

Emerging role of the peroxisome proliferator-activated receptor-gamma in hepatocellular carcinoma

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Abstract: Hepatocellular carcinoma (HCC) is the major leading cause of cancer death worldwide. Hepatitis B virus, hepatitis C virus, alcohol consumption, non-alcoholic fatty liver disease, and diabetes are the major risks for developing HCC. Until now, recurrence and metastasis are the major cause of death in HCC patients. Therefore, identification of new effective molecular targets is an urgent need for treatment of HCC. Peroxisome proliferator-activated receptor γ (PPAR γ) is a ligand-activated nuclear receptor which could be activated by PPAR γ agonists such as thiazolidinediones, and natural PPAR γ ligand (such as 15-deoxy- Δ 12,14-prostaglandin J₂, 15d-PGJ₂). Increasing in vitro and in vivo evidence has demonstrated that PPAR γ agonists exhibit an inhibitory role on tumor cell growth, migration, and invasion, suggesting that PPAR γ activation may play an important role in the regulation of growth of HCC. It has been reported that PPAR γ activation by thiazolidinediones or overexpression of PPAR γ by virus-mediated gene transfer has shown growth inhibitory effects in hepatoma cells, but the expression level of PPAR γ in HCC tissues still remains conflicting. Notably, a novel PPAR γ agonist, honokiol, has recently been found to activate the PPAR γ /RXR heterodimer, and has also exhibited significant anti-cancer effects in hepatoma cells. In the present review, we summarized studies on the role and the molecular regulation of PPAR γ in HCC development in vitro and in vivo. PPAR γ has the potential to be a therapeutic target for future treatment of HCC.

Keywords: hepatocellular carcinoma, peroxisome proliferator-activated receptor γ , thiazolidinediones, honokiol, microRNA

Hepatocellular carcinoma

Liver cancer is the second leading cause of cancer death in males worldwide.¹ Hepatocellular carcinoma (HCC) is the major subtype among liver cancers. Eighty percent of HCC occurred in Asia and Africa, where the high prevalence of hepatitis B virus (HBV) and hepatitis C virus (HCV) infections is highly correlated to inflammation of the liver, leading to the subsequent development of HCC.² Additionally, patients with diabetes not only have an increased risk of HCC³ but also have a poorer survival rate after curative therapy for HCC.⁴ On the other hand, obesity, dietary aflatoxin B1 exposure, and excessive alcohol consumption are also major risk factors for the development of HCC.^{5,6} Moreover, liver cirrhosis is a well-known risk factor for HCC (Figure 1). Inflammatory cytokine-induced signaling plays an important role in liver cirrhosis⁷ and carcinogenesis of HCC.⁸ In western countries, 30%–40% of HCC cases are attributable to non-alcoholic fatty liver disease (NAFLD) or metabolic syndrome.^{9,10} Until now, surgical resection has been the best treatment for HCC, especially for patients with a tumor \leq 5 cm in diameter. In addition, liver transplantation provides another option

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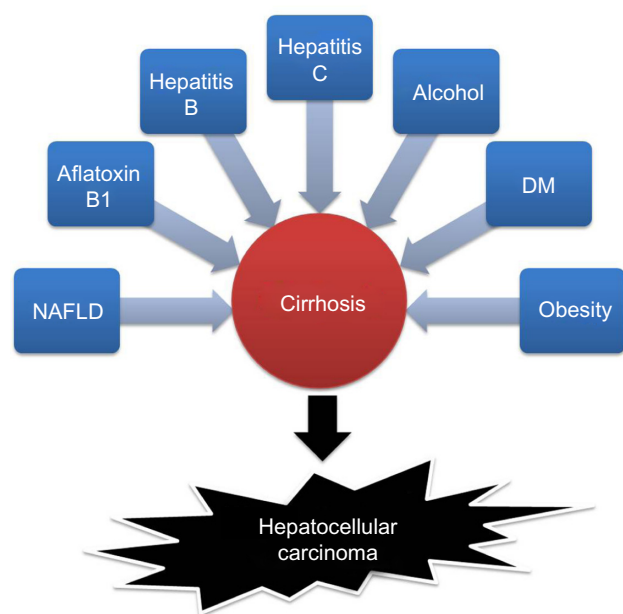


Figure 1 Risk factors for hepatocellular carcinoma development.
Abbreviations: NAFLD, non-alcoholic fatty liver disease; DM, diabetes mellitus.

for curing HCC but is limited by the number of available donors.¹¹ Recurrence and metastasis are the major causes of increased mortality after treatments such as transcatheter arterial chemoembolization, chemotherapy, and radiotherapy.^{12,13} Therefore, understanding the pathogenesis and development of HCC can help us to identify new therapeutic targets and to develop new therapeutic agents or strategies to increase the effectiveness of treatment for patients with HCC.

Targeted therapy has significantly advanced treatment of HCC as chemotherapy for HCC has been met with limited success for many years as a result of high expression of multidrug resistance in HCC.^{14,15} In the past few years, a small multikinase (vascular endothelial growth factor [VEGF] receptor, platelet-derived growth factor receptor, and Raf kinases) inhibitor, sorafenib (Nexavar®, Bayer and Onyx Pharmaceuticals, South San Francisco, California, USA), was the first targeted therapeutic agent that showed a significant increase in the survival of HCC patients.^{16–18} It has long been known that HCC tissue has VEGF expression.¹⁹ Most recently, it has been reported that decreased plasma VEGF, a major mediator of angiogenesis in HCC, with a level >5% at 8 weeks after sorafenib treatment was highly associated with favorable overall survival in advanced HCC patients.²⁰ It is interesting that the VEGF level in these patients showed an increase at 4 weeks after sorafenib treatment and this may have resulted from sorafenib-induced hypoxia in tumor cells leading to increased secretion of VEGF. However, only those patients who had a >5% decreased VEGF level at 8

weeks after starting sorafenib treatment had favorable overall survival. The mechanism for this observation is not clear but it is likely that decreased secretion of VEGF from tumor cells may be important. This report demonstrated that sequential measurement of VEGF is important for the prediction of sorafenib treatment's efficacy in advanced HCC patients.²⁰

In addition to targeted therapy, there are many anti-inflammatory herbal medicines such as baicalein,²¹ curcumin,²² quercetin,²³ resveratrol,²⁴ and honokiol^{25–27} which also exhibited an inhibitory role on cell growth, migration, and invasion of HCC.²⁸ Recently, a natural biphenolic compound honokiol was found to function as a novel non-adipogenic peroxisome proliferator-activated receptor γ (PPAR γ) agonist of adipocyte.²⁹ Notably, PPAR γ is not only involved in anti-inflammatory pathways, but also exerted cell growth inhibition and anti-metastasis in HCC.³⁰ Moreover, synthetic PPAR γ agonists, thiazolidinediones (TZDs), also showed anti-tumor effects on HCC.³⁰ Consequently, the function, molecular pathways, and herbal medicine involved in PPAR γ signaling pathways will be novel targets for potential treatment of HCC.

Molecular structure of PPAR γ

PPAR γ is a ligand-activated nuclear hormone receptor. Once ligand binding has occurred, PPAR γ heterodimerizes with retinoid X receptor (RXR) and this complex subsequently recruits coactivators or corepressors, to regulate the target genes related to lipid and glucose metabolisms as well as inflammation.³¹ Human PPAR γ protein is composed of four functional domains including an N-terminal A/B domain (containing activation function 1, AF1), a DNA-binding domain containing two zinc fingers, a hinge domain, and a C-terminal ligand binding domain (LBD) (containing AF2) (Figure 2).³² The A/B domain is associated with ligand-independent

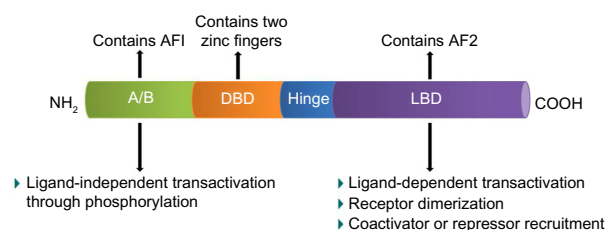


Figure 2 Schematic diagram of the functional domain of PPAR γ .

Notes: PPAR γ is composed of four different domains. The N-terminal A/B domain (containing activation function 1) is responsible for ligand-independent transactivation through phosphorylation. The DNA-binding domain contains two zinc fingers. The hinge domain allows independent movement of the next and last domain of PPAR γ (ligand binding domain). The C-terminal ligand binding domain (containing activation function 2) is responsible for ligand-dependent transactivation and is the crucial regulatory domain for the heterodimerization which is subsequently capable of recruiting coactivators and corepressors necessary for the transcriptional regulation.

Abbreviations: AF, activation function; DBD, DNA-binding domain; LBD, ligand-binding domain; PPAR, peroxisome proliferator-activated receptor.

transactivation through phosphorylation.³³ This phosphorylation decreased transcriptional activity of PPAR γ by mitogen-activated protein kinase³⁴ and 5'-AMP-activated protein kinase to repress ligand-dependent effects including lipid and glucose metabolisms.³⁵ The LBD is responsible for ligand-dependent transactivation and is the crucial regulatory domain for the heterodimerization which is capable of recruiting coactivators and corepressors necessary for the subsequent transcriptional regulation (Figure 3).³⁶ PPAR γ can be activated by the natural ligands such as 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂ (15d-PGJ₂),³⁷ lysophosphatidic acid,³⁸ and nitrolinoleic acid.³⁹ PPAR γ can also be activated by synthetic ligands including the TZDs such as rosiglitazone, pioglitazone, ciglitazone, and troglitazone.^{40,41} There are two PPAR γ isoforms expressed in mouse⁴² and human,⁴³ γ_1 and γ_2 , with different length of N-terminus. PPAR γ_2 has an additional 28 amino acids at its N-terminus resulting from different promoter use and alternative RNA splicing.⁴² PPAR γ_1 is expressed in various tissues including adipose tissue, heart, skeletal muscle, liver, kidney, intestines, colon,

kidney, pancreas, and spleen.⁴⁴ Whereas PPAR γ_2 is expressed mainly in adipose tissue.^{45,46} A previous study reported that PPAR γ_1 and PPAR γ_2 have distinctive activation capacities of insulin, the authors isolated PPAR γ_1 and PPAR γ_2 N termini with activation domains, and the activation capacities of PPAR γ_2 was 5–6 fold greater than that of PPAR γ_1 .³³ A better understanding of the molecular structure of PPAR γ will provide more opportunities to design ligands to regulate the receptor-mediated biological activities.

Expression of PPAR γ in HCC

The expression of PPAR γ in human HCC tissues showed conflicting results from previous studies. Koga et al found there was no significant difference of PPAR γ protein expression between HCC tissues and adjacent non-tumorous liver tissues from five patients with HCV-associated HCC.⁴⁷ Interestingly, Schaefer et al found increased expression of PPAR γ protein in tumors from 20 HCC patients by immunohistochemistry but no expression in non-tumorous liver distal to the tumor.⁴⁸ In contrast, Yu et al found a significantly

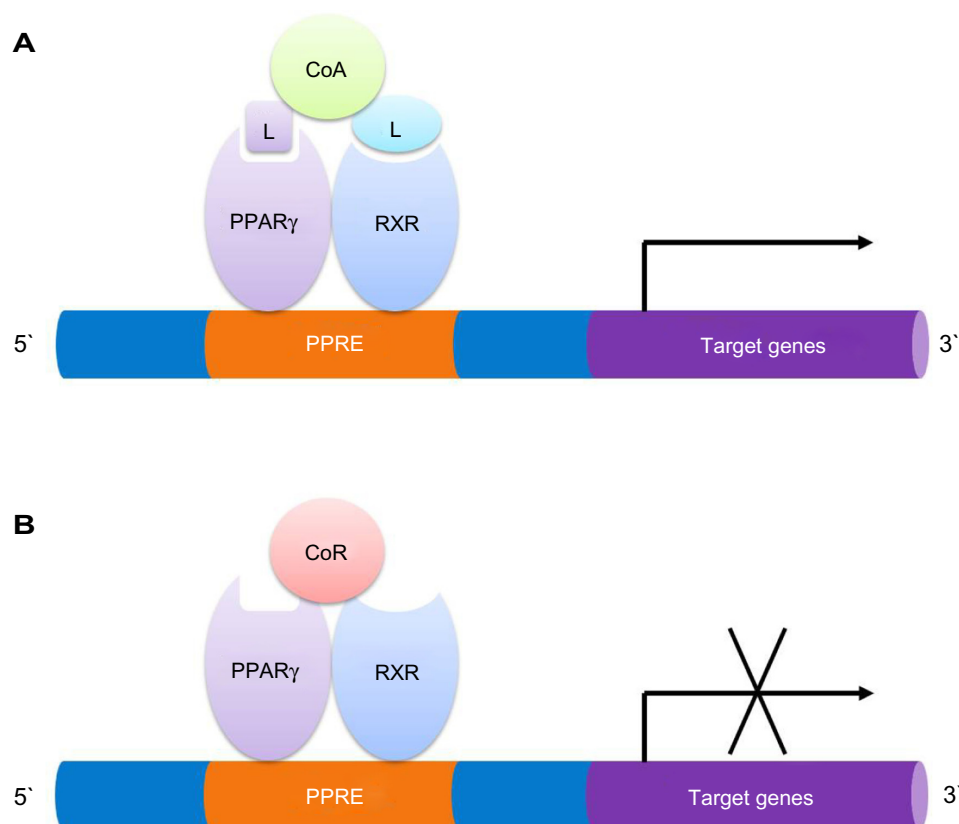


Figure 3 Binding of PPAR γ :RXR heterodimers to the PPAR response element.

Notes: In an activation state, once ligand binding has occurred, PPAR γ heterodimerizes with RXR and this complex subsequently recruits coactivators, which have histone acetylase activity promoting the transcription for target genes related to lipid and glucose metabolisms as well as inflammation. (A) In an inactive state, PPAR γ interacts with the corepressor in the absence of ligands, and this complex has histone deacetylase activity to suppress transcription (B).

Abbreviations: PPAR, peroxisome proliferator-activated receptor; RXR, retinoid X receptor; L, ligand; CoA, coactivator; CoR, corepressor; PPRE, peroxisome proliferator-activated receptor response element.

reduced PPAR γ expression in HCC tissues compared with adjacent non-tumorous liver in 20 HCC patients, as assessed by Western blot.⁴⁹ More recently, Lin et al found a significant increase in mRNA expression of PPAR γ by reverse transcription polymerase chain reaction (RT-PCR) in tumor tissues compared with normal liver in 16 HCC patients. In addition, they also examined the expression of PPAR γ in HepG2, Hep3B, Huh-7, and HA22T hepatoma cell lines by RT-PCR and Western blot analyses. The protein level (Hep3B>Huh-7=HA22T>HepG2) of PPAR γ did not show exactly the same patterns as compared with mRNA level (Hep3B>HepG2>Huh-7>HA22T).⁵⁰ Therefore, studies using a larger sample size are required in order to elucidate the functional importance and the clinical significance of PPAR γ in HCC. A better understanding of the expression of PPAR γ in HCC tissues may help us to elucidate its role in the pathogenesis of HCC.

Functions of PPAR γ and PPAR γ ligands in HCC

Deficiency and overexpression of PPAR γ in the progression of HCC

The role of PPAR γ in hepatocarcinogenesis was examined by Yu et al using PPAR γ -deficient (PPAR $\gamma^{+/-}$) and wild-type (PPAR $\gamma^{+/+}$) mice in a diethylnitrosamine-induced HCC model. They found increased hepatocellular carcinogenesis in PPAR $\gamma^{+/-}$ mice in the diethylnitrosamine-induced HCC model as compared to PPAR $\gamma^{+/+}$ mice, and also found that rosiglitazone decreased the incidence of HCC in PPAR $\gamma^{+/+}$ mice compared to PPAR $\gamma^{+/-}$ mice, indicating that PPAR γ acted as a tumor suppressor in hepatocarcinogenesis.⁵¹ Furthermore, adenovirus-mediated mouse PPAR γ overexpression in Hep3B hepatoma cells showed significant inhibition of cell growth, increased cell apoptosis through intrinsic (caspase-3, 7, 9, Bax, and poly[ADP-ribose] polymerase) and extrinsic (Fas, tumor necrosis factor- α , and caspase-8) pathways, and cell cycle arrest in G2/M phase through phosphorylation of G2/M phase inhibitors cell division cycle 25 c (cdc25c) and cdc2. In addition, PPAR γ overexpression decreased cell viability after rosiglitazone treatment in Hep3B cells.⁵¹ Recently, overexpression of mouse PPAR γ in MHCC97L and BEL-7404 hepatoma cells treated with rosiglitazone showed a significant inhibition of migration and invasion through upregulation of E-cadherin and tissue inhibitor of metalloproteinase (TIMP) 3 and downregulation of heparanase (HPSE), matrix metalloproteinase (MMP) 9, and MMP13.⁵² Most recently, adenovirus-mediated PPAR γ gene transfer in

Hep3B cells exhibited a dramatic increase of Glu/Asp-rich carboxyl-terminal domain, 2 (CITED2) expression in mRNA and protein levels, resulting in G1-S phase arrest and cell growth inhibition.⁵³ However, it will be more interesting if the direct effect of PPAR γ ligands on the expression of CITED2 is known in the PPAR γ transfected cells. Apparently, PPAR γ appears to play a tumor suppressor role in HCC (Figure 4).

PPAR γ agonists inhibit cell growth and induce apoptosis in HCC

Both natural and synthetic PPAR γ agonists exhibited growth inhibitory effects in hepatoma cells. TZDs are synthetic PPAR γ agonists, used as insulin sensitizers for the treatment of patients with type II diabetes mellitus (DM). PPAR γ agonists exert their functions through PPAR γ -dependent and PPAR γ -independent pathways.^{54,55} Many studies have reported that TZDs had growth inhibitory effects, such as troglitazone that not only inhibited cell proliferation through increasing p21 and p27⁴⁷ but also induced apoptosis in hepatoma cells mediated through PI3K-Akt pathway.⁵⁶ Additionally, troglitazone suppressed COX-2 expression

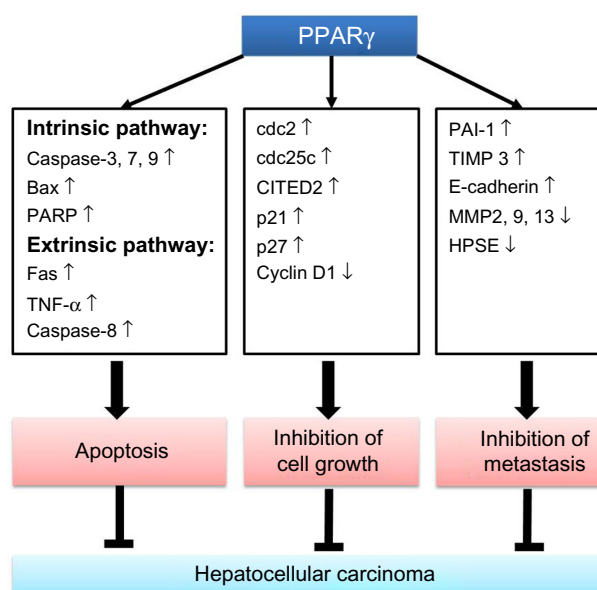


Figure 4 PPAR γ signaling in hepatocellular carcinoma.

Notes: Activation of PPAR γ triggers apoptosis, and inhibition of cell growth and metastasis in hepatocellular carcinoma. Cell growth inhibition is induced by increased levels of cell cycle arrest-related proteins such as cdc25c, cdc2, p21, p27, and CITED2, and/or decreased levels of cell cycle promotion-related proteins such as cyclin D1. Apoptosis is induced through intrinsic (increased Bax and PARP, and activated caspase 3, 7, 9) or extrinsic (increased Fas and TNF- α , and activated caspase-8) pathways. Inhibition of metastasis is induced through upregulation of TIMP3, PAI-I, and E-cadherin, and/or downregulation of MMP2, 9, 13, and HPSE.

Abbreviations: PPAR γ , peroxisome proliferator-activated receptor γ ; PARP, poly(ADP-ribose) polymerase; TNF- α , tumor necrosis factor- α ; cdc, cyclin division cycle; CITED2, Glu/Asp-rich carboxyl-terminal domain, 2; HPSE, heparanase; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of matrix metalloproteinase; PAI-I, plasminogen activator inhibitor-I.

and increased p27 expression to inhibit cell growth in Hep3B and Huh7 hepatoma cells.⁴⁹ Moreover, rosiglitazone upregulated phosphatase and tensin homolog (PTEN) and downregulated COX-2 expression via PPAR γ signaling pathway, leading to the growth inhibition in BEL-7402 and Huh7 cells.⁵⁷ A natural ligand of PPAR γ , 15d-PGJ₂, induced apoptosis via activation of NF κ B in HepG2 and SK-Hep1 cells.⁵⁸ Furthermore, 15d-PGJ₂ and pioglitazone induced apoptosis possibly through caspase-dependent pathways in HBV-associated cell lines.⁵⁹ It is interesting that HepG2 and Hep3B cells are less invasive as compared to other hepatoma cells such as QGY7703 cells,⁶⁰ further investigation using overexpression of PPAR γ in low endogenous PPAR γ expression hepatoma cells may provide more valuable information. These results together suggest that activation of PPAR γ can induce cell growth inhibition and apoptosis in hepatoma cells.

PPAR γ ligands inhibit migration, invasion, and metastasis in HCC

PPAR γ agonists not only have cell growth inhibitory effects but can also inhibit migration, invasion, and metastasis of hepatoma cells. Rosiglitazone inhibited migration through upregulation of PTEN accompanied with decreased phosphorylation of Akt and focal adhesion kinase in hepatoma cell line BEL-7404.⁶¹ Rosiglitazone and troglitazone inhibited cell migration via upregulation of E-cadherin expression in HepG2 cells.⁵⁴ A recent study demonstrated that activation of PPAR γ by rosiglitazone decreased lung metastasis in an orthotopic HCC mouse model.⁵² PPAR γ agonist GW1929 decreased invasion ability via upregulation of plasminogen activator inhibitor-1 (PAI-1) in HepG2 and Hepa1-6 cells. However, the invasion ability was restored after pretreatment of PPAR γ antagonist GW9662 in Hepa1-6 cells.⁶² Interestingly, PPAR γ antagonists GW9662 and T0070907 also inhibited cell migration and invasion through induction of vimentin degradation and inactivation of focal adhesion kinase in poorly differentiated hepatoma cell lines SH-J1 and HLE with high expression of PPAR γ .⁶³ At present, the detailed mechanism of PPAR γ antagonist-induced inhibition of migration in HCC cells is not clear. It is possible that an off target effect exists for PPAR γ antagonist in HCC cells, but further experiments using PPAR γ knockdown HCC cells will be able to clarify whether this effect is dependent on PPAR γ or not. Together, these results clearly showed that both PPAR γ agonists and antagonists exhibited an inhibitory role on migration and invasion in hepatoma cells, the detailed molecular mechanism of PPAR γ upregulated genes

in relation to migration and invasion still needs further investigation.

Honokiol serves as a novel non-adipogenic PPAR γ agonist

Honokiol, a natural biphenolic compound derived from the stem and bark of the plant *Magnolia officinalis*, exhibits anti-angiogenic and pro-apoptotic activity in cancer cells.⁶⁴ Recent studies found that honokiol, a natural rexinoid, can serve as an RXR agonist in the activation of RXR heterodimers.^{65,66} In addition, honokiol potentiated the PPAR γ agonist rosiglitazone induced activation of PPAR γ /RXR heterodimers.⁶⁷ Most recently, one study has found that honokiol directly bound to purified PPAR γ LBD and served as a novel non-adipogenic PPAR γ agonist to stimulate glucose uptake in 3T3-L1 adipocytes.²⁹ Additionally, it has been reported that honokiol possesses a wide variety of pharmacological actions with low toxicity such as anti-inflammatory (in lipopolysaccharide-stimulated human monocyte-derived dendritic cells through inhibition of NF- κ B signaling),⁶⁸ antioxidant,⁶⁹ anti-thrombosis,⁷⁰ and neuroprotective.⁷¹ Moreover, its anti-tumor effects have been found in different types of cancers such as colorectal cancer,⁷² gastric cancer,⁷³ and HCC.²⁵⁻²⁷ Honokiol also exhibited an inhibitory role on hepatoma cells by activating the p38 mitogen-activated protein kinase and caspase 3 pathways to induce apoptosis in HepG2 cells,²⁵ upregulated Ras GTPase-activating-like protein expression to inhibit HepG2 cell migration.²⁶ Recently, it has been reported that honokiol inhibited STAT3 to reduce cell proliferation in HCC cells.²⁷ Collectively, results from these studies show that honokiol has the potential to become a novel agent to treat HCC.

Crosstalk between PPAR γ and TGF- β

Transforming growth factor- β (TGF- β) induces cell growth, cell migration, and epithelial to mesenchymal transition as a key driver to promote HCC progression.⁷⁴ There are two mechanisms: intrinsic and extrinsic activities involved in TGF- β activation pathway. Once the cell polarity has been lost, acquisition of motile ability, and epithelial to mesenchymal transition are considered as intrinsic changes of the tumor cells.⁷⁵ Extrinsic factors are involved in the changes of tumor microenvironment including angiogenesis, inflammation, and fibroblast activation.⁷⁴ Therefore, TGF- β can be considered as a therapeutic target in HCC. Interestingly, PPAR γ activation plays an inhibitory role to repress the expression of TGF- β via dephosphorylation of zinc finger transcription factor-9, and

this dephosphorylation was induced by PTEN-mediated p70 ribosomal S6 kinase-1 inhibition in mouse fibroblast cells.⁷⁶ However, there is no available information to present on the regulation between PPAR γ and TGF- β in HCC. Therefore, further investigations are needed to address this issue in HCC. In the pharmacological manipulation, the crosstalk between PPAR γ and TGF- β can be applied for treating TGF- β mediated fibrosis or cancer metastasis.

Interaction between microRNAs and PPAR γ in HCC

The function of microRNAs (miRNAs) in cancers has been studied extensively in recent years. miRNAs are a group of small (20–22 nucleotides) molecule, endogenous, and non-coding RNAs that play an important role in regulating gene expression by targeting mRNAs 3'-untranslated region for cleavage or translational repression to silence gene expression in eukaryotes.⁷⁷ miRNAs may serve as tumor suppressors or oncogenes which are involved in many biological functions including development, cell proliferation, differentiation, metabolism, and apoptosis.⁷⁸ Many studies have demonstrated that miRNAs are important regulators of gene expression in HCC cells. Recently, it has been reported that PPAR γ and RXR α were regulated by miR-27a in HCC cells to reduce lipid synthesis.⁷⁹ Additionally, miR-122 is liver-specific miRNA which is significantly reduced in HCC, and is correlated with poor prognosis and metastasis.⁸⁰ Most recently, it has been reported that restoration of miR-122 expression in HCC cells reduced AKT3 levels, inhibited cell migration and proliferation, and induced apoptosis, suggesting that miR-122 functions as a tumor suppressor.⁸¹ It has been reported that some miRNAs could target PPAR γ and mediate signal transduction of cellular functions in HCC cells. Interestingly, a recent study clearly showed that miR-122 can be upregulated in hepatoma cells via overexpression of PPAR γ by PPAR γ /RXR α complex.⁸² These studies together suggest that the reciprocal regulation between miR-122 and PPAR γ is worthy of further investigation.

Use of TZDs and the risk of HCC in patients with type II DM

Several cohort studies have reported that TZDs used in DM patients may influence the risk for HCC. The association between HCC and DM has been reviewed using epidemiological data. A previous study has shown that DM patients have a 2–3 fold increased risk of HCC from a population based case control study in the USA, despite the presence of other major HCC risk factors including HBV or HCV infections or alcoholic

liver disease.³ Similarly, results from a population-based cohort study conducted using the Taiwan National Health Insurance Research Database showed an increased incidence of HCC in DM patients. Moreover, this study also showed that the use of TZDs or metformin reduced the risk of HCC development in DM patients.⁸³ A similar study also showed a significant decrease of liver cancer incidence in type II DM patients treated with rosiglitazone or pioglitazone.⁸⁴ In contrast, a systematic review and meta-analysis using data from Medline, EMBASE, and Web of Science up to August 2012 showed that TZDs did not change the risk of HCC in patients with type II DM.⁸⁵ Whether this reduced risk is due to control of diabetes which reduces stress on the liver leading to reduced risk of cancer, or due to PPAR related effects is not clear at present and worthy of further investigation. These results together suggest that the underlying functional role of TZDs in HCC development in type II DM patients needs further investigation.

Conclusion

Current in vitro and in vivo evidence supports the fact that PPAR γ activation exerted an inhibitory role on cell growth, migration, and invasion in HCC cells. Most of the studies focused on the effects of TZDs (PPAR γ agonist) in HCC cells, while TZDs are currently only used for treatment of type II DM patients. TZDs have known adverse effects such as heart failure.⁸⁶ However, a combination of TZDs with chemotherapeutic drugs for local delivery via transcatheter arterial chemoembolization method may provide an alternative approach for HCC treatment. On the other hand, in view of the recent finding that honokiol has anti-cancer effects, the molecular mechanisms of honokiol-induced cell growth, apoptosis, migration, and invasion needs further investigation in hepatoma cells using in vitro and in vivo models. More importantly, studies focusing on the differential miRNAs expression in HCC, especially miR122 which was regulated by PPAR γ in hepatoma cells, may be a potential target for herbal drugs and PPAR γ agonists/antagonists. A better understanding of the molecular regulation on anti-cancer effects in PPAR γ -mediated signaling may contribute to the development of novel combination therapy for future treatment of HCC.

Disclosure

The authors have no conflicts of interest in this work.

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