PPARγ attenuates intimal hyperplasia by inhibiting TLR4-mediated inflammation in vascular smooth muscle cells

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Aims

Peroxisome proliferator-activated receptor γ (PPARγ) has been reported to attenuate intimal hyperplasia. This study aimed to test the hypothesis that PPARγ inhibits intimal hyperplasia through suppressing Toll-like receptor 4 (TLR4)-mediated inflammation in vascular smooth muscle cells.

Methods and results

TLR4−/− mice on a C57BL/6J background were used. Increased TLR4 and pro-inflammatory cytokines were observed in wire-injury-induced carotid neointima and in platelet-derived growth factor (PDGF)-activated vascular smooth muscle cells. The TLR4 deficiency protected the injured carotid from neointimal formation and impaired the cellular proliferation and migration in response to lipopolysaccharide and PDGF. Rosiglitazone attenuated intimal hyperplasia. Overexpression of PPARγ suppressed PDGF-induced proliferation and migration and inhibited TLR4-mediated inflammation in vascular smooth muscle cells, while PPARγ silencing exerted the opposite effect. Lipopolysaccharide counteracted the inhibitory effect of PPARγ on PDGF-induced proliferation and migration. Eritoran suppressed the proliferation and migration induced by PDGF and PPARγ silencing. Vascular smooth muscle cells derived from TLR4−/− mice showed impaired proliferation and migration upon PDGF activation and displayed no response to PPARγ manipulation.

Conclusion

PPARγ inhibits vascular smooth muscle cell proliferation and migration by suppressing TLR4-mediated inflammation and ultimately attenuates intimal hyperplasia after carotid injury.

Keywords

Intimal hyperplasia  ●  Peroxisome proliferator-activated receptor γ  ●  Vascular smooth muscle cell  ●  Toll-like receptor 4

1. Introduction

Intimal hyperplasia (IH) is not only an important pathological feature in atherosclerosis, but also a major cause of failure in vascular reconstructive procedures, such as angioplasty, vascular stenting, and transplant vasculopathy.1 IH is believed to be the consequence of vascular smooth muscle cell (VSMC) proliferation and migration from the media into the intima.2 The quiescent VSMCs in adult vessels can be activated and regain the capability of migration and proliferation in response to various vascular injuries, and ultimately contribute to the formation of neointima. Suppression of VSMC migration and proliferation appears to be an important strategy for the prevention of IH.

Accumulative evidence supports peroxisome proliferator-activated receptor γ (PPARγ) as a promising target for attenuation of IH. PPARγ regulates numerous genes that are involved in glucose and lipid metabolism, and thus modulates a variety of diseases, including diabetes, obesity, and atherosclerosis. PPARγ has been reported to retard the growth of VSMCs derived from spontaneously hypertensive rats,3 inhibit VSMC proliferation induced by platelet-derived growth factor (PDGF) and angiotensin II,4,5 and suppress VSMC migration initiated by matrix metalloproteinase.6 PPARγ ligand has been demonstrated to inhibit neointimal formation following wire-induced carotid injury in diabetic mice,7 and balloon catheter-induced vascular injury in Zucker rats.8 Clinical investigations also

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demonstrated the attenuation of IH by PPARγ ligands even in patients without diabetes. However, despite intensive research efforts, the precise mechanisms underlying the effect of PPARγ remain to be fully elucidated.

An inflammatory response is widely accepted as an essential event in VSMC activation and IH formation. Inhibition of the inflammatory response by PPARγ has been increasingly examined in recent studies. Toll-like receptor 4 (TLR4) is well known to play a critical role in initiating inflammation through increasing the production of pro-inflammatory factors, including interleukin (IL)-1, IL-6, interferon γ, monocyte chemoattractant protein-1, and tumour necrosis factor α (TNF-α). TLR4 is also reported to be involved in the modulation of VSMC proliferation and neoformation. For example, TLR4 activation by lipopolysaccharide (LPS) can induce neoformation or aggravate IH in different arterial injury models. In the present study, we test the hypothesis that PPARγ inhibits VSMC proliferation and migration by suppressing TLR4-mediated inflammation, and eventually inhibits the IH after wire injury.

2. Methods

2.1 Reagents

Dulbecco’s modified Eagle’s medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco BRL (Carlsbad, CA, USA). LPS (TLR4 ligand; Escherichia coli 026:B6) and PDGF-BB were obtained from Sigma-Aldrich (St Louis, MO, USA). Rosiglitazone (RSG) was from Eisai Inc. (Andover, MA, USA). Antibodies targeting TLR4, PPARγ, β-actin and smooth muscle α-actin (α-SMA) were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The small interfering RNA (siRNA) duplex targeting PPARγ was synthesized by Shanghai Biosia Company (Shanghai, China) and sequenced by Sunbio Biotechnology (Beijing, China).

2.2 Animals

Male TLR4+/− mice (B6.B10ScN-Tlr4−/−JthJ on a C57BL/6J background, stock number 007227) and wild-type mice (C57BL/6J, stock number 000664) were obtained at 8–10 weeks of age from the Jackson Laboratory (Bar Harbor, ME, USA). A wire-induced carotid injury model was established in 2000 by Lab Vision (Fremont, CA, USA). Nuclei were counterstained with Mayer’s haematoxylin.

2.3 Histopathology and immunohistochemistry

Morphometric analysis was performed on haematoxylin- and eosin-stained slides. The area of each vascular layer was measured by tracing the external elastic lamina (EEL), internal elastic lamina (IEL) and vessel lumen. Intimal area was determined by subtracting the lumen area from the area within the IEL. Media area was considered as the area between the EEL and IEL. The IH was assessed by calculating the ratio of intima to media area.

For immunohistochemistry, sections were deparaffinized and rehydrated by serial immersion in xylene, alcohol and water. Thereafter, 3% hydrogen peroxide was used to block endogenous peroxidase. Non-specific binding was blocked with 0.5% blocking solution. α-SMA was used as a protein marker for VSMCs and was applied at a dilution of 1:100 for 1 h at room temperature, and followed by amplification with anti-rabbit biotin–streptavidin–horseradish peroxidase and visualization with DAB chromogen. All the required reagents were obtained from Lab Vision (Fremont, CA, USA).

2.4 Cell culture

VSMCs were isolated from the thoracic aorta of mice using an explant technique and cultured in DMEM supplemented with 10% FBS, 100 U/mL penicillin and 100 mg/mL streptomycin in air supplemented with 5% CO2 at 37°C. In the second to sixth passages were used for experiments. To identify the role of TLR4 in VSMC activation, cells were incubated with LPS (100 ng/mL) for 6 h in the presence or absence of pre-treatment with the TLR4 inhibitor, eritoran (10 ng/mL), for 45 min. When the cells had grown to confluence, the medium was changed to serum-free medium for an additional 24 h before the experiments.

PPARγ manipulation was carried out by either gene silencing or adenovirus-mediated overexpression. The siRNA duplex targeting PPARγ was transfected into VSMCs using lipofectamine 2000 (Invitrogen, CA, USA) according to the manufacturer’s protocol. A non-related scrambled siRNA was used as a negative control. Adenovirus expressing PPARγ was generated using the ViraPower Adenoviral Expression System (Invitrogen, CA, USA) and then transfected into the cultured VSMCs for 24 h. VSMCs transfected with scrambled sequence, siRNA and PPARγ-expressing adenovirus were named PPARγ-scr, PPARγ-kd and PPARγ-ov, respectively. Experiments were performed after 48 h of transfection. Transfection rates of 60–70% of the cells were accepted for all the experiments.

2.5 Western blot analysis

Western blot analysis was performed as described previously. Briefly, protein samples were obtained either from homogenized arteries or cultured cells, and the protein concentration was determined. Protein samples (30 μg) were separated by SDS-PAGE, transferred to a nitrocellulose membrane, and incubated with appropriate primary antibodies. After incubation with secondary antibodies, the proteins were detected by enhanced chemiluminescence (NEN, MA, USA) and quantified using a Gel Doc 2000 Imager (Bio-Rad, CA, USA). Western blot quantification was performed by densitometry and normalized to β-actin.

2.6 Cell proliferation assay

To measure cell proliferation, VSMCs were seeded (3 × 10⁴ cells/mL) into 96-well plates and cultured in DMEM containing 10% FBS. PDGF (20 μg/L for 24 h) was added to the medium to induce proliferation of VSMCs. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to analyse cell proliferation after 3 days of culture. Briefly, a 20 μL aliquot of 5 mg/mL MTT solution was added to each well and incubated for 24 h. Supernatants were aspirated, and the resulting crystals were dissolved in 200 μL dimethyl sulfoxide.
Light absorbance at 570 nm was read on a microplate reader (Bio-Rad, CA, USA).

2.7 Cell migration assay
Cell migration was examined using modified Boyden chambers, as described previously. DMEM containing 0.4% FBS and PDGF (20 μg/mL) was added to the lower section of the Boyden chambers. VSMCs suspended in DMEM (5.0 × 10^5 cells/mL) were added to the upper section of the Boyden chambers. After incubation for 4 h at 37°C, the top surface of the filters was scraped and rinsed with phosphate-buffered saline. Cells on the underside of the filters were fixed with methanol and stained with DAPI 4,6-diamino-2-phenyl indole (Sigma-Aldrich, St Louis, MO, USA), and then counted from five high-power (×400) fields per well. The average was used as the migratory cell number.

2.8 Quantitative real-time PCR
Total RNA was extracted with an RNeasy kit (Qiagen, Düsseldorf, Germany). The RNA (1 μg) was reverse transcribed into cDNA using Revert Aid First Strand cDNA Synthesis Kit (Fermentas, St Leon-Rot, Germany), following the manufacturer’s instructions. Real-time PCR was carried out with the SuperScript III Platinum SYBR-Green One-Step qRT-PCR kit (Invitrogen, CA, USA) on an Mx3000P QPCR System (Stratagene, LaJolla, CA, USA), following the manufacturer’s instructions. Primers for TLR4, TNF-α, IL-1β, IL-6, and β-actin are shown in Table 1. Each cDNA was analysed with target gene and β-actin primer sets that approached 100% amplification efficiency, allowing direct comparison of threshold cycle (Ct) values to determine relative gene expression. The target gene signal was first normalized to β-actin and then expressed relative to the value obtained with control VSMCs by using the formula 2^-ΔΔCt.

2.9 Statistical analysis
Data are expressed as the means ± SEM of at least three independent experiments. Two-group comparison was performed using a t-test for independent samples. Multiple-group statistical analyses were performed by one-way ANOVA followed by least significant difference post hoc testing. Statistics were calculated with the GraphPad Prism 5 software package (GraphPad Software, La Jolla, CA, USA). Differences were considered statistically significant at P < 0.05.

3. Results

3.1 TLR4-mediated inflammation played an essential role in wire-injury-induced IH and in VSMC proliferation and migration
Wire injury elicited significant formation of neointima in carotid arteries of wild-type mice, identified by increased intima/media ratio. In contrast, TLR4^−/− mice displayed very low intima/media ratio in response to carotid injury. Eritoran pre-treatment inhibited the IH induced by wire injury in wild-type mice, but exerted no obvious effect on that in TLR4^−/− mice (Figure 1A).

Wire injury increased the expression of TLR4 and pro-inflammatory cytokine mRNA (IL-1β, IL-6 and TNF-α) in wild-type mice. In contrast, TLR4^−/− mice exhibited almost undetectable expression of TLR4 in the carotid arteries. Wire injury failed to induce the expression of TLR4 and cytokine mRNA in TLR4^−/− mice. Eritoran pre-treatment blocked the expression of cytokine mRNA induced by wire injury in wild-type mice but not in TLR4^−/− mice (Figure 1B). These data suggest an important role for TLR4-mediated inflammation in wire-injury-induced neointimal formation. In agreement with a previous study, VSMCs, identified by α-SMA positive immunostaining, were found to make a major contribution to the formation of neointima (Figure 1C).

The role of TLR4-mediated inflammation in VSMC proliferation and migration was tested in vitro. It was found that LPS induced wild-type VSMC proliferation and migration, and increased the expression of TLR4 and cytokine mRNA. Eritoran significantly reduced the proliferation and migration, and down-regulated the expression of cytokine mRNA induced by LPS in wild-type VSMCs (Figure 2A–C). In contrast, VSMCs derived from TLR4^−/− mice were resistant to LPS-induced proliferation and migration, and to inflammatory reaction (Figure 2A–C), suggesting a critical role of TLR4-mediated inflammation in VSMC proliferation and migration.

3.2 PPARγ inhibited VSMC proliferation and migration and attenuated neointimal formation
Rosiglitazone was used to activate PPARγ in vivo. It was found that RSG significantly impeded the increase of intima/media ratio following wire injury in wild-type mice, but exerted no obvious impact on the neointima in TLR4^−/− mice (Figure 1A and B).

The expression of PPARγ in cultured VSMCs was manipulated via gene silencing and adeno virus-mediated overexpression (Figure 3A). PDGF was used to induce VSMC proliferation and migration in vitro. The impact of PPARγ on VSMC proliferation and migration was subsequently detected. As shown in Figure 3B and C, cultured VSMCs in basal conditions displayed low levels of proliferation and migration, which were markedly elevated by PDGF. PPARγ overexpression inhibited whereas PPARγ silencing accelerated the VSMC proliferation and migration in basal conditions and in the setting of PDGF stimulation (Figure 3B and C).

3.3 PPARγ inhibited TLR4-mediated inflammation in vivo and in vitro
PPARγ was previously reported to suppress the inflammatory reaction. In agreement, we found that the elevated expression of

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**Table 1 Primers for real-time PCR**

<table>
<thead>
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<th>3' sequence</th>
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<tr>
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<td>GGG ACA ACA CAG CCT GGA TG</td>
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</table>
Figure 1 (A) Intimal hyperplasia (IH) in carotid arteries. Haematoxylin and eosin staining on cross-sections from representative injured carotid arteries are presented. Carotid wire injury induced IH with increased intima/media ratio in wild-type mice. TLR4$^{-/-}$ mice displayed a lower intima/media ratio in response to carotid injury. Eritoran and RSG inhibited the wire-injury-induced IH in wild-type mice but not in TLR4$^{-/-}$ mice. Lines indicate neointima. $^{*}P < 0.05$ vs. WT + sham; $^{#}P < 0.05$ vs. WT + In; and $^{\Delta}P < 0.05$ vs. TLR4$^{-/-}$ + sham. (B) Expression of TLR4 and cytokine mRNA in carotid arteries detected by western blot and RT-PCR. Wire injury induced TLR4 protein and mRNA expression, and elevated the mRNA level of IL-1β, IL-6, and TNF-α in wild-type mice but not in TLR4$^{-/-}$ mice. Eritoran pre-treatment blocked the expression of cytokine mRNA induced by wire injury in wild-type mice but not in TLR4$^{-/-}$ mice. (C) Accumulation of VSMCs in the neointima identified by α-SMA immunostaining. $n = 5–6$ mice per group. $^{*}P < 0.05$ vs. WT; and $^{#}P < 0.05$ vs. WT + In. Abbreviations: WT, wild-type mice; TLR4$^{-/-}$, TLR4$^{-/-}$ mice; In, carotid wire injury; sham, sham operation; RSG, rosiglitazone; and erit, eritoran.
TLR4 and cytokine mRNA induced by carotid injury in wild-type mice was significantly abrogated by the PPARγ agonist, RSG (Figure 4A). Cultured VSMCs in basal conditions displayed very low levels of TLR4 and cytokines that were up-regulated by PPARγ silencing. PDGF increased the expression of TLR4 and cytokine mRNA in cultured VSMCs, which were largely retarded by PPARγ overexpression and further improved by PPARγ silencing (Figure 4B).

### 3.4 TLR4 affected the PPARγ-modulated VSMC proliferation and migration

To identify the role of TLR4 in the process of PPARγ-modulated VSMC activation, we observed the impact of LPS and eritoran on VSMC proliferation and migration in the setting of PPARγ manipulation. It was found that PPARγ overexpression inhibited PDGF-induced VSMC proliferation and migration, which were reversed by LPS exposure (Figure 5A and B). On the contrary, PPARγ silencing further promoted the PDGF-induced proliferation and migration, which were significantly suppressed by the TLR4 inhibitor, eritoran (Figure 5C and D). These data suggested that TLR4 exerts an essential role in the PPARγ-modulated VSMC proliferation and migration.

We also detected the potential impact of TLR4 on PPARγ expression. It was shown that neither LPS nor eritoran exerted a detectable influence on the expression of PPARγ (Figure 5E).

### 3.5 TLR4 deficiency impaired the proliferative and migratory response of VSMCs to PPARγ manipulation

To further address the hypothesis that suppression of TLR4 is an important mechanism by which PPARγ inhibits VSMC proliferation and migration, we compared the proliferation and migration of normal VSMCs and TLR4−/− VSMCs in the setting of PPARγ
The major findings presented in this study are as follows. (1) TLR4-mediated inflammation plays a crucial role in wire-injury-induced IH and in VSMC proliferation and migration. TLR4 deficiency or inhibition protected injured carotid arteries from neointimal formation and impaired VSMC proliferation and migration induced by LPS and PDGF. (2) PPARγ activation by RSG attenuated wire-injury-induced IH and suppressed TLR4-mediated inflammation in vivo. PPARγ overexpression suppressed PDGF-induced VSMC proliferation and migration and inhibited TLR4-mediated inflammation, while PPARγ silencing exerted the opposite effects. (3) LPS counteracted the inhibitory effect of PPARγ on PDGF-induced VSMC proliferation and migration. In contrast, the TLR4 inhibitor, eritoran, suppressed the proliferation and migration induced by PDGF and PPARγ silencing. (4) TLR4+/− VSMCs showed impaired proliferation and migration upon PDGF stimulation, and displayed no response to PPARγ manipulation.

IH is the common pathological lesion of proliferative vascular diseases, including atherosclerosis, hypertension, and restenosis after angioplasty. IH is initiated by endothelial damage or loss. Thereafter, increased VSMC proliferation occurs within 1–3 days, followed by migration of VSMCs into the intima, which eventually contributes to neointimal formation. Accumulative evidence supports the inhibitory effect of PPARγ on IH and on VSMC proliferation and migration. In support of this notion, our study showed that the PPARγ agonist, RSG, protected wire-injured carotid arteries from IH, and that PPARγ overexpression reduced whereas PPARγ silencing promoted the proliferation and migration of cultured VSMCs. Intensive research efforts have been focused on potential mechanisms responsible for the effect of PPARγ. It has been reported that PPARγ inhibits VSMC proliferation through down-regulating inflammatory genes, preventing the G1 to S phase transition, and inhibiting telomerase activity. In the present study, we tested the hypothesis that PPARγ inhibits VSMC proliferation and migration and attenuates IH through suppressing the TLR4-mediated inflammation.

The inflammatory response is a major force in the pathophysiology of IH after vascular injury. TLR4 and its associated pro-inflammatory cytokines have been shown to be closely related to this process. TLR4 can promote a pro-inflammatory phenotype of VSMCs. LPS, a widely used exogenous ligand that activates TLR4, can induce neointimal formation in a femoral artery cuff model and aggravate balloon-injury-induced IH in rabbit aorta. In the present study, wire injury of the carotid artery promoted IH and elevated expression of TLR4 and cytokines, which is consistent with the in vitro findings that activating TLR4 by LPS induced VSMC proliferation and migration and an inflammatory reaction. TLR4 deficiency inhibited wire-injury-induced IH and impaired VSMC proliferation and migration in response to LPS and PDGF, suggesting that TLR4 is required for VSMC activation and IH formation. Furthermore, pro-inflammatory cytokines downstream of TLR4 have also been demonstrated to participate in IH. Carotid balloon injury increased IL-6 expression and promoted VSMC migration. Blockade of GP130, a signal transducer of IL-6, inhibited VSMC migration and blocked neointimal formation. The
Figure 4 PPARγ inhibited the TLR4-mediated inflammatory reaction. Expressions of TLR4 protein and cytokine mRNA were detected by western blot and RT-PCR, respectively. (A) Carotid wire injury increased the expression of TLR4 and cytokine mRNA, including IL-1β, IL-6, and TNF-α, which were abrogated by the PPARγ agonist, RSG. *P < 0.05 vs. WT; and #P < 0.05 vs. WT + In. Abbreviations: WT, wild-type mice; In, carotid wire injury; and RSG, rosiglitazone. (B) PPARγ silencing up-regulated the expression of TLR4 and cytokine mRNA in cultured VSMCs. Increased expressions of TLR4 and cytokine mRNA induced by PDGF were largely reduced by PPARγ overexpression and further improved by PPARγ silencing. *P < 0.05 vs. VSMCs in basal conditions; and #P < 0.05 vs. VSMCs in the presence of PDGF only. Abbreviations: ov, PPARγ overexpression; kd, PPARγ silencing.
wire-injury-induced IH was exacerbated by elevated plasma TNF-α and was attenuated by TNF-α inhibition.26 Iliac balloon injury increased the IL-1β expression, and the IL-1β level 6 days after injury displayed a strong positive correlation with the extent of stenosis at 28 days postsurgery.27 The present study used eritoran, a synthetic analogue of the lipid A component of LPS, to inhibit the inflammatory reaction in vivo, and showed a significant suppression of mRNA expression of IL-1β, TNF-α, and IL-6 induced by wire injury. This finding agrees with previous studies showing that eritoran inhibits the production of inflammatory cytokines, including TNF-α, IL-6, IL-1β, macrophage inflammatory protein 1α, and macrophage inflammatory protein 2.28 Eritoran pretreatment also inhibited wire-injury-induced IH in wild-type mice, further suggesting the essential role of TLR4 and its associated pro-inflammatory cytokines in IH. According to previous studies, mitogen-activated protein kinase, a well-known signalling pathway that promotes cell proliferation,29 matrix metalloproteinases,30 and reactive oxygen species,31 may be associated with the effect of TLR4 on IH, although the precise mechanism is still a matter of debate.

A significant activation of TLR4 was observed in the present study after carotid injury, which may be attributed to the elevated endogenous TLR4 ligands, including HSP60, fibronectin, and hyaluronan. Reportedly, carotid catheter-induced injury in rats induced fibronectin expression in the neointima.32 Balloon catheter-induced injury increased the hyaluronan production in the aorta of rats, accompanied by increased intima-media thickness.33 We therefore speculated that carotid wire-induced injury up-regulated endogenous ligands and then activated TLR4 in vivo in the absence of LPS.

We next detected the impact of PPARγ on TLR4-mediated inflammation. Emerging evidence supports the cross-talk between PPARγ and TLR4. RSG can down-regulate TLR4 expression in the thoracic...
aorta in angiotensin II-infused rats.\textsuperscript{34} The PPARγ agonist, pioglitazone, decreases LPS-induced expression of TLR4 and cytokines.\textsuperscript{35} In turn, activation of TLR4 by LPS down-regulates PPARγ mRNA in peritoneal macrophages.\textsuperscript{36} The reasons for this discrepancy remain unclear. Our study showed that PPARγ silencing up-regulated whereas PPARγ overexpression down-regulated the expression of TLR4 and cytokines in PDGF-challenged VSMCs. Consistently, RSG decreases LPS-induced expression of TLR4 and cytokines.\textsuperscript{35} In turn, manipulation, whether overexpression or silencing, had no detectable impact on proliferation and migration in wild-type VSMCs. Compared with wild-type VSMCs, TLR4\textsuperscript{−/−} macrophages.\textsuperscript{36} The reasons for this discrepancy remain unclear. Our study showed that PPARγ silencing up-regulated whereas PPARγ overexpression down-regulated the expression of TLR4 and cytokines in PDGF-challenged VSMCs. Consistently, RSG decreases LPS-induced expression of TLR4 and cytokines.\textsuperscript{35} In turn, manipulation, whether overexpression or silencing, had no detectable impact on proliferation and migration in wild-type VSMCs. Compared with wild-type VSMCs, TLR4\textsuperscript{−/−} VSMCs showed an impaired proliferative and migratory response to PPARγ manipulation. PPARγ overexpression and silencing respectively inhibited and promoted the PDGF-induced proliferation (A) and migration (B) in wild-type VSMCs. In contrast, PDGF failed to increase the proliferation (A) and migration (B) in TLR4\textsuperscript{−/−} VSMCs. PPARγ manipulation exerted no detectable impact on proliferation (A) and migration (B) in TLR4\textsuperscript{−/−} VSMCs. $n = 5$. *$p < 0.05$ vs. wild-type VSMCs in basal conditions; and #$p < 0.05$ vs. wild-type VSMCs with PDGF stimulation only. Abbreviations: ov, PPARγ overexpression; kd, PPARγ silencing.

As a potent mitogen and chemoattractant, PDGF is widely used to induce VSMC proliferation and migration.\textsuperscript{17} In the present study, PDGF induced VSMC proliferation and migration paralleled with increased expression of TLR4 and cytokines, indicating a potential role of PDGF in inflammation. This finding is consistent with the study of Deng et al.\textsuperscript{22} showing that PDGF induced expression of IL-1α, IL-1β, and IL-6 in cultured human coronary artery smooth muscle cells.\textsuperscript{37}

There are some limitations to our study. Firstly, some effects of PPARγ agonists on VSMCs are PPARγ independent. We therefore used a genetic approach in vitro as a complement to RSG to identify the effect of PPARγ. Secondly, PPARγ can regulate the actions of VSMCs by improving insulin resistance and dyslipidaemia, and therefore inhibit IH. Obviously, the suppression of TLR4-mediated inflammation by PPARγ is one of the important pathways. Nonetheless, our findings provide the first evidence that PPARγ suppresses VSMC proliferation and migration by inhibiting TLR4-mediated inflammation, and thus attenuates IH following carotid injury. We therefore provide further insight into the mechanisms by which PPARγ regulates IH, and offer TLR4 as a promising target for the prevention and treatment of IH.

**Conflict of interest:** none declared.

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**References**


PPARγ inhibits TLR4 and attenuates intimal hyperplasia