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REVIEW

Curcumin Targets Multiple Pathways to Halt Hepatic Stellate Cell Activation: Updated Mechanisms In Vitro and In Vivo

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Abstract Nonalcoholic steatohepatitis (NASH) is the advanced form of nonalcoholic fatty liver disease, which is often accompanied by obese and/or type II diabetes mellitus. Approximately one-third of NASH patients develop hepatic fibrosis. Hepatic stellate cells are the major effector cells during liver fibrogenesis. Advanced liver fibrosis usually proceeds to cirrhosis and even hepatocellular carcinoma, leading to liver failure, portal hypertension and even death. Currently, there are no approved agents for treatment and prevention of liver fibrosis in human beings. Curcumin, the principal curcuminoid of turmeric, has been reported to show antitumor, antioxidant, and anti-inflammatory properties both in in vitro and in vivo systems. Accumulating data shows that curcumin plays a critical role in combating liver fibrogenesis. This review will discuss the inhibitory roles of curcumin and update the underlying mechanisms by which curcumin targets in inhibiting hepatic stellate cell activation.

Keywords Leptin signaling · Lipid metabolism · Hepatic stellate cell · Liver fibrosis · Therapeutic strategy · Curcumin · Phytochemicals

Abbreviations

AGEs	Advanced glycation end-products
AGE-Rs	AGE receptors
AMPK	AMP-activated protein kinase
CCL4	Carbon tetrachloride
C/EBP-α	CCAAT/enhancer-binding protein-α
ECM	Extracellular matrix
ERK	Extracellular signal-regulated kinases
FA	Fatty acid
GLUTs	Glucose transporters
HCC	Hepatocellular carcinoma
HSCs	Hepatic stellate cells
IL-6	Interleukin 6
IRS	Insulin receptor substrates
JAK	Janus kinase
LOX-1	LDL receptor-1
MAPK	Mitogen-activated protein kinase
MMPs	Matrix metalloproteinases
NAFLD	Nonalcoholic fatty liver disease
NASH	Nonalcoholic steatohepatitis
NF-kB	Nuclear factor-kappaB
Ob-R	Leptin receptor
PDGF-βR	Platelet-derived growth factor-β receptor
PI3K	Phosphoinositide 3-kinases
PPARs	Peroxisome proliferator-activated receptors
RAGE	Receptor for AGEs
RXRs	Retinoid X receptors
SREBP-1c	Sterol regulatory element-binding protein-1c
STAT-3	Signal transducer and activator of transcription
T2DM	Type II diabetes mellitus
TGs	Triglycerides
TGFβ	Tissue growth factor β
TIMPs	Metalloproteinases
TLRs	Toll-like receptors
TNF-α	Tumor necrosis factor alpha

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Introduction

Obesity and type II diabetes mellitus (T2DM) have been urgent public health concerns worldwide in recent years. Obese and/or type II diabetes mellitus patients are often coupled with non-alcoholic fatty liver disease (NAFLD). Nonalcoholic steatohepatitis (NASH) is the advanced form of NAFLD, featured by steatohepatitis. Approximately one-third of NASH patients develop hepatic fibrosis and even cirrhosis [1].

Liver fibrosis is featured by the excessive accumulation of extracellular matrix (ECM) proteins including collagen in the extracellular spaces. It might occur in most types of chronic liver diseases, including NASH. Advanced liver fibrosis results in cirrhosis and even hepatocellular carcinoma (HCC), leading to liver failure, portal hypertension and even death. The liver lobule consists of parenchymal cells (PCs) and non-parenchymal cells (NPCs). The latter includes endothelial cells, kupffer cells, natural killer cells, dendritic cells and hepatic stellate cells (HSCs) [2]. Activated HSCs are the major source of collagen products, which leads to the imbalance of formation and degradation of ECM in tissues. Additionally, portal fibroblasts, and myofibroblasts of bone marrow origin also contribute to collagen production in the injured liver [2]. These cells are activated by fibrogenic cytokines such as TGF-beta1, angiotensin II and leptin [3], and regulated by pro-inflammatory cytokines such as nuclear factor-kappaB (NF-kB), tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) [3–5]. Recent research demonstrated that advanced liver fibrosis in patients could be reversed, which has stimulated researchers to develop antifibrotic drugs [3, 6]. Potential antifibrotic therapies are aimed at inhibiting the activation of fibrogenic cells, inducing the apoptosis of activated HSCs and/or preventing the deposition of ECM proteins. Currently, no approved agents for treatment and prevention of liver fibrosis in human beings are available.

Curcumin is the principal curcuminoid of turmeric. The curcuminoids are natural phenols that are responsible for the yellow color of turmeric. Turmeric has been used historically as a component of Chinese traditional medicine for thousands of years. In the latter half of the 20th century curcumin was identified as the agent responsible for most of the biological activity of turmeric [7]. As of 2008, numerous clinical trials in humans focused on studying the effect of curcumin on various diseases, such as multiple myeloma, pancreatic cancer, myelodysplastic syndromes, colon cancer, psoriasis, and Alzheimer's disease [8]. In both in vitro and animal studies, curcumin has shown antitumor [9–11], antioxidant [12], and anti-inflammatory properties [13]. This review will focus on the inhibitory roles played by curcumin and its underlying mechanisms in liver fibrogenesis in vivo and in vitro.

Curcumin Improves HSC Activation in CCl4-Induced Fibrotic Animal Models

HSCs are the main effectors during liver fibrogenesis. HSC activation initiates liver fibrosis regardless of etiology. The carbon tetrachloride (CCl4)-fibrotic animal model is commonly used to investigate the procedure of HSC activation in vivo. It is well documented that curcumin plays a critical role against CCl4 -induced liver fibrosis in mice and rat models, suggesting an inhibitory role of curcumin in targeting HSC activation in vivo, which is regarded as a direct and potential therapeutic approach. As shown in Table 1, CCl4-induced liver fibrosis models administered with curcumin for 4–8 weeks showed reduced liver damage and lowered α-SMA and procollagen expression in the livers. Curcumin takes action by targeting multiple sites in those models, such as tissue growth factor β (TGFβ) [14, 15], platelet-derived growth factor-β receptor (PDGF-βR) [16], toll-like receptors (TLRs) [17], matrix metalloproteinases (MMPs) [16, 18], peroxisome proliferator-activated receptors (PPARγ) [19], inflammatory cytokines [17, 19–21], apoptotic pathway [22, 23] and microRNAs [24]. Curcumin may synergistically combine with acupuncture [16], saikosaponin A [21] and its analogs [25]. Although the underlying mechanisms remain largely elusive, it is accepted that curcumin may target multiple pathways to stem hepatic stellate cell activation.

Curcumin Blocks Leptin Signaling Pathway in Hepatic Stellate Cells

Accumulating evidence has shown that leptin and its receptor play critical roles in the development of hepatic fibrosis that is triggered by hepatic stellate cell activation in animal models [26–28] and humans [29–31] with NASH. These observations collectively indicate the significance and essential nature of leptin and Ob-R in the activation of hepatic stellate cells.

An abnormally enhanced level of leptin activates Ob-R and its downstream signaling pathways in HSCs, which induce oxidative stress [32–36], cell proliferation [37], and overproduction of ECM [33, 34, 36, 38], leading to the activation of HSCs. An in vitro study shows that curcumin abrogates the stimulatory effects of leptin by interrupting leptin signaling via inhibiting the phosphorylation of Ob-R and suppressing Ob-R gene expression [36]. The latter is mediated by stimulating PPARγ activity and attenuating oxidative stress [36]. Also, curcumin eliminated stimulatory effects of leptin on HSC activation via increasing AMPK activity and regulating intracellular lipids in HSCs [39]. Moreover, curcumin prevented leptin from elevating

Table 1 Curcumin ameliorates CCl4-induced liver fibrosis

Author	Setting/model	Treatment course	Reported effect
Yao et al. [14]	CCl4-induced rat fibrosis model	6 weeks	Alpha-SMA and collagen deposition ▼, Sma7 ▲ TGFβ1, Smad2, p-Smad2, Smad3, and CTGF ▼
Zhang et al. [16]	CCl4-induced rat fibrosis model		PDGF-βR/ERK signal ▼, serum PDGF and CTGF ▼ MMP-9 and ECM degradation ▲
Tu et al. [17]	CCl4-induced rat fibrosis model	6 weeks	TNF-α, IL-6, MCP-1, and ECM ▼ Number of activated HSCs ▼ HMGB-1, TLR4 and TLR2 ▼
Bassiouny et al. [20]	CCl4-induced rat fibrosis model	200 mg/kg, dietary	TNF-α, NF-kB, IL-6 ▼
Morsy et al. [18]	CCl4-induced rat fibrosis model	200 mg/kg, dietary	ALT, AST, bilirubin, and TGF-α ▼ Serum MMP-13 and reduced GSH ▲
Wu et al. [21]	CCl4-induced rat fibrosis model	0.005 %, dietary, 8 weeks	Collagen deposition ▼, IL-10 ▲ NF-kB, TNF-alpha, IL-1beta, and IL-6 ▼
Shu et al. [22]	CCl4-induced rat fibrosis model	50–200 mg/kg, dietary, 8 weeks	Liver fibrosis score ▼ Ratio of activated HSCs ▼, apoptosis index ▲
Priya et al. [23]	Isolated HSCs from CCl4-induced rat fibrosis model		Caspase-3 activity ▲ Apoptosis of activated HSCs ▲
Reyes-Gordillo et al. [15]	Four-week BDL or CCl4-induced rat fibrosis model	100 mg/kg, dietary, 2 months	ALT, TGFβ ▼
Hassan et al. [24]	CCl4-induced mice fibrosis model	5 mg/mouse/day Once daily 4 weeks	microRNAs (−199 and −200)
Fu et al. [19]	CCl4-induced rat fibrosis model	200–400 mg/kg, dietary, 8 weeks	GSH and GSH/GSSG ▲, LPO ▼ PPAR-γ ▲, IFN-γ, TNF-α and IL-6 ▼

▲ up-regulation, ▼ down-regulation

levels of intracellular glucose in activated HSCs, leading to the inhibition of HSC activation [40]. The same group recently reported that curcumin contributes to the inhibition of HSC activation by eliminating the AGE-caused activation of leptin signaling in activated HSC [32]. Those observations provide novel insights into mechanisms of curcumin in inhibiting leptin-induced HSC activation in vitro. Further research needs to confirm the inhibitory roles of curcumin in leptin-induced HSC activation in in vivo systems. It apparently indicates that curcumin may exert as a therapeutic candidate for the treatment and prevention of liver fibrogenesis induced by hyperleptinemia which was commonly accompanied with NASH, obesity and/or T2DM (Fig. 1).

Curcumin Regulates Intracellular Glucose and Its Derivatives in Hepatic Stellate Cells

Approximately 15–40 % of NASH patients develop hepatic fibrosis [41]. T2DM- and NASH-associated hepatic fibrosis currently is the subject of significant scientific and clinical interest, and will remain so in the future. The correlation of hyperglycemia with the presence of liver fibrosis in NASH patients has been clinically described [42]. T2DM could be a predictor of worsening hepatic fibrosis. Hyperglycemia is suggested as a harmful prognostic factor in the evolution of NASH towards fibrosis. However, little attention has been paid to impacts of hyperglycemia on HSC activation and on NASH-associated hepatic fibrogenesis.

Glucose transport across the plasma membranes of mammalian cells is carried out by two distinct processes: facilitative transport, mediated by a family of facilitative glucose transporters (GLUTs); and sodium-dependent transport, mediated by the Na+/glucose co-transporters (SGLT). GLUTs are important for maintaining glucose metabolism homeostasis and are molecular targets of antidiabetic drugs [43]. In the liver, glucose transporter-2 (GLUT2) and glucose transporter-4 (GLUT4) are the major GLUTs responsible for glucose transportation into hepatocytes [44, 45]. Due to its low affinity and high capacity, GLUT2 transports glucose in a large range of physiological concentrations of glucose, whereas GLUT4 action is extensively regulated by insulin-activated phosphoinositide 3-kinases (PI3K) [44, 45]. In the liver, GLUT2 is translocated from the cytoplasm to the plasma membrane in response to high levels of plasma glucose and is the

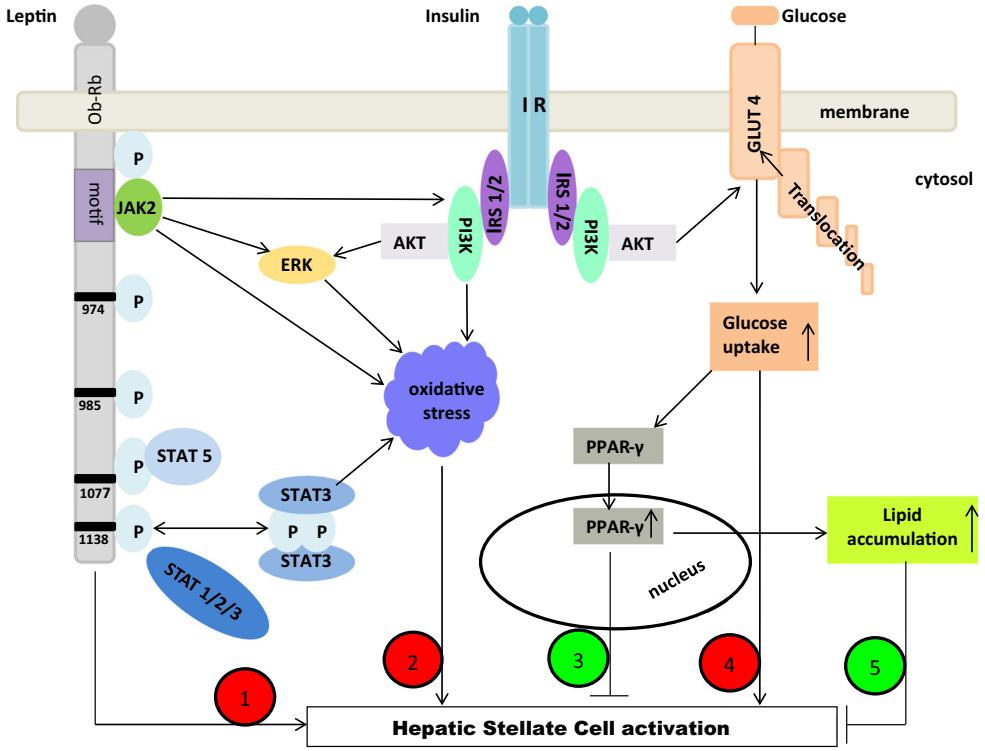

Fig. 1 Multiple targets by curcumin in activated hepatic stellate cells. Curcumin inhibits HSC activation via *1* blocking leptin signaling pathway, *2* fighting against oxidative stress, *3* inducing PPARγ gene expression and its transactivity, *4* suppressing GLUT4 translocation from cytosol to membrane and hence lowering glucose uptake, and *5* promoting intracellular lipid accumulation

primary carrier to transport plasma glucose into hepatocytes [44, 45]. An abnormally high level of intracellular glucose could be deleterious to cellular functions in some types of cells [46].

Published data showed that hyperglycemia stimulated activation of hepatic stellate cells and curcumin removed this action in vitro [47]. Extensive studies suggested that curcumin decreased intracellular glucose level of HSCs by suppressing membrane translocation and gene expression of glucose transporter-2 [47]. The same group also reported that curcumin blocked translocation of glucose transporter-4 by interrupting the insulin receptor substrates (IRS)/PI3K/AKT signaling pathway, a crosslink between leptin and insulin pathway, and increased glucokinase activity by increasing AMP-activated protein kinase (AMPK) activity and suppressing PKA activity, leading to increased conversion of glucose to G-6-P and lowered glucose levels in HSCs [40]. The latter was mediated by activating PPARγ and attenuating oxidative stress.

Hyperglycemia is a high-risk factor for the development of nonalcoholic steatohepatitis (NASH) [48], which is a poorly studied complication of T2DM. Hyperglycemia facilitates the non-enzymatic formation of advanced glycation end-products (AGEs), which are a heterogeneous group of molecules formed by non-oxidative and oxidative reactions of sugars with proteins and/or lipids [49]. AGEs accumulate in tissues and circulation during aging, as well as diabetic, chronic renal failure and liver fibrogenesis [50], leading to inflammation and pathogenesis [51]. One of the mechanisms by which glycated proteins are converted into AGEs is involved in oxidation reaction. One study shows that the formation of AGEs from Amadori products occurs partly because of oxidation [52]. Therefore agents with anti-oxidation properties that can prevent further oxidation of Amadori products may halt the accumulation of AGEs [53]. Khan et al. [54] reported that curcumin had the properties of anti-glycation. In vitro studies imply that AGEs play a critical role in HSC activation, which can be diminished by curcumin [32, 55, 56]. Effects of AGEs are mediated by their receptor system, which could be generally divided into two categories, such as receptor for AGEs (RAGE) and AGE receptors (AGE-Rs, also called OST-48).

On the one hand, RAGE, a member of the immunoglobulin superfamily of cell surface molecules, mediates effects of AGEs and involves oxidative stress, cell growth and inflammation [57]. The interaction of AGEs and RAGE may stimulate the activation of a diverse array of signaling cascades, including MAPKs, JAK/STAT, and PI3K [58]. It has been reported that RAGE is up-regulated

in cultured HSCs [59] and AGEs induce cell proliferation of HSCs [60]. AGEs stimulated the activation of HSCs in vitro by inducing cell proliferation and stimulating expression of genes relevant to HSC activation [55]. The phytochemical curcumin eliminated the stimulating effects of AGEs and inhibited the gene expression of RAGE by attenuating oxidative stress and stimulating the activity of PPARγ in HSCs [56].

On the other hand, AGE-Rs, e.g., AGE-R1, are responsible for detoxification and clearance of AGEs [61]. In contrast to a dramatic increase in expression of RAGE in diabetes with high levels of AGEs [62], the abundance of AGE-R1 is significantly reduced in diabetic organs, e.g., kidney [63], suggesting a possible inverse relationship between AGEs-mediated cell injury and low expression of AGE-R1. In addition to its participation in AGE removal, AGE-R1 negatively regulates AGE pro-inflammatory signal processing [61]. Studies demonstrated that curcumin inhibited AGEs stimulated HSC activation at least partially by inducing AGE-R1 gene expression. This process was likely mediated by inhibiting extracellular signal-regulated kinases (ERKs) activity, inducing gene expression of PPARγ and stimulating its transactivity [55]. Furthermore, curcumin eliminated the effects of AGEs on the divergent regulation of gene expression of RAGE and AGE-R1 in HSCs by interrupting the AGE-caused activation of leptin signaling, leading to the inhibition of HSC activation [32].

Taken together, AGEs might be regarded as one of the mechanisms by which HSCs are activated in high glucose conditions, which probably can, at least partially, explain why liver fibrosis is highly associated with type II diabetes mellitus. Curcumin has a potential to fight against AGE involved HSC activation. However, no evidence is available to show the role of curcumin in regulating gene expression of RAGE and AGE-R1 in vivo.

Curcumin Modulates Lipid Metabolism in Hepatic Stellate Cells

HSCs were previously called fat storing cells [64]. During hepatic injury, quiescent HSCs undergo profound phenotypic changes, including enhanced cell proliferation, loss of lipid droplets, de novo expression of α-smooth muscle actin, and excessive production of extracellular matrix. This process is called HSC activation. Freshly isolated HSCs in culture gradually and spontaneously become fully activated [65], mimicking the process seen in vivo, which provides a good model for elucidating underlying mechanisms of HSC activation and studying potential therapeutic intervention of the process [66, 67]. Accumulating evidence supports the proposal that recovering the accumulation of lipids could inhibit HSC activation [68, 69].

Lipid homeostasis is tightly controlled, via biosynthesis and cellular uptake, by a group of proteins. Several transcription factors, notably sterol regulatory element-binding protein-1c (SREBP-1c), PPARγ, and CCAAT/enhancer-binding protein-α (C/EBP α) have emerged as master regulators in lipogenesis as well as in lipid uptake and metabolism [70]. Interaction, cooperation, and cross talk have been observed among those regulators [71, 72]. It has been proposed that the process of HSC activation may be similar to that of adipocyte dedifferentiation, causally associated with transcriptional regulation of genes relevant to lipid accumulation [68, 69]. In vitro research demonstrated that curcumin could increase intracellular lipid accumulation in HSC via inducing expression of lipogenesis related genes, such as SREBP-1c, PPARγ, and C/EBP α, leading to an inhibition of HSC activation [39]. Similarly, the effect of curcumin on lipid metabolism has been also observed in HepG2 cells [73]. Despite the observation that curcumin paradoxically promotes lipid accumulation and inhibits HSC activation in vitro, curcumin and its water-soluble derivative displays an effective improvement in the lipid metabolism and delays the progression of hepatic fibrosis in rats and mice with steatohepatitis [74–76].

AMPK is a sensor of cellular energy homeostasis [77]. It is activated by rising AMP and falling ATP by a complex mechanism that results in an ultrasensitive response. The activation of AMPK by pharmacological agents presents a unique challenge, given the complexity of the biology, but holds a considerable potential to reverse the metabolic abnormalities [78]. In skeletal muscles, AMPK stimulates glucose transport and fatty acid (FA) oxidation. In the liver, it decreases glucose output, leading to lowered blood glucose levels in hyperglycemic individuals [79]. AMPK may play a key role in regulating the activation of SREBP-1 and lipogenesis [80]. The process of HSC activation is accompanied by depletion of intracellular lipid droplets, loss of lipid storage capacity, and suppression of expression of transcription factors, including SREBP-1, PPARγ, and C/EBPα [66, 68]. In vitro experiments demonstrated that curcumin inhibited HSC activation by activating AMPK activity, leading to the induction of the expression of genes relevant to lipid accumulation and to the elevation of the levels of intracellular fatty acids (FAs) and triglycerides (TGs) [39]. Interestingly, activation of AMPK by curcumin shows different functions in other cell types, such as hepatoma cells [81], HT-29 colon cancer cells [82], and 3T3-L1 adipocytes [75]. These observations collectively suggested that curcumin might show distinct effects on regulating gene expression and on lipid accumulation depending on cell types. Therefore, additional experiments are necessary to elucidate the mechanisms by which curcumin activates AMPK and shows distinct effects in different cell types.

Table 2 Curcumin distinctively regulates gene expression of TIMPs and MMPs in hepatic stellate cells in vitro and in vivo

MMPs and TIMPs	Effects	Species	Dosage	Manners/term	References
TIMP-1	↓	C57BL/6 mice	25 mg/mouse, IP every other day	In vivo/4 weeks	[113]
TIMP-1, TIMP-2	↓	T6 HSCs	78.125 mg/ml in medium	In vitro/24 h	[116]
PDGF-induced TIMP-1 and 2	(–)	Wistar S-D rats	300 mg/kg/day, by gavage	In vivo/12 weeks	[117]
TIMP-1 and 2	↓				
MMP-13 and -7	↑	Hamsters	1 % Curcumin (w/w), dietary	In vivo/3–6 months	[114]
TIMP-1 and -2	↓				
MMP-2, MMP-9	↑	Wistar S-D rats	20 mg/kg body weight, dietary	In vivo/45 days	[115]
MMP-13	↑	CCl4-induced rat fibrosis	200 mg/kg, dietary	In vivo	[18]

↑ Up-regulation; (–) no effect; ↓ down-regulation. *IP* intraperitoneal

Kang et al. [83] reported that curcumin inhibited low-density lipoprotein (LDL)-induced activation of HSCs by suppressing expression of LDL receptor, removed the role of oxidized LDL in activating HSCs by lowering gene expression of lectin-like oxidized LDL receptor-1 (LOX-1) [84], and attenuated expression of SREBP-2 by reducing the activity of specific protein-1, resulting in inhibition of HSC activation [85]. Recently, Kuo and colleagues reported that curcumin protects hepatocytes from high free fatty acid (HFFA)-induced lipoapoptosis and mitochondrial dysfunction, which partially occurs through the regulation of mitochondrial biogenesis [86].

Curcumin Balances Formation and Degradation of ECM Via Regulating TIMPs and MMPs

Fibrosis results due to increased deposition of ECM proteins which are predominantly synthesized by activated HSCs. The accumulation of these proteins is controlled by the rate of their synthesis and degradation. Physiologically, balance of ECM in liver is sustained by a group of enzymes called MMPs and their specific inhibitors, tissue inhibitors of metalloproteinases (TIMPs). Once secreted, MMP activity is regulated by the binding of TIMPs [87]. Overall, all MMPs are inhibited by at least one of the specific endogenous TIMPs once they are activated [87]. During chronic liver injury, HSCs are activated and differentiate into a fibroblast-like phenotype. The balance between MMPs and TIMPs is broken, excessive ECM accumulates in the extracellular spaces and finally fibrosis occurs.

The family of MMPs consists of 23 different members [88], but only a few are expressed in liver tissue and associated with activation of HSCs. They are MMP-1 [88], MMP-2 [89–95], MMP-3 [89, 96–99], MMP-9 [100, 101], and MMP-13 [89, 100, 102, 103]. Upregulation of the above MMPs may facilitate activation of hepatic stellate cells, leading to liver fibrosis.

In the TIMP family, four subtypes have been identified: TIMP-1, TIMP-2, TIMP-3, and TIMP-4. TIMP-1 and TIMP-2 are mainly produced by HSCs [104, 105]. TIMP-1 is also produced by Kupffer cells and hepatocytes especially in early stages of liver injury [102, 106–108], plays a putative role in tissue fibrosis [104, 109], and is also capable of inhibiting programmed cell death of HSCs, mediated via inhibition of pro-MMP activation and MMP activity [110, 111]. TIMP-2 is essential for MMP-2 activation in mice [112].

Published data suggested that curcumin targets the above two protein families that are responsible for fibrogenesis and fibrolysis, respectively, for anti-fibrotic therapeutic interventions: upregulation of MMP activity or downregulation of TIMP activity (Table 2). Curcumin downregulates TIMP-1 and TIMP-2 in vivo [18, 113–115] and in vitro [116], and upregulates MMP-2 [115], MMP-7 [114], MMP-9 [115] and MMP-13 [18, 114], resulting in the degradation of fibrillar collagens, the main components in ECM, leading to inhibition of HSC activation. It is also reported that curcumin has no effect on PDGF-induced TIMP-1 and TIMP-2 expression in rats [117].

Curcumin Activates PPAR-γ Signaling Pathway in Activated Hepatic Stellate Cells

PPARs belong to the superfamily of nuclear receptors [118]. PPARs form heterodimers with retinoid X receptors (RXRs) and these heterodimers regulate transcription of various genes. Three isoforms of PPARs are identified: PPAR-α, PPAR-δ, and PPAR-γ, of which PPAR-γ is the most well documented [119]. PPAR-γ is involved in fatty acid storage and glucose metabolism. The genes activated by PPARγ stimulate lipid uptake and adipogenesis by fat cells. PPARγ knockout mice fail to generate adipose tissue when fed a high-fat diet [120].

PPARγ is highly expressed in quiescent HSCs with a large amount of lipid droplets in the normal livers [25, 68, 121]. However, expression of PPAR-γ and its activity are dramatically reduced with HSC activation in vitro and in vivo [25, 68, 121], which is accompanied by an increase

Table 3 Curcumin inhibits activated HSCs in vitro via PPARγ signaling

Author	Agent	Setting/model	Treatment course	Reported effect
Lin et al. [55]	PGJ2/PD98059	In vitro AGEs-induced Activated HSCs from rats	0–30 μM, 24 h	Induces AGE-R1 expression Inhibits ERK signaling Induces PPARγ expression and transactivity
Tang et al. [39]	pPPRE-TK-Luc	In vitro leptin-induced Activated HSCs from rats	0–30 μM, 24 h	Stimulates AMPK activity Induces PPARγ activity and expression Upregulates C/EBP-α and SREBP-1 Increases lipid accumulation and levels
Kang et al. [85]	PPARγ cDNA SREBP-2 SP-1 promoter	In vitro LDL-induced Activated HSCs from rats	0–30 μM, 24 h	Activates PPARγ Reduces SP-1 trans-activation activity Reduces SREBP-2 expression
Kang et al. [84]	LOX-1 cDNA Lox-1 promoter PGJ2 Rosiglitazone	In vitro LDL-induced Activated HSCs from rats	0–30 μM, 24 h	Reduces lox-1 expression Inhibits Wnt signaling Simulates PPARγ
Kang et al. [83]	pLDLR-Luc	In vitro LDL-induced Activated HSCs from rats	0–30 μM, 24 h	Simulates PPARγ Increases SREBP-1c, reduces SREBP-2 Inhibits LDLR expression Reduces cellular cholesterol Increases cellular FA and TG
Tang et al. [36]	Ob-R promoter PPARγ cDNA PD98235/PGJ2	In vitro leptin-induced Activated HSCs from rats	0–30 μM, 24 h	Inhibits leptin and its signaling pathway Simulates PPARγ Attenuates oxidative stress
Fu et al. [19]	CCl4	In vivo CCl4-induced Fibrosis in rats	200–400 mg/kg By gavage for 8 weeks	Induces PPARγ Prevents liver injury Suppress inflammation Lowers PDGF and TGF-β Attenuates oxidative stress
Zheng et al. [124]	TGF-β PD68235	In vitro cultured rat HSCs	0–30 μM, 24 h	Activates PPARγ activity Blocks TGF-β signaling, inhibits TGF-βR Induces apoptosis, reduces ECM
Xu et al. [125]	PGJ2/PD68235 Troglitazone	In vitro cultured rat HSCs	0–30 μM, 24 h	Activates PPARγ activity Inhibits HSC growth and proliferation Induces activated HSC apoptosis
Lin et al. [56]	PGJ2 Rosiglitazone	In vitro AGEs-induced Activated HSCs from rats	0–30 μM, 24 h	Suppresses RAGE expression Attenuates oxidative stress Induces PPARγ expression and transactivity

in cell growth and proliferation, loss of lipid droplet and vitamin A-storing capability, expression of α-SMA and type I collagen-alpha 1 and deposition of excessive ECM in extracellular space. Extensive data indicate that induction of PPARγ activity by its agonists reduces HSC proliferation and α1 (I) collagen production [68, 122]. Moreover, forced expression of PPARγ via adenoviral vector-mediated system draws the morphology of activated HSC back to the quiescent phenotype [123]. Additionally, PPAR-γ ligands inhibit cell proliferation and collagen-1(I) expression in primary HSCs [68]. The dramatic reduction in the level of PPAR-γ is accompanied by the process of HSC activation [68, 25, 121]. It is, therefore, implied that targeting PPAR-γ signaling is a potential therapeutic strategy in prevention and treatment of liver fibrosis.

Increasing data, including ours and others, shown in Table 3, demonstrate the role of curcumin in inhibition of liver fibrosis through dramatically inducing the expression of PPAR-γ at levels of transcription and translation as well as revived PPAR-γ trans-activating activity in activated HSC. Furthermore, activation of PPAR-γ by curcumin resulted in inhibition of transcription factor NF-kB trans-

activating activity. On the other hand, blocking PPAR-γ activation by a specific PPAR-γ antagonist caused a marked reduction in inhibition of activated HSC proliferation. Together, our results have indicated that PPAR-γ activation by curcumin plays critical and significant roles in inhibition of activated HSC.

Conclusions

Liver fibrosis is triggered by activation of hepatic stellate cells, the major source of collagen products, resulting in an imbalance of formation and degradation of ECM in tissues. So far, no approved agents for treatment and prevention of liver fibrosis in human beings are available. Antifibrogenic agents that are involved in inhibiting HSC activation is of high priority and is urgently needed. Increasing evidence has shown that curcumin has antitumor, antioxidant, and anti-inflammatory properties. Our results and others, both in vitro and in vivo, demonstrate that curcumin plays a role in inhibiting HSC activation by blocking leptin signaling, regulating intracellular glucose and its derivatives and modulating lipid metabolism, as well as balancing formation and degradation of ECM. These results provide novel insights into therapeutic mechanisms of curcumin in inhibiting HSC activation and intervening liver fibrogenesis associated with NAFLD and/or NASH.

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Conflicts of interest None.

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