to complete regression of hepatocellular carcinoma [156, 157]. Furthermore, senescent human HSCs showed less fibrogenic phenotype and expressed decreased ECM proteins [158]. Senescence of activated HSCs also contributed to the clearance of profibrogenic HSCs in the fibrotic liver, and p53 and nature killer cells played a critical role [159]. These observations advance the understanding toward fibrosis reversal complimentary to the apoptotic mechanisms, but how HSC senescence is initiated, and how relevant these findings in rodents is to liver fibrosis pathogenesis in humans remain to be defined. More recently, there has been clinical evidence that cellular senescence in the liver of children with end-stage liver disease was associated with damage rather than corresponding to an age-dependent phenomenon [160]. Further studies are needed to assess the hypothesis that these senescence markers correlate with liver disease progression.

Several recent studies have described the cross-regulation of PPARγ system in cellular senescence. Gan et al. reported that PPARγ activation enhanced SA-β-gal and p16INK4a expression, induced G1 arrest and delayed cell growth in human diploid fibroblasts, and thereby accelerated cellular senescence. PPARγ bound to the p16
promoter and induced its transcription, but phosphorylation of PPARγ decreased with increased cell passage [161]. A later in vitro investigation demonstrated a negative feedback and self-regulation loop, in which ligand-activated PPARγ interacted with human SIRT1 (silent information regulator type 1) and inhibited its activity, for regulation of senescence in human embryonic lung diploid fibroblasts, suggesting a new post-translational modification that may affect cellular senescence. And acetylation of PPARγ was shown to increase with increasing cell passage number in this study [162]. Recently, a knockout study defined the role of senescence marker protein 30 (SMP30), which is highly expressed in liver and functions to protect hepatocytes from apoptosis by promoting vitamin C synthesis, in liver fibrogenesis. Neither quiescent HSCs nor activated ones expressed SMP30, but SMP30 depletion upregulated PPARγ expression and attenuated liver fibrosis, implying that PPARγ antagonism to SMP30 could inhibit hepatocyte senescence for anti-fibrotic potential [163]. Consistent with this, deficiency of growth hormone receptor/binding protein led to significant PPARγ upregulation in liver and showed extended life-spans in mice, indicating the potential of an antiaging role for PPARγ [164]. Altogether, current data actually cannot draw a definite conclusion for PPARγ modulation of cellular senescence, which is presumably dependent on cell types or pathologic circumstances. Since HSC senescence has been identified in fibrogenesis, there is a special need to elucidate how PPARγ interferes with the senescence signaling in activated HSCs with the aim of identifying mechanisms that can be selectively targeted to promote HSC clearance while leaving its functions intact in other liver cells.

7. Implications and future directions

Continuous unraveling of PPARγ biology and molecular basis underlying liver fibrogenesis has established the pivotal role for PPARγ system in cross-regulation of the signaling transductions and cellular events implicated in liver fibrogenic mechanisms. We here provide a general mode for PPARγ regulation of signal pathways mediated by several growth factors and adipokines discussed in the above sections (Fig. 2). These novel insights portray a new paradigm of liver fibrogenesis. There are already efforts underway to translate these discoveries into potential therapies. PPARγ gene therapy and thiazolidinedione PPARγ ligands have indeed shown therapeutic benefits for liver fibrosis treatment in both laboratory contexts and clinical patients with chronic liver diseases [31, 165].

We need more molecular investigations into the interplay of PPARγ and new signaling events implicated in liver fibrosis to support the rationale for PPARγ-targeted anti-fibrotic therapy. Liver fibrosis has no longer been considered to be a single and irreversible disease, but rather a broader category subdivided by progressive status, reversibility, and complication with metabolism syndrome such as hyperinsulinemia [166]. The liver is an insulin sensitive organ that plays a critical role in regulating glucose homeostasis. Elevated insulin signaling stimulates HSC activation by inducing mitogenesis and collagen synthesis [167]. Given that PPARγ is also a critical regulator in metabolic syndrome, it is interesting to speculate that PPARγ has interactions with insulin pathway in HSCs, which may affect liver fibrogenic mechanisms. Furthermore, the liver has been identified as an important immunoregulatory organ and innate immune and inflammatory systems are critically involved in liver pathology. Intact Toll-like receptor (TLR)-4 signaling triggered by lipopolysaccharide has been identified in HSCs and mediates key responses including inflammatory phenotype, fibrogenesis and anti-apoptotic properties [168]. Further clarification of the PPARγ interactions with TLR signaling in HSCs and other liver cells can uncover novel mechanisms of fibrogenesis and facilitate the development of therapeutic strategies.

As we have discussed, the experimental results on PPARγ cross-regulation of signaling events provide stimulatory impulses for the development of effective antifibrotics targeting signal transduction. However, these signaling pathways exist in numerous cell types in the body and have impacts on a considerable number of genes. For this reason, it may be necessary to develop agents that selectively

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**Fig. 2.** This diagram summarizes the PPARγ regulation of some key signal transduction pathways implicated in liver fibrogenesis. Several growth factors and adipokines that mediate the activation of intracellular signaling pathways through binding to specific receptors are involved in the dynamic process of fibrogenesis. Pathways transmitted by PDGF, TGF-β, and leptin are profibrogenic, whereas pathways transmitted by HGF and adiponectin are antifibrogenic. PPARγ as a pivotal molecular has different regulatory effects on these cascades in the context of liver fibrogenesis, and shows antifibrotic potential. Blue arrows indicate positive regulation, whereas red connectors denote negative regulation. Dashed lines indicate certain downstream cascades performed by some cytokines and transcriptional factors transmitting the signals to the nucleus.
regulate the interplay of PPARy and signalling specific in certain cell types such as HSCs in order to prevent or at least limit unwanted side effects. Moreover, with respect to cellular senescence, PPARy seems to protect hepatocytes form senescence [163], which could be beneficial for fibrosis resolution. How precisely PPARy modulates senescence signaling in HSCs would be another potential area of research for the future. Noteworthy, many appreciated discoveries discussed in this review are obtained from studies in vitro, and have not yet been explored in animals or humans. We thus should also dedicate more research to define these cellular and molecular mechanisms in vivo for further validating the potential drug targets and searching for therapeutic approaches. Basic and preclinical studies into PPARy-mediated mechanisms in liver fibrogenesis are clearly encouraging and it is an intriguing task to investigate if these novel molecular insights will allow the beneficial therapies for the millions of patients suffering from chronic liver diseases worldwide.

Conflict of interest
The authors disclose no conflicts.

Author contributions
Feng Wang was the major drafter of the manuscript; Yin Lu provided essential assistance in data analysis, and administrative and technical support; and Shizhong Zheng was responsible for the study concept and critical revision of the manuscript for important intellectual content.

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