Peroxisome proliferator-activated Receptor γ Ligands Inhibit Retinoblastoma Phosphorylation and G₁ → S Transition in Vascular Smooth Muscle Cells*

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Peroxisome proliferator-activated receptor γ (PPARγ) is a member of the nuclear receptor superfamily that is activated by binding certain fatty acids, eicosanoids, and insulin-sensitizing thiazolidinediones (TZD). The TZD troglitazone (TRO) inhibits vascular smooth muscle cell proliferation and migration both in vitro and in vivo. The precise mechanism of its antiproliferative activity, however, has not been elucidated. We report here that PPARγ ligands inhibit rat aortic vascular smooth muscle cell proliferation by blocking the events critical for G₁ → S progression. Flow cytometry demonstrated that both TRO and another TZD, rosiglitazone, prevented G₁ → S progression induced by platelet-derived growth factor and insulin. Movement of cells from G₁ → S was also inhibited by the non-TZD, natural PPARγ ligand 15-deoxy-12,14Δ prostaglandin J₂ (15d-PGJ₂), and the mitogen-activated protein kinase pathway inhibitor PD98059. Inhibition of G₁ → S exit by these compounds was accompanied by a substantial blockade of retinoblastoma protein phosphorylation. TRO and rosiglitazone attenuated both the mitogen-induced degradation of p27kip1 and the mitogenic induction of p21cip1. 15d-PGJ₂ and PD98059 inhibited both the degradation of p27kip1 and the induction of cyclin D1 in response to mitogens. These effects resulted in the inhibition of mitogenic stimulation of cyclin-dependent kinases activated by cyclins D1 and E. These data demonstrate that PPARγ ligands are antiproliferative drugs that act by modulating cyclin-dependent kinase inhibitors; they may provide a new therapeutic approach for proliferative vascular diseases.

Proliferation of vascular smooth muscle cells (VSMC) plays a key role in the development of restenosis and in the progression of atherosclerosis (1, 2). Injury to the endothelium results in the migration of underlying VSMC into the intimal layer of the arterial wall, where they proliferate and synthesize extracellular matrix components. Although many growth factors induce the proliferation of VSMC (3), platelet-derived growth factor (PDGF) is an important mitogenic and chemotactic regulator of VSMC, since blocking antibodies to PDGF inhibits neointimal formation in rat models of arterial injury (4). Insulin is also a potent mitogen for VSMC, and physiologic hyperinsulinemia enhances the mitogenic effects of PDGF (5). In response to vascular injury, quiescent VSMC (G₀) must transit through the G₁ phase of the cell cycle and enter into the S phase to undergo DNA replication.

Progression through the cell cycle requires the formation and activation of cyclin and cyclin-dependent kinase (CDK) complexes (6). Progression through the G₁ phase requires cyclin D/CDK4, cyclin E/CDK6, and cyclin E/CDK2 holoenzymes. Functional cyclin A/CDK2 complexes are required for DNA synthesis (S phase), and subsequently, cyclin A/CDK2 and cyclin B/CDK2 pairs are assembled and activated during G₂ phase and mitosis (M phase), respectively. Activation of the G₁ phase cyclin-CDK complexes results in the phosphorylation of retinoblastoma gene products (Rb) (7). Rb proteins are critical negative regulators of cell cycle progression by controlling gene expression mediated by E2F transcription factors (7). E2F-regulated genes encode proteins required for S phase DNA synthesis (8). In the absence of its being phosphorylated by CDKs, Rb binds and sequesters E2F, thereby preventing transcriptional activation of target genes. CDK-phosphorylated Rb releases E2F that permits the induction of E2F-dependent genes. CDK inhibitors (CDKIs), p21cip1 (9, 10), p27kip1 (11–13), and p15/p16ink4c (14), regulate this process by inhibiting cyclin/CDK activity and phosphorylation of Rb, resulting in G₁ arrest (15). Progression through the mammalian cell cycle is regulated by the balance between the levels and activities of cyclin-CDK complexes, the growth-promoting transcriptional factors they regulate, CDKIs and other growth suppressor proteins.

Thiazolidinediones (TZDs) are high-affinity ligands for peroxisome proliferator-activated receptor γ (PPARγ). PPARγ is expressed in VSMC (16, 17), and ligands for this nuclear receptor inhibit VSMC proliferation induced by several different.
FIG. 1. PPARγ ligands and PD98059 prevent mitogen-induced G1 → S progression in RASMC. A, quiescent RASMC (0.4% FBS for 24 h) were stimulated by treatment with PDGF (20 ng/ml) and insulin (1 μM). Cells were preincubated with troglitazone (10 μM), rosiglitazone (10 μM), 15d-PGJ2 (5 μM), and PD98059 (30 μM) for 30 min prior to addition of mitogens. 24 h after stimulation, DNA was stained with propidium iodide (P1), and 1 × 10^6 cells were analyzed by flow cytometry. A shows representative DNA histograms for quiescent RASMC (0.4% FBS for 24 h) (a), RASMC stimulated with PDGF and insulin (P + I) (b), RASMC stimulated in the presence of TRO (c), RSG (d), 15d-PGJ2 (e), and PD98059 (f), respectively. The x and y axes represent the intensity of propidium iodide fluorescence and cell number, respectively. The data are representative of three separate experiments. B, PPARγ ligands and PD98059 inhibit mitogen-induced G1 → S progression in a dose-dependent manner. Results are the mean of three independent experiments. Mean ± S.E. is expressed as percentage of S phase transition.
mitogens *in vitro* (18, 19) and intimal hyperplasia *in vivo* (18). These observations suggest that activation of PPARγ interferes with the function of a fundamental component of the cell cycle machinery. The specific mechanism by which PPARγ inhibits VSMC proliferation, however, remains to be determined. We have previously shown that PPARγ ligands inhibit ERK MAPK-dependent mitogenic signaling pathways in VSMC at a step downstream of ERK activation (18). Induction of cyclin D and G₁ → S progression of nonvascular cells has also been shown to require activation of ERK and MAPK. The purpose of this study was to examine the effect of PPARγ ligands on cell cycle regulators in rat aortic smooth muscle cells and to delineate the mechanism of their antiproliferative activity.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Treatment with Growth Factor and Reagents—** Rat aortic smooth muscle cells (RASMC) were prepared from thoracic aorta of 2-3-month-old Harlan Sprague-Dawley rats by using the explant technique. The cells were cultured in Dulbecco’s modified Eagle’s medium containing 10% FBS (Irvine Scientific, Santa Ana, CA), 100 units/ml penicillin, 100 μg/ml streptomycin, and 200 μm L-glutamine. Human coronary artery smooth muscle cells (CASM, purchased from Clonetics, San Diego, CA) were cultured in smooth muscle cell growth medium-2 containing 5% FBS, 50 μg/ml gentamicin, 50 ng/ml amphotericin-B, and 5 μg/ml bovine insulin (all purchased from Clonetics). For all experiments, early passaged (5–8) RASMC or CASMC were grown to 60–70% confluency and made quiescent by serum starvation (0.4% FBS) for at least 3 times. After appropriate treatments, cells were washed with cold PBS and solubilized on ice in lysis buffer (50 mM Tris, pH 8.0; 250 mM NaCl; 0.5% Nonidet P-40; 1 μg/ml leupeptin; and 1 mM phenylmethylsulfonyl fluoride). Insoluble materials were cleared through centrifugation at 4 °C for 10 min at 12,000 rpm. Protein concentrations were determined, and protein was suspended in 1 ml of lysis buffer and immunoprecipitated by incubating with agarose-conjugated anti-cyclin D1 (sc-450AC, Santa Cruz Biotechnology) or cyclin E rabbit IgG (sc-481, Santa Cruz Biotechnology) overnight. Immunoprecipitants were washed three times with kinase buffer (150 mM NaCl; 1 mM EDTA; 50 mM Tris-HCl, pH 7.5; and 0.1% Triton X-100) and then treated with 20 mM Tris-HCl, pH 7.5; 10 mM MgCl₂; 10 mM dithiothreitol; 1 mM ATP; and 1 mM EGTA. Resuspended complexes were incubated for 15 min at 37 °C with 0.5 μg of soluble Rb (sc-4112, Santa Cruz Biotechnology) or histone-H1 (Upstate Biotechnology, Inc.) and with 3 μg of γ-32P]ATP. Samples were analyzed by SDS-polyacrylamide gel electrophoresis, and the dried gel was exposed on film with an intensifying screen at −80 °C overnight and quantitated by densitometry.

**Statistics—** Analysis of variance with paired or unpaired t tests was performed for statistical analysis, as appropriate. Values of *p* < 0.05 were considered to be statistically significant. Data are expressed as mean ± S.E.

**RESULTS**

**PPARγ Ligands and PD98059 Block the Progression of VSMC into S Phase—** TZD PPARγ ligands TRO and RSG, a non-TZD PPARγ ligand 15d-PGJ₂, and a MEK inhibitor PD98059 all inhibited cell cycle progression, as determined by flow cytometry. Subconfluent RASMC accumulated in G₂ after serum starvation for 24 h (70.74% in G₀/G₁ phase and 16.14% in S phase; Fig. 1A). Quiescent RASMC were induced to enter S phase by stimulation with the competence factor PDGF (20 ng/ml) and a progression factor insulin (1 μM). The population of G₀/G₁ cells decreased substantially (43.91%; Fig. 1B) with a concomitant increase in RASMC in S phase (48.45%; Fig. 1B). TRO and RSG inhibited G₁ → S progression as reflected by the higher percentage of G₀/G₁ cells (65.17% in Fig. 1C and 62.14% in Fig. 1D, respectively) and by the lower percentage of S phase cells (19.59% in Fig. 1C and 21.68% in Fig. 1D, respectively). Movement of G₀/G₁ cells was also inhibited by 15d-PGJ₂ and the MAPK pathway inhibitor PD98059, with an increase in the population of G₀/G₁ cells (66.29% in Fig. 1E and 66.34% in Fig. 1F, respectively) and with a concomitant decrease in S phase cells (19.25% in Fig. 1E and 20.66% in Fig. 1F, respectively). All PPARγ ligands tested, as well as PD98059, prevented mitogen-induced G₁ → S progression in a dose-dependent manner (Fig. 1B). Inhibition of ~80% was observed at 10 μM for TRO and RSG, 5 μM for 15d-PGJ₂, and 30 μM for PD98059.

**PDGF (20 ng/ml) + Insulin (1μM)**

![Figure 1](image)

**PPARγ Ligands Inhibit Mitogen-induced Rb Phosphorylation**

Sonicated in solubilization buffer (20 mM Tris-HCl, pH 7.5; 150 mM NaCl; 1 mM EDTA; 1 mM EGTA; 1% Triton X-100; 2.5 mM sodium pyrophosphate; 1 mM sodium vanadate; 10 μg/ml each aprotinin and leupeptin; 2 mM phenylmethylsulfonyl fluoride). Cell lysates were cleared by centrifugation, and protein concentrations were determined by the Lowry assay (Bio-Rad). Cell lysates containing 50 μg of protein were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Protein was transferred electrophoretically to a nitrocellulose membrane (Hybond, Amersham Pharmacia Biotech). After blocking in 20 mM Tris-HCl, pH 7.6, containing 150 mM NaCl, 0.1% Tween 20, and 2% (v/v) non-fat dry milk, blots were incubated with specific antibodies against total Rb (14001A Pharmingen), phospho-Rb Ser-807/Ser-811 (9308S, New England Biolabs, Beverly, MA), cyclin D1 (sc-481, Santa Cruz Biotechnology), cyclin E (sc-753, Santa Cruz Biotechnology), CDK2 (sc-6248, Santa Cruz Biotechnology), CDK4 (sc-749, Santa Cruz Biotechnology), CDK6 (sc-7181, Santa Cruz Biotechnology), CDKI p27kip1 (sc-1641, Santa Cruz Biotechnology), and p21Waf1/cip1 (sc-6246, Santa Cruz Biotechnology) at 1:2000 concentration. Immunoreactive bands were visualized by incubation with peroxidase-conjugated anti-rabbit IgG or anti-mouse IgG antibody (1:1000 dilution) (Amersham Pharmacia Biotech). The antigen-antibody complexes were detected using ECL (Amersham Pharmacia Biotech). Quantification of the Western blots was done by densitometry.

**Immunocomplex Kinase Assay—** Cyclin D1-CKD complex activity and cyclin E-CDK activity were measured as described previously (20). Briefly, after appropriate treatments, cells were washed with cold PBS and solubilized on ice in lysis buffer (50 mM Tris, pH 8.0; 250 mM NaCl; 0.5% Nonidet P-40; 1 μg/ml leupeptin; and 1 mM phenylmethylsulfonyl fluoride). Insoluble materials were cleared through centrifugation at 4 °C for 10 min at 12,000 rpm. Protein concentrations were determined, and protein was suspended in 1 ml of lysis buffer and immunoprecipitated by incubating with agarose-conjugated anti-cyclin D1 (sc-450AC, Santa Cruz Biotechnology) or cyclin E rabbit IgG (sc-481, Santa Cruz Biotechnology) overnight. Immunoprecipitants were washed three times with kinase buffer (150 mM NaCl; 1 mM EDTA; 50 mM Tris-HCl, pH 7.5; and 0.1% Tween 20). CDK activities present in the immunoprecipitants were determined by reuspension in kinase buffer (50 mM Tris-HCl, pH 7.5; 10 mM MgCl₂; 10 mM dithiothreitol; 1 mM ATP; and 1 mM EGTA). Resuspended complexes were incubated for 15 min at 37 °C with 0.5 μg of soluble Rb (sc-4112, Santa Cruz Biotechnology) or histone-H1 (Upstate Biotechnology, Inc.) and with 3 μg of γ-32P]ATP. Samples were analyzed by SDS-polyacrylamide gel electrophoresis, and the dried gel was exposed on film with an intensifying screen at −80 °C overnight and quantitated by densitometry.

**Statistics—** Analysis of variance with paired or unpaired t tests was performed for statistical analysis, as appropriate. Values of *p* < 0.05 were considered to be statistically significant. Data are expressed as mean ± S.E.
tion—To elucidate the mechanism by which PPARγ ligands inhibit G1 → S progression, we examined their effect on Rb phosphorylation. Rb migrates in an SDS-polyacrylamide gel as multiple, closely spaced bands reflecting varying degrees of phosphorylation. After 24 h mitogenic stimulation with PDGF + insulin, a mobility shift of Rb was observed indicative of increased phosphorylation in RASMC. All PPARγ ligands tested, as well as PD98059, inhibited the mobility shift (Fig. 2A).

In some experiments, Rb from RASMC treated with PPARγ ligands appeared to migrate through gels with a slightly faster mobility than that observed for Rb in G0/G1-arrested cells. Enhanced mobility of Rb after PPARγ activation could result from dephosphorylation of hypophosphorylated Rb present in G0/G1 cells. To explore this finding further, we performed similar experiments using human CASMC. An advantage of using CASMC is the availability of antibodies that recognize site-specific phosphorylations on Rb. Several of these antibodies were tried, unsuccessfully, on RASMC. In CASMC, a phosphospecific antibody was used to assess the phosphorylation status of Ser-807/Ser-811 in Rb, which mediates CDK-dependent regulation of Rb function (21, 22). PPARγ ligands, as well as PD98059 at highest concentration tested, inhibited the mitogen-induced phosphorylation at Ser-807/Ser-811 (Fig. 2B, a). Importantly, even at 20 μM, TRO and RSG did not reduce Ser-807/Ser-811 phosphorylation to levels lower than that detected in G0/G1 cells (data not shown). No evidence of PPARγ ligand-induced dephosphorylation of hypophosphorylated Rb (i.e. band migrating faster than those detected in G0/G1 cells) was observed when an antibody recognizing total human Rb was used to analyze CASMC (Fig. 2B, a).

In combination, these experiments strongly suggest that activation of PPARγ, or inhibition of ERK-MAPK activity, blocks only mitogen-induced Rb phosphorylation and has no effect on its basal phosphorylation in G0/G1.

Effects of PPARγ Ligands on Expression of Early G1 Cyclins and CDKs in VSMC—To understand the mechanism by which PPARγ ligands inhibit Rb phosphorylation, we examined their effect on the expression of CDKs and their cyclin partners for which Rb is a major physiological substrate. CDK2 levels were low in quiescent cells, increased after 24 h mitogenic stimulation, and did not change with any of these compounds (Fig. 3A). Quiescent RASMC expressed both CDK4 and CDK6 which did not change after either mitogenic stimulation or treatment with any of these compounds (Fig. 3A). We next examined the effect of these compounds on protein expression of G1 phase cyclins D1 and E. Both cyclins D1 and E were expressed at low levels in quiescent RASMC and increased after 24 h stimulation with PDGF + insulin. Treatment with TRO and RSG had no effect on the induction of cyclin D1 by mitogens, whereas addition of 15d-PGJ2 and PD98059 to mitogen-stimulated VSMC attenuated induction of cyclin D1 levels by 3.7 and 68 ± 7.7% at the maximum concentration tested, respectively (p < 0.05 versus PDGF/insulin alone, Fig. 3, A and B). Mitogenic induction of cyclin E was not affected by any agent (Fig. 3A).

Effects of PPARγ Ligands on CDKI Expression in VSMC—The CDKI p27kip1 inhibits the activities of cyclin E-CDK2 and cyclin D1-CDK4 complexes (11, 12). Down-regulation of p27kip1 during G1 in response to mitogens is important for maximal activation of G1 cyclin/CDK holoenzymes (23). We therefore investigated the effect of PPARγ ligands and PD98059 on p27kip1 expression after mitogenic stimulation. Western analysis of quiescent RASMC revealed substantial p27kip1 protein. Expression of p27kip1 decreased markedly after 24 h stimulation with PDGF + insulin (PDGF + insulin alone, 32.5 ± 4.7% of quiescent cells, p < 0.01 versus quiescent cells). All PPARγ ligands and PD98059 significantly attenuated mitogen-induced down-regulation of p27kip1 (PDGF + insulin + 10 μM TRO, 63 ± 8.5% of quiescent cells; 10 μM RSG, 64 ± 8.2% of quiescent cells; 5 μM 15d-PGJ2, 75 ± 6.0% of quiescent cells; 30 μM PD98059, 57 ± 6.1% of quiescent cells; all p < 0.01 versus PDGF + insulin alone) (Fig. 4A). All tested compounds atten-
mitogenic induction of p21 (Fig. 4 A). Separate experiments. Expression of p21 cip1 kip1 treatment with PDGF (20 ng/ml) and insulin (1 μM). Cells were preincubated with TRO (10 μM), RSG (10 μM), 15d-PGJ2 (5 μM), PD98059 (30 μM), and Me2SO (control) for 30 min prior to addition of mitogens. After 24 h cyclin D1- and cyclin E-associated kinase were immunoprecipitated with anti-cyclin D1 (A) or anti-cyclin E (B) antibody from 750 (A) and 100 μg (B) of total cellular lysate. The kinases dependent on cyclin D1 (A) and anti-cyclin E (B) assays were performed as described under “Experimental Procedures” four times with similar results.

FIG. 5. PPARγ ligands and PD98059 inhibit cyclin D1- and cyclin E-dependent kinase activities. Quiescent VSMC were stimulated with PDGF (20 ng/ml) and insulin (1 μM) and inhibited mitogen-stimulated induction of p21 cip1 kip1 (both p < 0.05 versus PDGF + insulin alone; Fig. 4C). This inhibition was dose-dependent for both ligands (Fig. 4D). In contrast, 15d-PGJ2 and PD98059 had no effect (Fig. 4C).

Effects of PPARγ Ligands and PD98059 on Cyclin D1-associated CDK and Cyclin E-associated CDK Activities—To determine whether various PPARγ ligands and PD98059 can regulate CDK activity, we measured the effects of these compounds on mitogen-stimulated cyclin D1-associated and cyclin E-associated CDK, respectively. By using glutathione S-transferase-Rb fusion protein and purified histone H1 proteins as substrates for cyclin D1-associated CDK and cyclin E-associated CDK, we found that stimulation with PDGF (20 ng/ml) + insulin (1 μM) increased activity of both CDKs (Fig. 5, A and B). All these PPARγ ligands and PD98059 inhibited the induction of cyclin D1-dependent kinase activity (PDGF + insulin + 10 μM TRO, 58 ± 3.7% inhibition, p < 0.01 versus PDGF + insulin alone; 10 μM RSG, 53 ± 4.5% inhibition, p < 0.05 versus PDGF + insulin alone; 5 μM 15d-PGJ2, 73 ± 4.6% inhibition, p < 0.01 versus PDGF + insulin alone; 30 μM PD98059, 77 ± 7.4% inhibition, p < 0.01 versus PDGF + insulin alone) (Fig. 5A). Similarly, all the compounds inhibited the induction of cyclin E-dependent kinase activity (10 μM TRO, 73 ± 7.3% inhibition; 10 μM RSG, 67 ± 7.8% inhibition; 5 μM 15d-PGJ2, 82 ± 8.0% inhibition; 30 μM PD98059, 87 ± 9.4% inhibition; all p < 0.01 versus PDGF + insulin alone) (Fig. 5B).

**DISCUSSION**

The principal finding of this study is that PPARγ ligands inhibit VSMC proliferation by attenuating the activity of several key cell cycle regulators that control G1 → S progression. All PPARγ ligands prevented mitogen-induced phosphorylation of Rb by inhibiting cyclin D1- and cyclin E-dependent kinase activity. Attenuation of mitogen-induced p21 cip1 kip1 degradation...
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dation by PPARγ ligands is likely the major mechanism ultimately resulting in the inhibition of Rb phosphorylation. Indeed, depletion of p27kip1 by antisense oligodeoxynucleotides promotes cell growth (28), and mice with targeted disruption of the p27kip1 gene have enhanced growth with enlargement of the pituitary, adrenals, and gonads (29, 30). A recent study demonstrated that overexpression of p27kip1 inhibited serum-stimulated DNA synthesis in VSMC (28). Furthermore, in a porcine balloon-injury model, p27kip1 demonstrated that overexpression of p27kip1 in PDGF-stimulated fibroblasts requires its phosphorylation by cyclin E-CDK2 (41). We observed that all PPARγ ligands inhibited cyclin E-dependent kinase activity, which may be the mechanism by which they attenuate p27kip1 degradation. This effect could result from an inhibition of mitogen-induced ERK signaling by PPARγ as we previously identified. Alternatively, PPARγ may regulate p27kip1 turnover through a novel action to block Rho signaling. However, we found that PPARγ activation did not decrease mitogen-enforced Rho protein levels. In addition, it is unlikely that a nuclear receptor like PPARγ would affect Rho movement from the cytosol to the plasma membrane, which is important for activation of the Rho pathway (43). Additional experiments are required to establish whether the Rho pathway is targeted by PPARγ.

Quiescent VSMC expressed high levels of p27kip1, but p21cip1 was not detectable. Mitogenic stimulation with the combination of PDGF and insulin increased expression of p21cip1 during G1 → S transition. Up-regulation of p21cip1 during G1 at first glance is paradoxical, given that it and other CDKIs function to regulate negatively cyclin-CDK activity. Several recent reports, however, have revealed that CDKI modulation of the cell cycle is complex and involves both positive and negative regulation by CDKIs (23–26, 44). Threshold levels of p21cip1 have been shown to be required for the formation of functional cyclin D1-CDK4 complexes (44). Higher concentrations of p21cip1, however, inhibited cyclin D1-CDK4 activity consistent with its more traditionally recognized CDKI function (44). The potential for p21cip1 to regulate CDKs positively is supported by a recent genetic study showing that primary mouse embryonic fibroblasts from p27kip1/p21cip1 double knockout animals failed to assemble detectable amounts of cyclin D1-CDK complexes (27). At physiological concentration (10 μM), the PPARγ ligands TRO and RSG inhibited mitogen-induced p21cip1, but 15d-PGJ2 and the MAPK pathway inhibitor had no effect. Since we saw similar effects of TRO and RSG to inhibit p21cip1 induction, it is possible that this also plays a role in the inhibition of G1 → S transition by PPARγ. PPARγ blockade of p21cip1 induction by mitogens may result from the ability of these nuclear receptors to inhibit the function of transcription factors, such as NFκB, STAT, and AP-1, via the mechanism of transrepression (45, 46).

Recently, Morrison and Farmer (47) showed that activation of ectopically expressed PPARγ in 3T3-L1 fibroblasts inhibited their growth and promoted their differentiation into adipocytes, which was associated with a concomitant increase in mRNA and protein for CDKIs, p18ink4c and p21cip1. There was no effect of PPARγ activation on p27kip1 in these cells. Inhibition of cell cycle progression frequently is a prerequisite to terminal differentiation. Regulatory mechanisms causing cell cycle arrest during differentiation, however, appear to differ from those governing the exit of quiescent cells from G1 → S. For example, during adipocyte differentiation PPARγ-mediated growth arrest did not require a functional pRB (48). In contrast, our data strongly implicate PPARγ-dependent inhibition of Rb phosphorylation as the mechanism by which PPARγ ligands prevent quiescent VSMC from exiting G1. PPARγ ligands also inhibit growth of tumor cells (49–51) and growth of endothelial cells to prevent angiogenesis (52). The impact of

2 S. Wakino, U. Kintscher, S. Kim, F. Yin, W. A. Hsueh, and R. E. Law, unpublished results.
PPARγ ligands on the cell cycle in these cell types remains to be determined.

Recent studies have illustrated the feasibility of targeting specific cell cycle regulators in cardiovascular cells as an alternative antiproliferative therapy (53). A wide range of antiproliferative drugs has been tested as means to prevent restenosis and vein graft neointimal formation. One alternative to drug therapy is the use of modified viruses designed to carry a cell cycle regulatory gene directly into the arterial wall. Infection of porcine femoral or rat carotid arteries with an adenoviral vector designed to express a nonphosphorylatable, constitutively active form of Rb inhibited neointima formation in animal balloon-injury models (54). Our recent studies have shown that TRO inhibited intimal hyperplasia after balloon injury of aortae in the rat (18). The present study suggests that the prevention of the reduction of p27kip1 levels by TRO in vivo may contribute, at least in part, to its activity to inhibit the vascular injury response. The observation that PPARγ ligands inhibit important cell cycle processes activated by growth factors produced in response to vascular damage may provide a new oral therapeutic approach for proliferative vascular disease such as restenosis and atherosclerosis.

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