Research Article

Peroxisome Proliferator-Activated Receptor γ Regulates the Expression of Lipid Phosphate Phosphohydrolase 1 in Human Vascular Endothelial Cells

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Lipid phosphate phosphohydrolase 1 (LPP1), a membrane ectophosphohydrolase regulating the availability of bioactive lipid phosphates, plays important roles in cellular signaling and physiological processes such as angiogenesis and endothelial migration. However, the regulated expression of LPP1 remains largely unknown. Here, we aimed to examine a role of peroxisome proliferator-activated receptor γ (PPARγ) in the transcriptional control of LPP1 gene expression. In human umbilical vein endothelial cells (HUVECs), quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) demonstrated that activation of PPARγ increased the mRNA level of LPP1. Chromatin immunoprecipitation assay showed that PPARγ binds to the putative PPAR-responsive elements (PPREs) within the 5′-flanking region of the human LPP1 gene. Genomic fragment containing 1.7-kilobase of the promoter region was cloned by using PCR. The luciferase reporter assays demonstrated that overexpression of PPARγ and rosiglitazone, a specific ligand for PPARγ, could significantly upregulate the reporter activity. However, site-directed mutagenesis of the PPRE motif abolished the induction. In conclusion, our results demonstrated that PPARγ transcriptionally activated the expression of LPP1 gene in ECs, suggesting a potential role of PPARγ in the metabolism of phospholipids.

1. Introduction

Lipid phosphate phosphohydrolases (LPPs), also known as phosphatidate phosphohydrolase-2 (PAP-2), are the Mg²⁺-independent and N-ethylmaleimide-insensitive N-glycosylated integral membrane ectophosphohydrolase [1, 2]. LPPs catalyze the dephosphorylation of a range of lipid phosphates, such as lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P) [3, 4]. Extracellular LPA and SIP bind to the G-protein-coupled receptors (GPCRs) and exert a number of pathophysiological actions, such as angiogenesis, platelet activation, inflammation, smooth muscle cells (SMCs) proliferation and migration, and cardiovascular remodeling [4, 5]. LPPs hydrolyze these lipid phosphates to terminate their signaling actions or generate new signaling molecules [6]. Three isoforms of LPPs (LPP1, LPP2, and LPP3) have been found [7]. LPP1 negatively regulates lysophospholipid signalings by degrading the bioactive lysophospholipids released from platelets and modulates their effects on the cell proliferation, migration, inflammation, coagulation, and wound healing [5, 6]. The activity of LPP1 is mainly regulated through de novo expression rather than posttranslational modification such as phosphorylation. Expression of LPP1 was induced by androgens in human prostatic adenocarcinoma cells and decreased in ovarian cancers [8, 9]. However, transcriptional mechanism underlying the regulation expression of the LPP1 remains largely unclear.

Peroxisome proliferator-activated receptors (PPARs) are a family of ligand-activated nuclear receptors and transcription factors [10]. Among three PPAR isoforms (α, β/δ, and γ), PPARγ is predominantly expressed in adipose tissue and also in vasculature including vascular smooth muscle cells (VSMCs) and endothelial cells (ECs) [11, 12]. PPARγ forms a heterodimer with RXR and binds to the PPAR response elements (PPREs) in the promoter region of target genes [13]. When activated by various natural and synthetic ligands such as prostaglandin metabolite 15d-PGJ2 [14] and the
insulin sensitizer rosiglitazone [15], PPARγ transactivates the gene expression and regulates adipogenesis [16] and insulin response [17]. In addition, PPARγ possesses antiatherogenic and anti-inflammatory actions in ECs [18, 19]. Therefore, we attempted to examine a role of PPARγ in the regulation of LPP1 gene expression in ECs.

2. Materials and Methods

2.1. Cell Culture and Reagents. Human umbilical vein endothelial cells (HUVECs) were cultured as previously described [20]. Bovine aortic endothelial cells (BAECs) were harvested from bovine aorta and maintained in DMEM with 10% FBS [21]. Rosiglitazone, GW501516, and GW9662 were obtained from Cayman Chemical. Polyclonal rabbit anti-PPARγ antibody was from Santa Cruz Biotechnology. Luciferase assay reagent, MMLV reverse transcriptase, Taq polymerase, restriction enzymes (XhoI, NheI), and DNA ligase were from Promega Corporation. Lipofectamine 2000 and Trizol reagent were obtained from Invitrogen. The QuickChange site-directed mutagenesis kit was from Stratagene Corporation.

2.2. Adenoviral Infection. Cells were infected with adenoviruses encoding the wild type human PPARα, β/δ, or γ1 (Ad-WT-PPARα or Ad-WT-PPARβ/δ, Ad-WT-PPARγ) together with Ad-tTA, which encodes a tetracycline-responsive transactivator. These viral constructs were previously described [22, 23]. Infected cells were maintained in the presence or absence of tetracycline (0.1 μg/mL, a tet-off expression) for 48 hours as described [20].

2.3. RNA Extraction and Real-Time Quantitative RT-PCR (qRT-PCR). Total RNA was extracted with Trizol reagent and reverse transcribed into cDNA with M-MLV reverse transcriptase with oligo-dT as a primer. Real-time PCR was performed with SYBR-green dye and Taq polymerase in the DNA Engine Opticon real-time system (Bio-Rad Laboratories Inc.). GAPDH was used as an internal control. The primer sequences are: LPP1 forward, 5'-CGAGGGATGGAATCTGTTAATGGGATCTGGTG-3' (5'-CTTGATATGCTCAACAGGTTCA and 5'-TCAGGT-GGTCTCCAGGAICT) with flanking sites of NheI and XhoI. The amplified product was subcloned into the pGL3-basic luciferase vector to generate the pGL3/LPP1-luc. The Quickchange site-directed mutagenesis kit was used to generate the pGL3/mLPP1-luc by disruption of the putative PPRE site (from −624 to −611 bp) with the use of the mutagenic primers: 5'-AAGGGATCTCGGTAAGGAGG-CCG(A)GTG(T)CCCAA(A)GCTG(T)CTTACCACG and 5'-CGAGGGATGGAATCTGTTAATGGGATCTGGTG-3'. The plasmids were transfected together with the pRSV-β-gal plasmid into BAECs by using Lipofectamine 2000. Luciferase activity was measured with the luciferase assay kit and normalized with β-galactosidase activity.

2.4. Plasmids, Mutation, Transfection, and Reporter Assay. The genomic fragment containing −218 to −221 bp upstream of the transcription start site of human LPP1 gene was PCR amplified from human genomic DNA with the primers (5'-CTTGATATGCTCAACAGGTTCA and 5'-TCAGGT-GGTCTCCAGGAICT) with flanking sites of NheI and XhoI. The amplified product was subcloned into the pGL3-basic luciferase vector to generate the pGL3/LPP1-luc. The Quickchange site-directed mutagenesis kit was used to generate the pGL3/mlPP1-luc by disruption of the putative PPRE site (from −624 to −611 bp) with the use of the mutagenic primers: 5'-TCAACTGCAAGCG-GTTCCTATAACAAAGG-3' and 5'-ATCATCCATCACCTGGTACCT-3'. The amplified product was subcloned into the pGL3-basic luciferase vector to generate the pGL3/mlPP1-luc. The Quickchange site-directed mutagenesis kit was used to generate the pGL3/mlPP1-luc by disruption of the putative PPRE site (from −624 to −611 bp) with the use of

| Table 1: The sequences of the primers for ChIP assay. |
|-----------------|-----------------|
| hLPP1 PPRE1     | 5'-AGGTGACGGTGATGGAA-3' |
|                 | 5'-CTCTTGTGATGAGCCCTT-3' |
| hLPP1 PPRE2     | 5'-AGGGCTCCATAACAAAGG-3' |
|                 | 5'-CTATCCATCCTGATACCTT-3' |
| hLPP1 PPRE3     | 5'-CGAGGATGGAATCTGTTAATGGGATCTGGTG-3' |
|                 | 5'-GAGCCCTTTCTCATCATTAGG-3' |

3. Results

3.1. PPREs Are Recurrent Motifs in the 5′-Flanking Region of Human LPP1 Gene. We analyzed the human LPP1 (5′-flanking (NC_000005.9) using MatInspector (http://www.genomatix.de/) and identified three putative PPRE motifs, respectively, located at −418 bp (AGGTCACAGTTGA), −548 bp (AATTCAACGGTGA), and −611 bp (AGGTCAAGGGCTT) upstream of the transcriptional start site of human LPP1 gene (Figure 1).

3.2. PPARγ Upregulates LPP1 Gene Expression in ECs. To examine whether PPARγ regulates LPP1, we infected HUVECs with Ad-WT-PPARγ together with Ad-tTA in the presence or absence of tetracycline (0.1 μg/mL) for 24 h. Then, cells were treated with the PPARγ ligand rosiglitazone (5 μM) for 24 h. The qRT-PCR results showed that mRNA level of LPP1 was significantly induced in ECs activated with rosiglitazone or overexpressing PPARγ. Rosiglitazone further augmented the induction by PPARγ overexpression (Figure 2).

To investigate whether other two isoforms of PPARs also have similar effects, we also infected the ECs with Ad-WT-PPARα or Ad-WT-PPARδ and treated with their specific
agonists fenofibrate (5 μM) and GW501516 (1 μM) for 24 h. LPP1 mRNA level were not affected by neither PPARα nor PPARβ/δ overexpression. Similarly, PPARα and PPARβ/δ agonists had no effect on LPP1 expression (Figure 2).

In order to examine whether the effect of rosiglitazone was specific for PPARγ, we used GW9662, a selective antagonist, to pretreat ECs before the exposure. As shown in Figure 3, PPARγ antagonism significantly attenuated the LPP1 induction by rosiglitazone. Taken together, these results indicated that LPP1 gene was induced by PPARγ activation.

3.3. PPARγ Binds to the PPRE in the Promoter of Human LPP1 Gene. Sequence analysis of the 5′-flanking region of human LPP1 gene revealed three putative PPRE motifs. To examine whether PPARγ binds to these regions, ChIP assay was performed with the anti-PPARγ antibody and IgG as control.

The results showed that PPARγ could bind to the PPRE located at −624/−611 bp upstream of the human LPP1 gene, while the two proximal sites at −481/−468 and −561/−548 had no binding (Figure 4). This result suggested that the PPRE at −624/−611 might mediate the induction by PPARγ.

3.4. PPARγ Increases the Promoter Activity of Human LPP1 Gene via Binding to PPRE. To further examine whether PPARγ promotes the LPP1 gene transcription activity, we constructed pGL3/LPP1-luc reporter driven by the fragment containing three putative PPREs (Figure 5(a)). BAECs were transfected with pGL3/LPP1-luc plasmid. Luciferase assay showed that the reporter activity was increased by rosiglitazone or overexpression of PPARγ (Figure 5(b)). However, site-directed mutagenesis of the PPRE at −624/−611 abolished the induction by PPARγ, indicating that the PPRE at −624/−611 is the cis-element mediating the PPARγ transactivation.
4. Discussion

In the present study, we have demonstrated a transcriptional mechanism regulating the expression of LPP1 gene. We provided novel evidence that PPARγ and its specific agonist rosiglitazone positively regulate the transcription of LPP1 gene in ECs. We also identified the PPRE motif within the regulatory region of the LPP1 gene that mediates the action of PPARγ.

Elucidation of the transcriptional regulation of LPP1 is of physiological importance because LPP1 is a key enzyme responsible for the catabolism of LPA and S1P which are the bioactive lipid phosphates [5]. Therefore, given the mass and extensive distribution of ECs, the induction of LPP1 expression may lead to a profound decrease in the concentrations of LPA and S1P, both in circulation and in the tissues, and exert important biological effects. Although PPARγ has its major roles in the regulation of adipogenesis and insulin sensitivity, it also has a variety of protective effects on cardiovascular functions ranging from atherogenesis to blood pressure regulation [24, 25]. The finding that PPARγ induces LPP1 expression in ECs may provide new insights into the molecular mechanisms underlying the cardiovascular effects of PPARγ modulation.

LPP1 is an ectoenzyme with its active sites located on the outer surface of plasma membrane. It degrades extracellular LPA to monoacylglycerol, which can be taken into cells to produce intracellular LPA by acylglycerol kinase.

Intriguingly, intracellular LPA was known to be an agonist for PPARγ [26, 27]. Thus, it is postulated that the regulation of LPP1 by PPARγ may act as positive loop for extracellular LPA degradation. In human atherosclerotic lesions, LPA was accumulated in the lipid core of the plaques and promoted the development of atherosclerosis in vivo [28, 29]. Lipoprotein-derived LPA promoted the atherosclerosis in animal models [30]. In contrast, PPARγ agonists thiazolidinediones (TZD) reduced atherosclerotic plaques both in diabetic patients and in animal models [31]. Therefore, whether the induction of LPP1 and ensuing decrease in LPA contribute to the antiatherosclerotic effect of PPARγ remains to be investigated. In addition, the effects of PPARγ activation on the protein level, enzymatic activity of LPP1, and extracellular levels of LPA and S1P need to be examined in the future.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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