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PPAR-γ agonist stabilizes KLF4 protein via activating Akt signaling and reducing KLF4 ubiquitination

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ABSTRACT

Peroxisome proliferator activated receptor γ (PPAR-γ) plays important roles in cell cycle regulation, differentiation and apoptosis. Krüppel-like factor 4 (KLF4) modulates vascular smooth muscle cell (VSMC) phenotype. Both KLF4 and PPAR-γ are involved in VSMC proliferation and differentiation. However, the actual relationship between KLF4 and PPAR-γ in VSMCs is not clear. In this study, we found that PPAR-γ agonist pioglitazone increases KLF4 protein levels but does not influence KLF4 gene transcription. PPAR-γ overexpression increases, while PPAR-γ knockdown reduces KLF4 expression, suggesting that the increase in KLF4 protein levels induced by pioglitazone is PPAR-γ-dependent. Further study showed that pioglitazone enhances KLF4 protein stability through reducing KLF4 ubiquitination. Furthermore, we demonstrated that stabilization of KLF4 by pioglitazone was related to the activation of Akt signaling pathway. Taken together, we revealed that PPAR-γ agonist pioglitazone stabilizes KLF4 protein via activating Akt signaling and reducing KLF4 ubiquitination, providing further insights into PPAR-γ and KLF4 in regulating each other's expression in VSMCs.

1. Introduction

Krüppel-like factor 4 (KLF4), a zinc-finger containing transcription factor, regulates a variety of cellular processes, including cell proliferation, differentiation, apoptosis, somatic cell reprogramming, as well as maintenance of tissue homeostasis by controlling the expression of a large number of genes with GC/GT-rich promoters [1]. In the vascular response to injury, KLF4 modulates endothelial inflammation, macrophage gene expression, and vascular smooth muscle cell (VSMC) proliferation and differentiation [2]. In VSMCs, KLF4 plays a key role in cell phenotypic switching by regulating the expression of VSMC marker genes and cell-cycle-regulatory genes.

The peroxisome proliferator-activated receptors (PPARs) are a subgroup of ligand-activated nuclear receptor superfamily, including three isoforms: PPAR-α, PPAR-β/δ, and PPAR-γ [3]. The most widely studied form among the three known forms of PPARs is PPAR-γ [4], which is mainly expressed in VSMCs, endothelial cells, adventitial fibroblasts, macrophages, and adipose tissue, where it controls adipocyte differentiation, energy homeostasis and genes that regulate insulin effects. PPAR isoforms form functional heterodimers with members of the retinoid X receptor (RXR) family of nuclear receptors. The PPAR/RXR heterodimer regulates expression of target genes by binding to the PPAR responsive elements (PPRE) [5]. PPAR-γ plays important roles in cell cycle regulation, differentiation, apoptosis and tumorigenesis [6]. In addition, increasing evidences have suggested potential anti-inflammatory and anti-atherogenic properties of PPAR-γ in monocyte/macrophages, endothelial cells, and VSMCs. Natural ligands of PPAR-γ are prostaglandins and oxidized linoleic acid, whereas synthetic ligands have pioglitazone and rosiglitazone [7]. PPAR-γ agonists can improve vascular remodelling and endothelial dysfunction by regulating oxidative stress and inflammation. These synthetic agonists have been widely used in clinical treatment of atherosclerosis, myocardial infarction and other cardiovascular diseases.

Regulation of proliferation and differentiation of VSMCs plays a critical role in blood vessel formation during embryogenesis and in pathologic states such as atherogenesis, restenosis, and hypertension. Both KLF4 and PPAR-γ are involved in VSMC proliferation and differentiation. Our previous study demonstrated that KLF4 and PPAR-γ could inhibit VSMC proliferation synergistically [8]. However, an actual relationship between KLF4 and PPAR-γ in regulating VSMC proliferation and differentiation had not yet been shown. In the present study, we sought to elucidate whether and how PPAR-γ regulates KLF4 expression in VSMCs.

2. Material and methods

2.1. Cell culture

VSMCs were isolated from the thoracic aorta of male Sprague-Dawley rats (80–100 g) as described previously [9]. VSMCs were

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maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) in a humidified atmosphere with 5% CO_2 at 37 °C. When cell growth reached 70–80% confluence, cells were incubated in serum-free DMEM for 24 h. When used, the cells were stimulated with pioglitazone (10 μM, Sigma, St. Louis, MO, USA) for different times. The NIH3T3 cells were maintained in high-glucose DMEM supplemented with 10% FBS.

2.2. Reagents

Pioglitazone (piog), WY14643, GW0742, GW9662 and cycloheximide (CHX) were purchased from Sigma–Aldrich, St.-Louis, MO. LY294002, SB431542 and PD98059 were from Promega, Madison, WI. All synthetic ligands were dissolved in DMSO. The final DMSO concentration in culture medium of all experiments was kept constant at 0.1%.

2.3. Western blotting

Crude proteins were extracted from VSMCs, resolved by SDS–PAGE and transferred onto a polyvinylidene difluoride membrane (Millipore, Billerica, MA, USA). Membranes were blocked with 5% milk in Tris-buffered saline with Tween 20 for 2 h at 37 °C and then incubated overnight at 4 °C with the following primary antibodies purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA): rabbit anti-KLF4, rabbit anti-PPAR-γ, rabbit anti-p-ERK, rabbit anti-ERK, rabbit anti-p-Akt, rabbit anti-Akt, rabbit anti-p-p38, rabbit anti-p38 or mouse anti-β-actin. After incubation with the appropriate secondary antibody, the immunoreactive signal of antibody-antigens was visualized using the Chemiluminescence Plus Western blot analysis kit (Santa Cruz).

2.4. Cell transfection

pCMV-FLAG plasmid was provided by Sigma-Aldrich. The PPAR-γ expression plasmid FLAG-PPAR-γ was created by the placement of PPAR-γ cDNA into the pCMV-FLAG-MAT-Tag-1 vector (Promega). VSMCs were transfected with these plasmids or siRNAs targeting rat PPAR-γ (siPPAR-γ) or non-specific siRNA (siControl) (Santa Cruz Biotechnology) using Lipofectamine reagent (Invitrogen) following the manufacturer's instructions, and then incubated in DMEM containing 10% FBS for 24 h followed by the application of pioglitazone.

2.5. Reporter gene assay

NIH3T3 cells were maintained in high-glucose DMEM supplemented with 10% FBS, were seeded in each well (3×10^4 cells/well) of a 24-well plate and grown for 24 h prior to transfection with

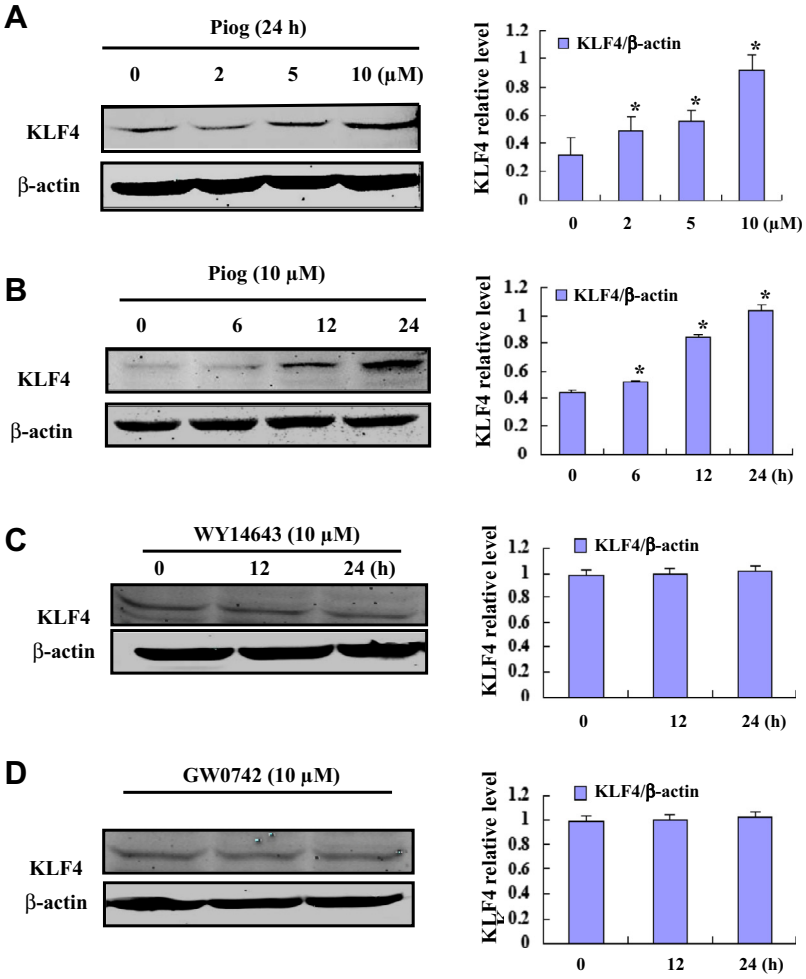

Fig. 1. Pioglitazone increases KLF4 protein levels in VSMCs. (A, B) Rat VSMCs were treated with pioglitazone at the indicated doses or the indicated times. Cells were collected and Western blotting was performed with anti-KLF4 antibody. β-actin was used as a control for equal protein loading. A representative result from three independent experiments is shown (left panel), whereas band intensities that were measured are shown on the right. *$p < 0.05$ versus the control group. (C, D) VSMCs were treated with WY14643 (10 μM) or GW0742 (10 μM) for the indicated times. Cells were collected and Western blotting was performed with anti-KLF4 antibody. β-actin was used as a control for equal protein loading. A representative result from three independent experiments is shown (left panel), whereas band intensities that were measured are shown on the right.

reporter plasmids or the control reporter plasmid pRL-TK. Cells were transfected using Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer's instructions. Twenty-four hours after treatment with pioglitazone, the cells were lysed, and luciferase assays were performed using a dual luciferase assay kit (Promega). Specific promoter activity was expressed as the relative ratio of firefly luciferase activity to Renilla luciferase activity. All promoter constructs were evaluated in a minimum of three separate wells per experiment.

2.6. RNA preparation and quantitative RT-PCR

Total RNA was isolated with TRIzol reagent (Invitrogen) according to the manufacturer's instructions. Quantitative PCR of KLF4 was performed using Platinum SYBR Green quantitative PCR Super-Mix UDG Kit (Invitrogen). As an internal control, the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene primers were used for RNA template normalization. All of the PCRs were performed in triplicate. The relative expression level was calculated using the following equation: relative gene expression = $2^{-(\Delta CtSample - \Delta CtControl)}$. The following primers were used: KLF4, 5′-CGGGAAGGGAGAAG ACACTGC-3′ (sense) and 5′-GCTAGCTGGGGAAGACGAGGA-3′ (antisense); GAPDH, 5′-ACCACAGTCCATGCCATCAC-3′ (sense) and 5′-TTC ACCACCCTG TTGCTGTA-3′ (antisense).

2.7. Immunofluorescent staining

VSMCs were fixed in 4% paraformaldehyde and permeabilized with 0.1% Triton X-100, incubated with anti-KLF4 antibody, and

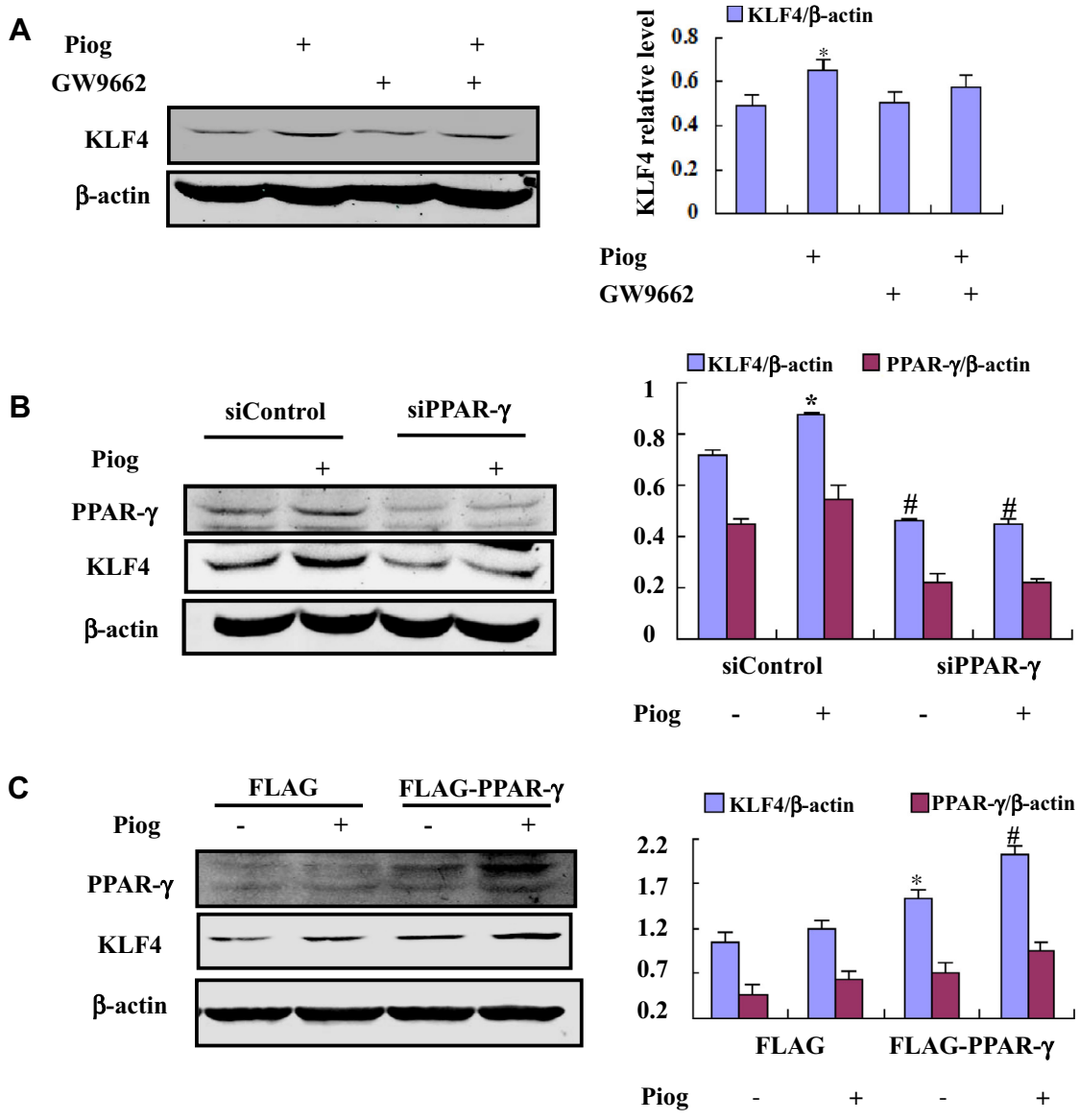

Fig. 2. The increase in KLF4 protein induced by pioglitazone is PPAR-γ-dependent. (A) VSMCs were treated with pioglitazone (10 μM), or preincubated with GW9662 (20 μM) for 2 h and then treated with pioglitazone (10 μM) for 24 h. Cells were collected and Western blotting was performed using anti-KLF4 and anti-β-actin antibodies, respectively. A representative result from three independent experiments is shown (left panel), whereas band intensities that were measured are shown on the right. $^{*}p < 0.05$ versus pioglitazone-untreated group. (B) VSMCs were transfected with siRNA targeting PPAR-γ (siPPAR-γ) or control siRNA (siControl) for 24 h and then treated with or without pioglitazone (10 μM) for another 24 h. Cells were collected and Western blotting was performed using anti-KLF4, anti-PPAR-γ and anti-β-actin antibodies, respectively. A representative result from three independent experiments is shown (left panel), whereas band intensities that were measured are shown on the right. $^{*}p < 0.05$ versus pioglitazone-untreated group, $^{\#}p < 0.05$ versus siControl-transfected group. (C) VSMCs were infected with FLAG-PPAR-γ or empty vector FLAG for 24 h and then treated with or without pioglitazone (10 μM) for another 24 h. Cells were collected and Western blotting was performed using anti-KLF4, anti-PPAR-γ and anti-β-actin antibodies, respectively. A representative result from three independent experiments is shown (left panel), whereas band intensities that were measured are shown on the right. $^{*}p < 0.05$ versus FLAG-infected group, $^{\#}p < 0.05$ versus pioglitazone-untreated group.

further stained with FITC-conjugated secondary antibody or TRITC-phalloidin. Staining of 4′6-diamidino-2-phenylindole (DAPI) was used to visualize nuclear localization. Confocal microscope was performed with the Confocal Laser Scanning Microscope Systems (Leica).

2.8. Statistical analyses

Data presented as bar graphs are the mean ± standard error of the mean (SEM) of ⩾3 independent experiments. Statistical analyses were performed using Student's *t*-test. The results were considered statistically significant at $P < 0.05$.

3. Results

3.1. Pioglitazone increases KLF4 protein levels in VSMCs

To examine the effect of pioglitazone on KLF4, we firstly analyzed the expression of KLF4 in VSMCs treated with pioglitazone. Western blotting showed that pioglitazone significantly induced KLF4 expression in a time- and dose-dependent manner (Fig. 1A and B). In addition, laser scanning confocal microscope was used to detect KLF4 expression and similar results were obtained (Suppl. Fig. 1A and B). In contrast, PPAR-α agonist, WY14643, and PPAR-β agonist, GW0742, did not affect KLF4 expression (Fig. 1C and D). These results indicate that PPAR-γ agonist pioglitazone can specifically increase KLF4 levels in VSMCs.

3.2. The increase in KLF4 protein levels induced by pioglitazone is PPAR-γ -dependent

Because PPAR-γ agonist is known to regulate gene expression in a PPAR-γ-dependent or independent manner [10,11], we sought to clarify whether pioglitazone-induced KLF4 expression was PPAR-γ-dependent. Thus, we tested KLF4 expression in VSMCs treated with or without pioglitazone antagonist GW9662, the results showed that induction of KLF4 expression by pioglitazone was blocked by the PPAR-γ antagonist GW9662 (Fig. 2A). Furthermore, VSMCs transfected with PPAR-γ-specific siRNA (siPPAR-γ) or with PPAR-γ expression vector FLAG-PPAR-γ were stimulated with or without pioglitazone, and then Western blotting was performed to detect KLF4 expression. We found that in cells where PPAR-γ was knocked down by transfecting siPPAR-γ, KLF4 expression was markedly reduced and no longer affected by pioglitazone treatment (Fig. 2B). In contrast, overexpression of PPAR-γ largely promoted KLF4 expression, which was further increased by pioglitazone treatment (Fig. 2C). These results indicated that pioglitazone induces KLF4 expression in a PPAR-γ-dependent manner.

3.3. Pioglitazone does not affect KLF4 gene transcription

Next, we sought to determine whether pioglitazone increased the transcription of KLF4 gene. VSMCs were treated with pioglitazone for 24 h and then KLF4 mRNA was detected by real-time PCR analysis. Interestingly, we found that the expression of KLF4 mRNA was not affected by pioglitazone treatment (Fig. 3A). To further characterize the role of PPAR-γ in pioglitazone-induced KLF4 expression, we examined the effect of PPAR-γ on KLF4 promoter activity by luciferase reporter gene assays. As shown in Fig. 3B, PPAR-γ overexpression could not activate KLF4 promoter activity. In addition, we test whether liganded PPAR-γ could activate KLF4 transcription, the results showed that pioglitazone did not affect KLF4 promoter activity in NIH3T3 cells transfected with PPAR-γ expression plasmids (Fig. 3C). These results demonstrated that PPAR-γ does not regulate KLF4 gene transcription, no matter whether cells were treated with pioglitazone.

3.4. Pioglitazone enhances KLF4 protein stability by reducing KLF4 ubiquitination

The fact that pioglitazone increases KLF4 protein levels, but does not influence its mRNA expression implies that pioglitazone might enhance KLF4 protein stability. To test this possibility, we used the protein synthesis inhibitor cycloheximide (CHX) to block new protein synthesis to probe the effect of pioglitazone on KLF4 protein stability in VSMCs. As a result, treatment of VSMCs with CHX time-dependently reduced KLF4 protein level (Fig. 4A). However, KLF4 protein levels remained high up to 12 h after CHX treatment in pioglitazone-treated cells (Fig. 4A), suggesting that pioglitazone increases KLF4 protein levels through enhancing protein stability in VSMCs. Because protein stability is regulated by ubiquitination, we sought to examine whether pioglitazone affected KLF4 ubiquitination. As shown in Fig. 4B, a significant decrease in ubiquitin-conjugated KLF4 was observed in response to pioglitazone stimulation. Taken together, these results suggest that pioglitazone enhances KLF4 protein stability by reducing KLF4 ubiquitination.

3.5. Stabilization of KLF4 by pioglitazone is related to the activation of Akt signaling

To test which signal pathway mediates KLF4 protein stability in response to pioglitazone, we observed the effect of pioglitazone on

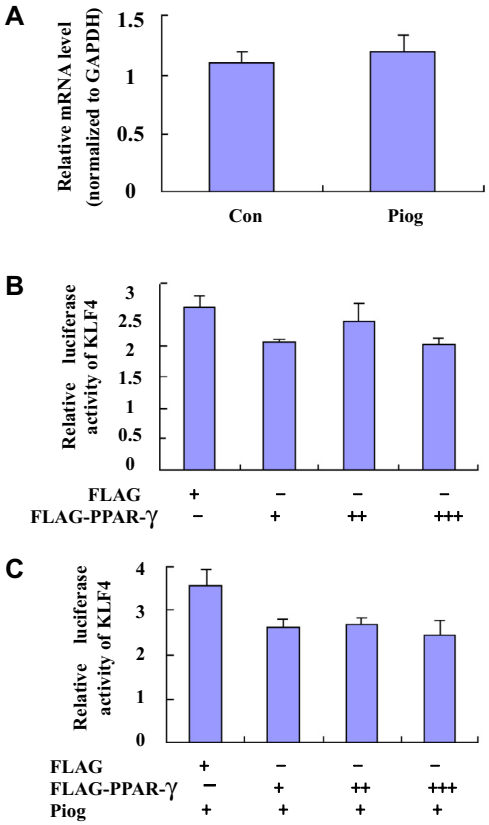

Fig. 3. Pioglitazone does not affect KLF4 gene transcription. (A) VSMCs were treated with 10 μM pioglitazone for 24 h. Total RNA was prepared, and the level of KLF4 mRNA was examined by qRT-PCR and presented relative to GAPDH mRNA (means ± SEM; $n = 3$). (B, C) NIH3T3 cells were transfected with different concentration of PPAR-γ expression plasmid FLAG-PPAR-γ, along with pGL3 basic (control), pRL-TK and KLF4 promoter-reporter constructs, 24 h after transfection, cells were treated with or without 10 μM pioglitazone for 24 h. Cells were lysed, and luciferase activity was measured using the dual luciferase reporter assay system. Data represent relative KLF4 promoter activity normalized to Renilla luciferase. Error bars represent means ± SEM from three independent experiments.

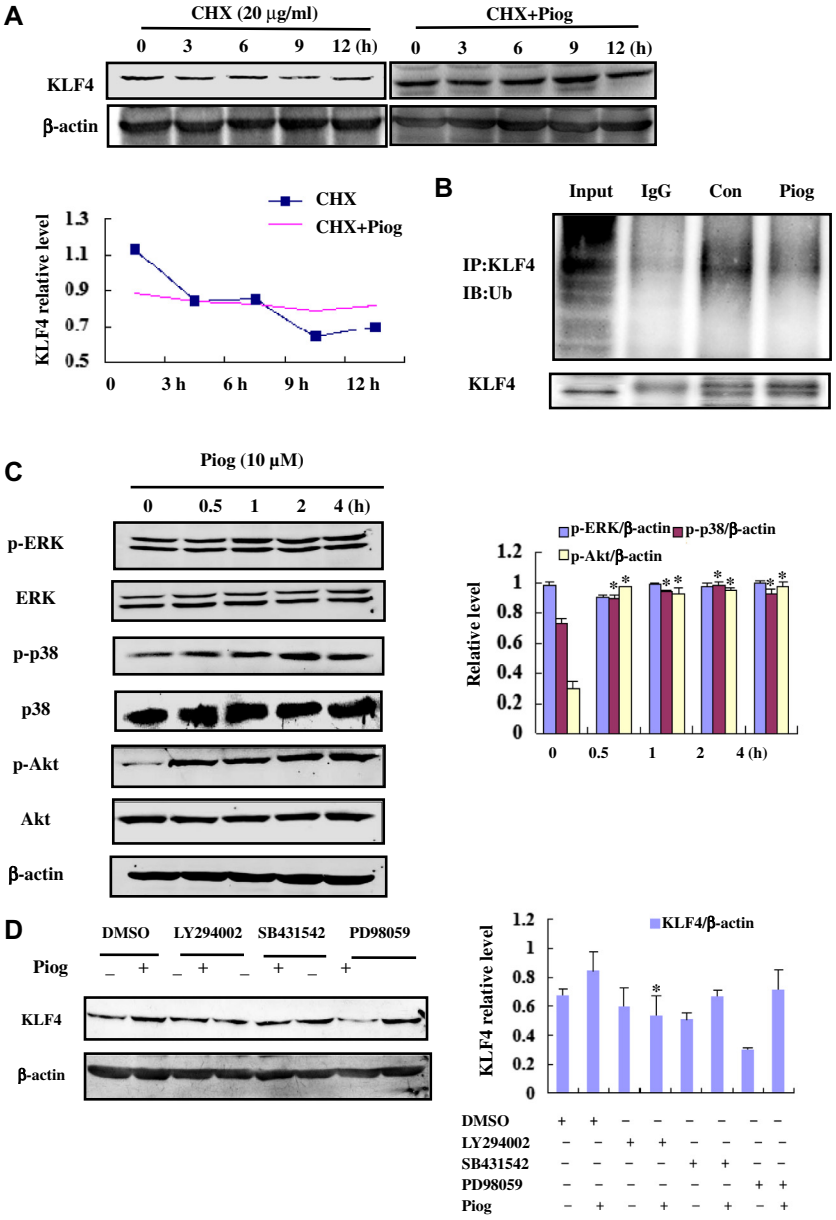

Fig. 4. Pioglitazone enhances KLF4 protein stability via reducing KLF4 ubiquitination and activating Akt signaling. (A) VSMCs were treated with 20 μg/ml of cycloheximide (CHX) for the indicated times, followed by treatment with or without pioglitazone for the indicated times. The cells were collected and KLF4 protein levels were measured by immunoblotting. A representative result from three independent experiments is shown (upper panel), whereas band intensities that were measured are shown on the lower panel. (B) VSMCs were collected 4 h after pioglitazone stimulation. KLF4 was immunoprecipitated by anti-KLF4 antibody coupled with protein A beads. The ubiquitin-conjugated KLF4 in the immunoprecipitated complex was detected by immunoblotting using anti-ubiquitin antibody. KLF4 protein levels in the whole cell lysates were measured by immunoblotting. (C) VSMCs were treated with 10 μM pioglitazone for the indicated times. Total cell lysates were analyzed by Western blotting using antibodies to p-ERK, ERK, p-p38, p38, p-Akt and Akt. β-actin was used as a control for equal protein loading. A representative result from three independent experiments is shown (left panel), whereas band intensities that were measured are shown on the right. $^{*}p < 0.05$ versus the control group (0 h). (D) VSMCs were pretreated with LY294002 (25 μmol/L), SB431542 (10 μmol/L), or PD98059 (25 μmol/L) for 2 h, followed by a 24 h incubation with or without pioglitazone (10 μM). Cells were collected and KLF4 expression was examined by Western blotting using anti-KLF4 antibody. β-actin was used as a control for equal protein loading. A representative result from three independent experiments is shown (left panel), whereas band intensities that were measured are shown on the right. $^{*}p < 0.05$ versus pioglitazone-alone-treated group.

the phosphorylation of ERK, p38, and Akt by Western blotting. As shown in Fig. 4C, the levels of phospho-p38 and phospho-Akt were increased within 30 min and persisted to 4 h after pioglitazone treatment, whereas ERK phosphorylation was not affected by pioglitazone under same experimental condition. The level of expression of these molecules did not change during this time course. To assess the importance of p38 and Akt signaling in stabilizing KLF4 protein, VSMCs were pretreated either with the Akt inhibitor, LY294002, the p38 inhibitor, SB431542, or the ERK inhibitor, PD98059, for 2 h before exposure to pioglitazone. Pharmacological inhibition of Akt blocked pioglitazone-induced increase of KLF4 levels, whereas inhibition of ERK and p38 did not affect KLF4 increase induced by pioglitazone (Fig. 4D). These results suggest that Akt signaling pathway mediates the pioglitazone-induced KLF4 protein stability in VSMCs.

4. Discussion

Several groups have reported that PPAR-γ is highly expressed within the atherosclerotic plaque and the neointima after balloon injury [12], where it regulates gene expression of some key

proteins involved in vascular inflammation and proliferation. PPAR-γ ligands, such as thiazolidinediones (pioglitazone and rosiglitazone), are potent and selective activator of PPAR-γ. Recent studies showed that activation of PPAR-γ by its ligands inhibits cellular proliferation, promotes differentiation, induces apoptosis and inhibits angiogenesis by trans-repression of pro-inflammatory and pro-oxidant genes in the vascular system [13]. The cardiovascular protective effect of PPAR-γ prompted researchers to postulate that PPAR-γ may play an important role in VSMCs. Our previous studies showed that proliferation inhibition of VSMCs is governed by the activity of a transcription factor network [14]. KLF4 is one component of such a network. We demonstrated that KLF4 overexpression inhibits VSMC proliferation induced by PDGF-BB and endothelial injury [14]. Both KLF4 and PPAR-γ are involved in VSMC proliferation and differentiation. However, whether and how KLF4 is regulated by PPAR-γ in VSMCs is unknown. In the present study, we found that PPAR-γ agonist pioglitazone stabilized KLF4 protein in VSMCs by activating Akt signaling and reducing KLF4 ubiquitination.

Previous studies showed that some PPAR-γ agonists induced KLF4 expression in a receptor-dependent manner [15]. Consistent with this finding, we show that pioglitazone specifically increased KLF4 protein level by stabilizing KLF4 protein. KLF4 expression was upregulated by the overexpression of PPAR-γ and downregulated in the absence of PPAR-γ, suggesting partial regulation of KLF4 by PPAR-γ activation. Santiago found that pioglitazone induced VSMC apoptosis in a PPAR-γ-dependent mechanism [16]. But in colon cancer cells, Chen and Tseng demonstrated that a highly specific activator of PPAR-γ, 15d-PGJ2, upregulated KLF4 expression independently of PPAR-γ through activation of the MAPK signaling pathway [17]. The differences regarding the role of PPAR-γ agonists could be attributable to different cell conditions and may provide an explanation for different action of PPAR-γ agonists in different cells.

Li and Zhou reported that PPAR-γ agonists induced cell cycle arrest through transcriptional regualtion of KLF4 in colon cancer cells [18], but it is unclear whether PPAR-γ agonists regulate the expression of KLF4 in VSMCs. Our data clearly demonstrated that pioglitazone upregulated KLF4 protein level, but did not influence the mRNA transcription in VSMCs. Additionally, our data showed that pioglitazone enhanced the KLF4 protein stability by activating the Akt signaling and reducing KLF4 ubiquitination. In contrast, Li revealed that PPAR-γ regulated the expression of KLF4 by binding directly to the PPAR response element (PPRE) within the KLF4 promoter [18]. The PPRE resides at −1657 bp to −1669 bp upstream of the KLF4 ATG codon, which is essential for the transactivation of KLF4 expression by PPAR-γ ligands [18]. Therefore, further work will be necessary to elucidate the effect of PPAR-γ agonists on KLF4 gene expression and their mechanism of action in VSMCs.

KLF4 is an important regulator that mediates the aberrant behavior of VSMCs in the pathogenesis of vascular diseases. KLF4 can be activated through several signal pathways, including p38, MEK1/ERK, and PI3-kinase/Akt-mediated signal pathways [19]. Our experiments have shown that the inhibition of PI3-kinase/Akt completely blocked the increase of pioglitazone-induced KLF4 protein in VSMCs. But Chen and Tseng concluded that 15d-PGJ2 upregulated KLF4 expression through activation of p38 MAPK signaling pathway [17]. This could be attributable to the difference between colon cells and smooth muscle cells. Although we demonstrated that pioglitazone-induced increase of KLF4 protein levels was mediated by PI3-kinase/Akt-dependent pathway, it would be quite interesting to determine whether the KLF4 target genes are activated after pioglitazone stimulation in VSMCs.

It is well documented that KLF4 is one of the key regulatory factors both during blood vessel development and in vascular diseases. Several recent studies have suggested that PPAR-γ may function as a protective factor by improving vascular remodelling and endothelial dysfunction in vascular diseases [20]. In our study, it is the first time to demonstrate that PPAR-γ agonist pioglitazone can stabilize KLF4 protein via activating Akt signaling and reducing KLF4 ubiquitination in VSMCs. It will be helpful towards better understanding of the physiological and pathobiological role of PPAR-γ and KLF4 in regulating the proliferation and differentiation of VSMCs in the vasculature.

Author contributions

Sun Y, Zheng B, Zhang XH, He M, Guo ZW carried out the experiments, Wen JK reviewed the data and helped in the design and preparation of the manuscript.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.11.129.

References

[1] P.M. Evans, C. Liu, Roles of Krüppel-like factor 4 in normal homeostasis, cancer and stem cells, Acta Biochim. Biophs. Sin. 40 (2008) 554–564.
[2] B.B. McConnell, A.M. Ghaleb, M.O. Nandan, V.W. Yang, The diverse functions of Krüppel-like factors 4 and 5 in epithelial biology and pathobiology, BioEssays 29 (2007) 549–557.
[3] J. Vamecq, N. Latruffe, Medical significance of peroxisome proliferator-activated receptors, The Lancet 354 (1999) 141–148.
[4] M. Lehrke, M.A. Lazar, The many faces of PPARγ, Cell 123 (2005) 993–999.
[5] S.A. Kliewer, K. Umesono, D.J. Noonan, R.A. Heyman, R.M. Evans, Convergence of 9-cis retinoic acid and peroxisome proliferator signalling pathways through heterodimer formation of their receptors, Nature 358 (1992) 771–774.
[6] F. Yan, PPARs and cardiovascular disease, J. Med. Mol. Biol. 1 (2003) 376–378.
[7] B.M. Forman, P. Tontonoz, J. Chen, R.P. Brun, B.M. Spiegelman, R.M. Evans, 15-Deoxy-delta 12, 14-prostaglandin J2 is a ligand for the adipocyte determination factor PPAR gamma, Cell 83 (1995) 803–812.
[8] X.H. Zhang, B. Zheng, C. Gu, J.R. Fu, J.K. Wen, TGF-β1 downregulates AT1 receptor expression via PKC-δ-mediated Sp1 dissociation from KLF4 and Smad-mediated PPAR-γ association with KLF4, Arterioscler. Thromb. Vasc. Biol. 32 (2012) 1015–1023.
[9] M. Han, J.K. Wen, B. Zheng, Y. Cheng, C. Zhang, Serum deprivation results in redifferentiation of human umbilical vascular smooth muscle cells, Am. J. Physiol. Cell. Physiol. 291 (2006) C50–C58.
[10] M. Gurnell, D.B. Savage, V.K.K. Chatterjee, S. O'Rahilly, The metabolic syndrome: peroxisome proliferator-activated receptor γ and its therapeutic modulation, J. Clin. Endocrinol. Metab. 88 (2003) 2412–2421.
[11] S.S. Palakurthi, H. Aktas, L.M. Grubissich, R.M. Mortensen, J.A. Halperin, Anticancer effects of thiazolidinediones are independent of peroxisome proliferator-activated receptor γ and mediated by inhibition of translation initiation, Cancer Res. 61 (2001) 6213–6218.
[12] R.E. Law, S. Goetze, X.P. Xi, S. Jackson, Y. Kawano, L. Demer, M.C. Fishbein, W.P. Meehan, W.A. Hsueh, Expression and function of PPAR-γ in rat and human vascular smooth muscle cells, Circulation 101 (2000) 1311–1318.
[13] S. Benson, J. Wu, S. Padmanabhan, T.W. Kurtz, H.A. Pershadsingh, Peroxisome proliferator-activated receptor (PPAR)-γ expression in human vascular smooth muscle cells: inhibition of growth, migration, and c-fos expression by the peroxisome proliferator-activated receptor (PPAR)-γ activator troglitazone, Am. J. Hypertens. 13 (2000) 74–82.
[14] B. Zheng, M. Han, M. Bernier, X.H. Zhang, F. Meng, S.B. Miao, M. He, X.M. Zhao, J.K. Wen, Krüppel-like factor 4 inhibits proliferation by platelet-derived growth factor receptor β-mediated, not by retinoic acid receptor α-mediated, phosphatidylinositol 3-kinase and ERK signaling in vascular smooth muscle cells, J. Biol. Chem. 284 (2009) 22773–22785.
[15] M. Cekanova, S.H. Lee, M.F. McEntee, S.J. Baek, MCC-555-induced NAG-1 expression is mediated in part by KLF4, Eur. J. Pharmacol. 637 (2010) 30–37.
[16] S. Redondo, E. Ruiz, C.G. Santos-Gallego, E. Padilla, T. Tejerina, Pioglitazone induces vascular smooth muscle cell apoptosis through a peroxisome proliferator-activated receptor-γ, transforming growth

factor-β1, and a Smad2-dependent mechanism, Diabetes 54 (2005) 811–817.
[17] Z.Y. Chen, C.C. Tseng, 15-Deoxy-Δ12, 14 prostaglandin J2 up-regulates Krüppel-like factor 4 expression independently of peroxisome proliferator-activated receptor γ by activating the mitogen-activated protein kinase kinase/extracellular signal-regulated kinase signal transduction pathway in HT-29 colon cancer cells, Mol. Pharmacol. 68 (2005) 1203–1213.
[18] S. Li, Q. Zhou, H. He, Y. Zhao, Z. Liu, Peroxisome proliferator-activated receptor γ agonists induce cell cycle arrest through transcriptional regulation of Krüppel-like factor 4 (KLF4), J. Biol. Chem. 288 (2013) 4076–4084.
[19] H.X. Li, M. Han, M. Bernier, B. Zheng, S.G. Sun, M. Su, R. Zhang, J.R. Fu, J.K. Wen, Krüppel-like factor 4 promotes differentiation by transforming growth factor-β receptor-mediated Smad and p38 MAPK signaling in vascular smooth muscle cells, J. Biol. Chem. 285 (2010) 17846–17856.
[20] V. Subramanian, J. Golledge, T. Ijaz, D. Bruemmer, A. Daugherty, Pioglitazone-induced reductions in atherosclerosis occur via smooth muscle cell-specific interaction with PPAR-γ novelty and significance, Circ. Res. 107 (2010) 953–958.