Toxicology 363–364 (2016) 1–9

Contents lists available at ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol

Ligand activation of peroxisome proliferator-activated receptor-β/δ suppresses liver tumorigenesis in hepatitis B transgenic mice

Gayathri Balandaram[a], Lance R. Kramer[a], Boo-Hyon Kang[b], Iain A. Murray[a], Gary H. Perdew[a], Frank J. Gonzalez[c], Jeffrey M. Peters, Ph.D.[a,*]

[a] Department of Veterinary and Biomedical Sciences and The Center for Molecular Toxicology and Carcinogenesis, The Pennsylvania State University, University Park, PA, USA
[b] Chemon Nonclinical Research Institute, 240 Nampyeong-ro, Yangji-myeon, Cheoin-gu, Yongin-si, Gyeonggi-do, Republic of Korea
[c] Laboratory of Metabolism, National Cancer Institute, Bethesda, MD, USA

A R T I C L E I N F O

Article history:
Received 17 May 2016
Received in revised form 5 July 2016
Accepted 13 July 2016
Available online 15 July 2016

Keywords:
Peroxisome proliferator-activated receptor-β/δ
Liver cancer
Kupffer cell
Hepatitis B
Inflammation

A B S T R A C T

Peroxisome proliferator-activated receptor-β/δ (PPARβ/δ) inhibits steatosis and inflammation, known risk factors for liver cancer. In this study, the effect of ligand activation of PPARβ/δ in modulating liver tumorigenesis in transgenic hepatitis B virus (HBV) mice was examined. Activation of PPARβ/δ in HBV mice reduced steatosis, the average number of liver foci, and tumor multiplicity. Reduced expression of hepatic CYCLIN D1 and c-MYC, tumor necrosis factor alpha (*Tnfa*) mRNA, serum levels of alanine aminotransaminase, and an increase in apoptotic signaling was also observed following ligand activation of PPARβ/δ in HBV mice compared to controls. Inhibition of *Tnfa* mRNA expression was not observed in wild-type hepatocytes. Ligand activation of PPARβ/δ inhibited lipopolysaccharide (LPS)-induced mRNA expression of *Tnfa* in wild-type, but not in *Pparβ/δ*-null Kupffer cells. Interestingly, LPS-induced expression of *Tnfa* mRNA was also inhibited in Kupffer cells from a transgenic mouse line that expressed a DNA binding mutant form of PPARβ/δ compared to controls. Combined, these results suggest that ligand activation of PPARβ/δ attenuates hepatic tumorigenesis in HBV transgenic mice by inhibiting steatosis and cell proliferation, enhancing hepatocyte apoptosis, and modulating anti-inflammatory activity in Kupffer cells.

© 2016 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

PPARβ/δ is a member of the nuclear hormone receptor superfamily. Expression of PPARβ/δ is particularly high in skin, colon and liver and thought to be constitutively active in cells due to the presence of endogenous ligands (Bookout et al., 2006; Girroir et al., 2008; Modica et al., 2010). Consequently, PPARβ/δ dynamically modulates target gene expression through binding with peroxisome proliferator response elements (PPREs) of target genes. Nucleosome localization, chromatin structure, the presence/expression of co-repressors, co-activators, and enzymes that remodel chromatin all influence the binding of PPARβ/δ with DNA where it increases and decreases transcription of target genes (Khozoie et al., 2012). Additionally, PPARβ/δ can also modulate biological functions through protein–protein interactions with other transcription factors such as NF-κB (Planavila et al., 2005a), STAT3 (Kino et al., 2007) and ERK5 (Woo et al., 2006).

Ligand activation of PPARβ/δ can prevent liver damage caused by a number of insults in multiple models. For example, steatosis and inflammation induced by feeding a methionine and choline deficient diet are attenuated in mice by ligand activation of PPARβ/δ with GW501516 (Nagasawa et al., 2006). This protective effect was mediated by increasing oxidation of fatty acids and inhibition of pro-inflammatory cytokines in the liver (Nagasawa et al., 2006), although it was also suggested that ligand activation of PPARβ/δ inhibits lipogenesis and prevents hepatic steatosis (Qin et al., 2008). Hepatotoxicity caused by exposure to azoxymethane or carbon tetrachloride is exacerbated in *Pparβ/δ*-null mice as compared to controls (Shan et al., 2008a), and this effect appears

Abbreviations: ALT, alanine aminotransaminase; CCl_4, carbon tetrachloride; COX-2, cyclooxygenase-2; DBM, DNA binding domain mutant; ERK5, extracellular signal-regulated kinase 5; HBV, hepatitis B virus; LPS, lipopolysaccharide; NF-κB, nuclear factor κB; PARP, poly (ADP-ribose) polymerase; PBF, phosphate buffered formalin; PPARβ/δ, peroxisome proliferator-activated receptor-β/δ; PGE_2, prostaglandin E_2; STAT3, signal transducer and activator of transcription 3; TNF-α, tumor necrosis factor-α.

* Corresponding author at: Department of Veterinary and Biomedical Sciences and The Center for Molecular Toxicology and Carcinogenesis, 312 Life Sciences Building, The Pennsylvania State University, University Park, PA 16802, USA.
E-mail address: jmp21@psu.edu (J.M. Peters).

http://dx.doi.org/10.1016/j.tox.2016.07.010
0300-483X/© 2016 Elsevier Ireland Ltd. All rights reserved.

to be mediated by PPARβ/δ-dependent inhibition of NF-κB-dependent induction of pro-inflammatory cytokines following administration of the PPARβ/δ ligand GW0742 (Shan et al., 2008b). Activation of PPARβ/δ with another PPARβ/δ ligand KD3010 can also prevent inflammation and fibrosis in mouse liver in response to exposure to carbon tetrachloride (Iwaisako et al., 2012). Additional data supporting the notion that PPARβ/δ protects the liver by promoting anti-inflammatory activities is provided by studies showing markedly enhanced expression of many target genes associated with inflammation in *Pparβ/δ*-null mice as compared to controls (Sanderson et al., 2010). Since steatosis, inflammation and fibrosis are compounding risk factors for hepatocarcinogenesis, it is of interest to note that the role of PPARβ/δ in liver cancer has not been examined to date using an in vivo model.

Hepatocarcinogenesis is one of the leading causes of death in human cancer patients (Ferlay et al., 2015). Approximately 600,000 humans die every year due to liver cancer that develops from hepatitis B virus (HBV) infection (Franco et al., 2012). Chronic HBV infection induces persistent hepatocyte inflammation, oxidative DNA damage, uncontrolled cellular proliferation, fibrosis and cirrhosis, which eventually culminates in hepatocellular carcinoma (Park et al., 2007). Since steatosis and inflammation are two important mechanisms that mediate HBV-dependent hepatocellular carcinoma, and ligand activation of PPARβ/δ prevents hepatic steatosis and inflammation, the present study examined the hypothesis that ligand activation of PPARβ/δ inhibits liver cancer in HBV transgenic mice.

2. Material and methods

2.1. Mouse lines

Male C57BL/6J transgenic mice (Tg(Alb1-HBV)Bri44) that express the human HBV large envelope protein driven by the mouse albumin promoter (Chisari et al., 1987) and male C57BL/6J control mice were obtained from The Jackson Laboratory (Bar Harbor, ME). These HBV transgenic mice over-express the human HBV large envelope protein in hepatocytes, which leads to inflammation, hyperplasia and liver cancer (Chisari et al., 1989). Male wild-type and *Pparβ/δ*-null mice used for these studies have been previously described (Peters et al., 2000). A transgenic mouse line expressing a previously described PPARβ/δ isoform containing a DNA binding domain mutation (DBM) (Shi et al., 2002) was generated. The PPARβ/δ DBM isoform was produced by site directed mutagenesis of a mouse PPARβ/δ expression vector (pSG5-PPARβ/δ; kindly provided by Drs. Pallavi Devchand and Walter Wahli). The 90th and 93rd codons of this cDNA encoding critical cysteine residues in the DNA binding domain of PPARβ/δ were mutated from TGC to GCC, such that the resulting PPARβ/δ DBM expressed alanine residues rather than cysteine residues in the expressed protein. The mutant PPARβ/δ DBM expression vector was then characterized to verify that the PPARβ/δ DBM was incapable of binding with its cognate DNA response element, an event required for direct regulation of PPARβ/δ target genes. This characterization included: (1) examining the ability of the PPARβ/δ DBM to activate a luciferase reporter assay in wild-type and *Pparβ/δ*-null primary keratinocytes as previously described (Kim et al., 2006) except that a luciferase construct containing four copies of the rat acyl-CoA oxidase peroxisome proliferator response element (PPRE) was used for the reporter, (2) verifying that expression levels of the transfected wild-type PPARβ/δ and PPARβ/δ DBM were equivalent in transfected keratinocytes using quantitative western blot analysis as previously described (Girroir et al., 2008), (3) electrophoretic mobility shift analysis (EMSA) to determine whether the PPARβ/δ DBM can or cannot bind to a PPRE in the presence of its heterodimerization partner, retinoid X receptor-α (RXRα) as previously described (Kim et al., 2004), (4) determining whether in vitro translation efficiency of both wild-type PPARβ/δ and the PPARβ/δ DBM used for the EMSA was similar using a previously described method (Girroir et al., 2008), and (5) determining whether both the wild-type PPARβ/δ and the PPARβ/δ DBM can heterodimerize with RXRα using co-immunoprecipitation analysis as previously described (Sumanasekera et al., 2003). After this characterization, the cDNA encoding the mouse PPARβ/δ DBM cDNA was subcloned in frame into a vector with the CD68 promoter (kindly provided by Dr. Pamela Hankey-Giblin). This transgene vector was linearized, and microinjected into pronuclear stage embryos from homozygous *Pparβ/δ*-null mice at the Penn State Transgenic Mouse Facility (University Park, PA). After transfer to pseudo-pregnant CD-1 female mice, transgenic offspring were screened using Southern blot analysis of tail DNA isolated from the progeny using a previously described method (Peters et al., 2000) and a cDNA probe encoding part of the CD68 promoter and the PPARβ/δ DBM cDNA. The CD68 promoter is highly active in Kupffer cells. Confirmed transgenic offspring that incorporated the PPARβ/δ DBM cDNA into their genome were bred using a brother/sister mating strategy to obtain homozygous transgenic mice. Functional characterization of the PPARβ/δ DBM transgenic mice was performed as described below for examination of primary Kupffer cells.

2.2. Mouse treatments

All animal experiments were approved by the Institutional Animal Care and Use Committee at The Pennsylvania State University. To determine the effect of ligand activation of PPARβ/δ on tumorigenicity in HBV transgenic mice, 7-month-old male HBV transgenic mice were administered the specific PPARβ/δ agonist GW0742 at a dose of 2.5 mg/kg (5 days per week) for 8 months. Mice were dosed with a pellet composed of bacon-flavored transgenic dough (Bioserv, Inc., Piscataway, NJ) or the bacon-flavored transgenic dough mixed with GW0742 at a dose of 2.5 mg/kg (N = 5 mice per group). After 8 months of treatment, mice were euthanized by overexposure to carbon dioxide and exsanguination followed by cervical dislocation. The total number of visible tumors was counted for each mouse liver. Sections of liver tissue with and without visible tumors were fixed in 10% phosphate buffered formalin (PBF). Sections of liver without visible tumors were also snap frozen, stored at −80 °C until further use. Serum was isolated from whole blood. To determine the sub-chronic effect of ligand activation of PPARβ/δ in HBV transgenic mice, 6-month-old male mice were administered GW0742 at a dose of 5.0 mg/kg (5 days per week) for 3 weeks.

2.3. Histopathological analyses

Sections of liver tissue were fixed in 10% PBF overnight and transferred to 70% ethanol, prior to embedding in paraffin for histological analysis. Tissue sections were stained with hematoxylin and eosin. The total number of steatotic regions was quantified in the left lobe of representative liver sections using light microscopy. A pathologist scored the total number of liver tumor foci from representative liver sections.

2.4. RNA isolation and quantitative polymerase chain reaction (qPCR) analysis

RNA was extracted from whole liver tissue or cultured cells using Ribozol RNA extraction reagent (Amresco Inc., Solon, OH) and 1.25 μg of total RNA was reverse transcribed into cDNA using reverse transcription kit (Promega, WI,). qPCR reactions were

Download English Version:

https://daneshyari.com/en/article/2595403

Download Persian Version:

https://daneshyari.com/article/2595403

Daneshyari.com