Review Article
The Role of PPARγ in Hepatocellular Carcinoma

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Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related death worldwide. This cancer develops mainly in cirrhotic patients. The cirrhotic liver is considered to be a preneoplastic organ, suggesting the rationale for cancer prevention. PPARγ is a nuclear transcription factor whose activation leads to interaction in the metabolism of lipids, insulin sensitization of peripheral cells, anti-inflammatory action. It can also induce differentiation and inhibits proliferation of cancer cells. Until now, data using PPARγ ligands in HCC have demonstrated mainly in in vitro models that its activation could be due to an antiproliferative effect. PPARγ ligand administration has also been associated with a diminution of liver fibrosis in animal models, and potentially also on tumoral cell death. Soma data show that the favorable effect of natural and synthetized PPARγ agonists could also be independent of PPARγ activation. Furthermore, in some situations, PPARγ antagonists have also an anticancer effect. Therefore, we can conclude that the link between activation of the PPARγ pathway and an anticancer activity is suggested but until now not firmly established in HCC.

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1. INTRODUCTION

Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related death worldwide [1]. Its prognosis is poor, with up to 40% of patients diagnosed at an advanced stage, when only palliative treatments can be offered. Most patients that develop HCC have cirrhosis, considered as a preneoplastic organ. Strategies to prevent the development of HCC have been implemented, by preventing the appearance of cirrhosis (e.g., HBV vaccination and treatment) [2] or the reappearance of HCC in cirrhotic patients (e.g., interferon in HCV-related cirrhotic patients) [3].

PPARγ is a nuclear transcription factor, member of the nuclear hormone receptor superfamily, being activated by binding its ligand, then heterodimerizing with the Retinoid X receptor, to finally bind with specific response elements in the nucleus, called peroxisome proliferating response elements. Activation of PPARγ by agonists such as thiazolidinediones (TZDs) has been shown to have anticancer effect in vitro and in vivo in many cancer types [4]. However, as emphasized by articles in this special issue of PPAR research, in some situations, like in colon cancer models, PPARγ agonists can have tumor promoting effects [5].

Effect of PPARγ agonists on HCC has been studied in the last years both in cell cultures and in animal models. Here, we summarize the main findings of these works and attempt to define the role of PPARγ and its modulation in HCC.

2. EXPRESSION OF PPARγ IN RODENT AND HUMAN LIVER

PPARγ RNA and protein expression has been demonstrated in vitro in several cell lines, including Hep3B, HepG2, Huh7, and others [6–11]. Its expression was generally higher than positive control used but variable among cell lines, with mRNA levels not necessarily correlating protein expression.

In human HCC, reports from 3 papers show conflicting results: in the first paper, PPARγ protein expression was assessed by western-blot (WB) in 5 cirrhotic patients and showed no difference between HCC and nontumoral cirrhotic liver [12]. The second paper showed a constant overexpression of PPARγ protein in 20 HCCs and no expression in surrounding liver, assessed by immunohistochemistry (IHC). In this paper, no information was given regarding the presence of cirrhosis [13]. Finally, a recent report, analyzing
mRNA and protein expression (by WB) in 20 patients, with cirrhosis \((n = 12)\) and chronic hepatitis \((n = 8)\), showed a statistically significant decrease in PPAR\(\gamma\) expression in HCC compared to adjacent liver [14]. Furthermore, PPAR\(\gamma\) mRNA was less expressed in poorly differentiated HCC compared to well-differentiated ones. The latter [2] results, using 2 different methods (IHC versus WB) in different populations, can therefore not be compared, and certainly other analyses will be needed before we can draw any conclusions on PPAR\(\gamma\) expression in HCC and cirrhosis compared to normal liver, where it is known to be low [15].

3. EFFECT OF PPAR\(\gamma\) AGONISTS ON CELL PROLIFERATION

The effect of the natural PPAR\(\gamma\) agonist 15-deoxy-\(\Delta\)12,14-prostaglandin J2, and the synthetic agonists thiazolidinediones has been tested in many human HCC cell cultures [6, 7, 9, 11, 12, 14, 16, 17]. Overall, cell growth was inhibited at variable concentrations. Troglitazone, the most studied TZD, was shown to inhibit cell growth already at 5–10 \(\mu\)M concentrations, although some contradictory reports have been published [10]. Pioglitazone and ciglitazone, the other main TZDs tested, were less active, with concentrations from 50 to 100 \(\mu\)M needed to have significant growth inhibitory effect. The antiproliferative effect was shown to be due to a cell cycle arrest, with cells accumulating at the G1 phase. Koga et al. have performed the most thorough mechanistic study of cell cycle arrest, with cells accumulating at the G1 phase. They found that pioglitazone at a 200 ppm concentration, either by chronic administration of dimethylnitrosamine, carbon tetrachloride, or bile duct ligation. The authors demonstrated that, given early, TZDs could significantly reduce the development of liver fibrosis induced in rats, either by chronic administration of dimethylnitrosamine, carbon tetrachloride, or bile duct ligation. The authors could show that this antifibrotic effect was driven through the activation of PPAR\(\gamma\)-dependant decreased activation of hepatic stellate cells. These early results showing the importance of PPAR\(\gamma\) activation in the pathogenesis of fibrosis were confirmed by others [25, 26]. Though, we could show that the protective effect was very dependant on the rodent type [27] and the severity of fibrosis at the time of initiation of treatment [28].

4. ROLE OF PPAR\(\gamma\) IN THE PROGRESSION OF LIVER FIBROSIS

An important paper published in 2002 by Galli et al. [24] demonstrated that, given early, TZDs could significantly reduce the development of liver fibrosis induced in rats, either by chronic administration of dimethylnitrosamine, carbon tetrachloride, or bile duct ligation. The authors could show that this antifibrotic effect was driven through the activation of PPAR\(\gamma\)-dependant decreased activation of hepatic stellate cells. These early results showing the importance of PPAR\(\gamma\) activation in the pathogenesis of fibrosis were confirmed by others [25, 26]. Though, we could show that the protective effect was very dependant on the rodent type [27] and the severity of fibrosis at the time of initiation of treatment [28].

These data are thus to be interpreted with caution before going to clinical application. In humans, one randomised double-blind placebo-controlled study assessed the effect of pioglitazone versus placebo in patients with NASH [29]. One of the aims of the study was to assess fibrosis on liver biopsy before and after 6 months of a calorie-restricted diet with or without pioglitazone. The study failed to show differences in terms of fibrosis regression, assessed by histology, between groups \((P = 0.08)\), although patients in the pioglitazone group showed significantly less fibrosis after the 6-month regimen compared to before treatment \((P = 0.002)\).
5. **PPARγ MODULATION IN ASSOCIATION WITH CELL DEATH**

The role of PPARγ in apoptosis and anoikis is less clear. Several in vitro works report induction of apoptosis in HCC cell lines, constantly by troglitazone and not by others TZDs [9, 14, 30–32]. The main apoptotic mechanisms remain poorly understood, although some publications report an effect of troglitazone on the bax/bcl-2 balance [32], suggesting a role for the mitochondrial pathway. In another work, authors have analyzed the effect of the natural PPARγ agonist 15-deoxy-Δ12,14-prostaglandin J2 (15D-JG2) on apoptosis of SK-Hep1 and hepg2 cells. They indeed showed that given at a dose of 50 μMol, 15D-JG2 could induce apoptosis by caspase 3 activation. Using PPARγ antisense oligodeoxynucleotides, they demonstrated that apoptosis was induced independently of PPARγ expression. They also showed that 15d-PGJ2 inhibited NF-kB activation induced by TNFα when PPARγ was normally expressed or downregulated.

6. **IS THERE A ROLE FOR PPARγ INHIBITION IN CANCER?**

Although, as discussed earlier in the text, the vast majority of available data deals with PPARγ agonists, some authors analyzed the effect of PPARγ inhibitors on cancer cell growth. One publication reported the effect of PPARγ inhibitors on cell adhesion and anoikis [13]. On the contrary to the paper by Yu et al. [14], authors found overexpression of PPARγ in human HCC. They could show that these PPARγ inhibitors increased loss of adherence in vitrofollowed by caspase-dependent apoptosis, a finding reproduced using PPARγ siRNAs, reflecting the specificity of the PPARγ-dependent activation driving cell death. They also showed that inhibitors were at least 5-fold more potent in reducing cell number than troglitazone and rosiglitazone. Another study evaluated the effect of PPARγ agonists and antagonists on cell growth, migration, and invasion in four different HCC cell lines [33]. Authors could show that antagonists inhibited cell growth and migration more efficiently than PPAR agonists. They suggest that this effect could be due to Vimentin cleavage, thus interacting negatively with the cellular cytoskeleton.

7. **CONCLUSION**

Several in vitro and in vivo papers suggest a role of the PPARγ pathway in the prevention and treatment of HCC. PPARγ mRNA and protein expression are constantly found in cell lines. In normal liver, it is known to be expressed at low levels, ten times less than in adipose tissue. We do not know its actual level of expression, neither in cirrhotic nontumoral liver, nor in HCC, with conflicting results reported. Thiazolidinediones, which are active and specific PPARγ agonists, have shown antitumoral activity in cell lines and in animal models. This antitumor activity is due to inhibition of cell proliferation, by interfering with important cell cycle cyclins and cyclin-dependent kinase inhibitors, such as p27Kip1, but also by a proapoptotic action. The link between activation of the PPARγ pathway and the anticancer activity of TZDs is, however, not established. Finally, PPARγ antagonists have shown antitumor activity, by different mechanisms, mainly involving loss of cell adhesion, migration, and invasion.

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