Retinoids Inhibit Proliferation of Human Coronary Smooth Muscle Cells by Modulating Cell Cycle Regulators

Shu Wakino, Ulrich Kintscher, Sarah Kim, Simon Jackson, Fen Yin, Sunil Nagpal, Roshantha A.S. Chandraratna, Willa A. Hsueh, Ronald E. Law

Abstract—Retinoids inhibit rat vascular smooth muscle cell (VSMC) proliferation in vitro and intimal hyperplasia in vivo. We examined the mechanism of the antiproliferative effect of retinoids on human coronary artery smooth muscle cells (human CASMCs). The RAR ligands all-trans-retinoic acid (atRA) and ethyl-p-[(E)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)-l-propenyl]-benzoic acid (TTNPB); a pan-RXR/RAR agonist, 9-cis-retinoic acid (9cRA); and the RXR-selective ligand AGN4204 all inhibited DNA synthesis stimulated with platelet-derived growth factor and insulin (IC50: TTNPB 63 nmol/L, atRA 120 nmol/L, AGN4204 460 nmol/L, 9cRA 1.5 μmol/L). All retinoids blocked cell cycle progression as determined by flow cytometry and inhibited retinoblastoma protein (Rb) phosphorylation. TTNPB, atRA, and AGN4204 inhibited the mitogenic induction of cyclin D1, whereas 9cRA had no effect. None of the retinoids affected the expression of CKD 2, 4, or 6 or cyclin E. All retinoids attenuated mitogen-induced downregulation of CDKI p27kip1, a major negative regulator of Rb phosphorylation, partly through stabilizing p27kip1 turnover. These data demonstrate that retinoids have antiproliferative activity by modulating G1 → S cell cycle regulators in human CASMCs through inhibition of Rb phosphorylation and elevation of p27kip1 levels. (Arterioscler Thromb Vasc Biol. 2001;21:746-751.)

Key Words: retinoid ■ human coronary smooth muscle cell ■ p27kip1, Rb ■ cell cycle

Mechanical injury to the vessel wall induces the dedifferentiation, growth, and migration of medial smooth muscle cells (SMCs), which results in the formation of a neointima.1 Proliferation of vascular SMCs (VSMCs) plays a critical role in the development of restenosis after coronary angioplasty and in the progression of fatty streaks to atherosclerotic plaques.2,3 After injury to the endothelium, VSMCs migrate into the intimal layer of the arterial wall, where they transition from a quiescent state and reenter the cell cycle in response to growth factors and cytokines.1,4 Progression through the mammalian mitotic cycle is coordinated by expression and/or activation of multiple holoenzymes composed of a catalytic cyclin-dependent kinase (CDK) and a cyclin-regulatory subunit.5 Different cyclin/CDK complexes are temporally activated at specific phases of the cell cycle. Progression through the first gap phase (G1) requires cyclin D–dependent kinase (CDK4 and CDK6) and cyclin E/CDK2 activity.5 Functional cyclin A/CDK2 is assembled and activated in late Gi and during DNA synthesis (S phase).5 The kinase activity of the cyclin/CDK complexes can be negatively regulated by CDK-inhibitory proteins (CDKI), including p15INK4b, p16INK4a, p21cip1, and p27kip1.6 Cyclin/CDK holoenzymes phosphorylate the retinoblastoma tumor suppressor protein (Rb), resulting in inactivation of Rb and release of sequestered E2F transcription factors.7 E2F induces the transcription of genes encoding proteins that are required for S-phase DNA synthesis.8 These proteins have been shown to be upregulated in several animal models of angioplasty.9

All-trans-retinoic acid (atRA), a ligand for the nuclear receptor retinoic acid receptor (RAR), inhibits VSMC proliferation in vitro and neointima formation after mechanical injury.10–16 9-cis-Retinoic acid (9cRA), a pan-agonist for RAR and retinoid X receptor (RXR) families of nuclear receptors, also inhibits VSMC proliferation.10,12 The molecular mechanism by which retinoids exert their antiproliferative activity toward SMCs, however, has not been elucidated. Moreover, because VSMCs express at least 5 of the 6 isoforms that compose the RAR and RXR families,10 it is not clear which retinoid receptor(s) mediates the inhibition of VSMC growth. The purpose of the present study was to determine the mechanism by which retinoids inhibit VSMC proliferation by examining their effect on cell cycle regulators in human coronary artery SMCs (human CASMCs).

Methods

Platelet-derived growth factor (PDGF)-BB, DMSO, atRA, 9cRA, ethyl-p-[(E)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)-l-}
propenyl]-benzoic acid (TTNPB), and cycloheximide were purchased from Sigma Chemical Co. AGN4204 was kindly provided by Allergan Inc. Human recombinant insulin was obtained from Lilly, Bromodeoxyuridine (BrdU) labeling and detection kit II was from Boehringer Mannheim. Hybond enzyme-linked chemiluminescence nitrocellulose membrane, horseradish peroxidase–linked anti-rabbit and anti-mouse antibodies, and enzyme-linked chemiluminescence Western blotting detection reagents were from Amersham Pharmacia Biotech. Human CASMCs and SMC growth medium-2 (SmGM-2) were obtained from Clonetics.

**Cell Culture**

Early passage (fourth to ninth) human CASMCs were grown to 60% to 70% confluence in SmGM-2 containing 5% FBS, 2 ng/mL human basic fibroblast growth factor, 0.5 ng/mL human epidermal growth factor, 50 µg/mL gentamicin, 50 ng/mL amphotericin-B, and 5 µg/mL bovine insulin. For all data shown, individual experiments were performed from separate vials of CASMCs.

**Measurement of DNA Synthesis**

Incorporation of the thymidine analogue BrdU was measured as previously described. Human CASMCs were plated at 3.0×10⁴ cells on 24-well plates, and after serum starvation (0.4% serum) for 48 hours, cells were stimulated with growth factors (PDGF 20 ng/mL + insulin 1 µmol/L [P+I]) in the presence or absence of retinoids for the next 20 hours. Then 15 µmol/L BrdU was added, and incubation was continued for another 4 hours. Cell nuclei incorporating BrdU appeared brown and were counted in high-power fields. Data were based on 6 different experiments from 3 separate vials of CASMCs.

**Cell Cycle Distribution**

Flow cytometry was performed to analyze cell cycle distribution. Quiescent human CASMCs were pretreated for 30 minutes with each compound or vehicle (DMSO), followed by the addition of growth factors. After 24 hours, cells were stained and processed as previously described. DNA histogram analysis was performed with ModFitLT software (Becton Dickinson). Experiments were repeated at least 3 times.

**Western Blots**

Preparation of cell lysates and Western immunoblotting were performed as previously described. Specific antibodies against phospho-Rb Ser 807/811 (sc-481, Santa Cruz Biotechnology), cyclin D1 (sc-481, Santa Cruz), cyclin E (sc-753, Santa Cruz), cyclin A (sc-751, Santa Cruz), CDK 2 (sc-6248, Santa Cruz), CDK 4 (sc-749, Santa Cruz), CDK 6 (sc-7181, Santa Cruz), and CDKPI P27Kip1 (sc-1641, Santa Cruz) were used at a 1:200 concentration. Specific antibodies against RAR-α (sc-481, Santa Cruz), RAR-β (sc-753, Santa Cruz), RAR-γ (sc-751, Santa Cruz), RXR-α (sc-6248, Santa Cruz), RXR-β (sc-749, Santa Cruz), RXR-γ (sc-7181, Santa Cruz), elastin (clone BA-4, Sigma), and osteopontin (AF8108, R&D Systems Inc) were used at a 1:100 concentration. The Western blots were quantified by densitometry.

**Statistics**

ANOVA was performed for statistical analysis. Values of P<0.05 were considered to be statistically significant. Data were expressed as mean±SEM.

**Results**

**Phenotypic Characterization of Human CASMCs**

VSMCs isolated from tissue spontaneously change their phenotype when cultured in vitro. Quiescent medial VSMCs display a “contractile” phenotype characterized by high-level expression of contractile apparatus–associated proteins. When VSMCs switch from the contractile to the “synthetic” phenotype, levels of contractile proteins decrease, whereas genes that contribute to extracellular matrix production are upregulated. Human CASMCs expressed 2 markers of the synthetic phenotype, osteopontin and elastin, at relatively constant levels from passages 4 to 9 (data not shown). As in previous studies in rat VSMCs, human CASMCs expressed 5 of the 6 retinoid receptor subtypes, with only RXR-γ absent (data not shown).

**Retinoids Inhibit Mitogen-Induced DNA Synthesis in Human CASMCs**

The results given in Figure 1 show that all retinoids tested inhibited DNA synthesis in quiescent human CASMCs stimulated with P+I for 24 hours. All retinoids attenuated DNA synthesis in a dose-dependent manner with IC₅₀ of 63 nmol/L for TTNPB, 120 nmol/L for atrA, 460 nmol/L for AGN4204, and 1.5 µmol/L for 9cRA. Significant inhibitory effects were observed at 20 nmol/L for atrA and TTNPB and at 200 nmol/L for 9cRA and AGN4204. The RAR-selective ligands TTNPB and atrA were more potent inhibitors of DNA synthesis than the RXR-selective ligands AGN4204 and 9cRA. Interestingly, whereas AGN4204 had modest inhibitory effects at concentrations of 2 and 20 nmol/L (10±9.2% and 18±8.7% inhibition, respectively), enhanced antimitogenic activity was observed at 200 nmol/L and 2 µmol/L (42±10% and 65±13% inhibition, respectively), which approached those of atrA and TTNPB.

**Retinoids Block the Progression of Human CASMCs Into S Phase**

All retinoids inhibited cell cycle progression at the highest concentration tested (2 µmol/L) as determined by flow cytometry (Figure I, please see http://www.atvb.ahajournals.org). Subconfluent human CASMCs accumulated in G₁ after serum starvation for 24 hours (85.8% in G₀/G₁ phase and 10.2% in S phase, Figure I-A). Quiescent CASMCs were induced to enter S phase by stimulation with the competence factor PDGF (20 ng/mL) and a progression factor insulin (1 µmol/L [P+I]). The population of G₀/G₁ cells decreased substantially (70.6%, Figure I-B), with a concomitant increase in CASMCs in S phase (26.4%; Figure I-B). The RAR ligands TTNPB and atrA inhibited G₁→S progression, as reflected by the higher percentage of G₀/G₁ cells and by the lower percentage of S-phase cells.
Retinoids Inhibit Mitogen-Induced Rb Phosphorylation in Human CASMCs

To elucidate the mechanism by which retinoids inhibit the proliferation of human CASMCs, we examined their effect on Rb phosphorylation, because hyperphosphorylation of Rb is required for G1→S progression in most mammalian cells.7 Phospho-specific antibodies were used to assay the phosphorylation status of Ser 807/811 in Rb, which are important CDK-dependent functional sites in this G1 regulatory protein.20 All retinoids inhibited the mitogen-induced phosphorylation at Ser 807/811. TTNPB and atRA attenuated Rb phosphorylation at 20 nmol/L (46±6.6% and 33±9.4% inhibition, respectively, P<0.05 versus P+I alone, Figure 2A and 2B) to 2 μmol/L (86±5.4% and 71±8.6% inhibition, respectively, P<0.01 versus P+I alone, Figure 2A and 2B). AGN4204 had a modest inhibitory effect at 200 nmol/L (38±9.7% inhibition, P<0.01 versus P+I alone, Figure 2C) but substantially inhibited Rb phosphorylation at 2 μmol/L (68±8.0% inhibition: P<0.01 versus P+I alone, Figure 2C). 9cRA attenuated the phosphorylation of Rb significantly only at 2 μmol/L (48±17% inhibition, P<0.05 versus P+I alone, Figure 2D). These data are concordant with the potency of each retinoid to inhibit DNA synthesis and cell cycle progression, which suggests that their antiproliferative activity results from the prevention of Rb phosphorylation required for transit from G1 into S phase. Similar results were obtained with 10% serum used as a mitogen (Figure III, please see http://www.atvb.ahajournals.org).

Effects of Retinoids on G1 Cyclins and CDK Expression in Human CASMCs

To understand the mechanism by which retinoids 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 hours of mitogenic stimulation, and did not change with any of these compounds (data not shown). Quiescent human CASMCs expressed both CDK4 and CDK6, which did not change after either mitogenic stimulation or treatment with any of these compounds (data not shown). Cyclin D1 was expressed at low levels in quiescent CASMCs and increased 2.0±0.1-fold after 24 hours of stimulation with P+I (Figure IV, please see http://www.atvb.ahajournals.org). The RAR ligands TTNPB and atRA both inhibited the mitogenic induction of cyclin D1 protein (TTNPB: 2 nmol/L, 63±9.3% inhibition; 2 μmol/L, 79±11% inhibition, both P<0.01 versus P+I alone; atRA: 200 nmol/L, 58±7.0% inhibition, P<0.05 versus P+I alone; 2 μmol/L, 72±2.9% inhibition, P<0.01 versus P+I alone, Figure IV-A and IV-B). In contrast, RXR ligands had minimal effect on cyclin D1 expression. AGN4204 inhibited the induction of cyclin D1 only at 2 μmol/L, the highest concentration tested (71±9.7% inhibition, P<0.05 versus P+I alone, Figure IV-C), whereas 9cRA had no effect (Figure IV-D). None of the retinoids affected the mitogenic induction of cyclin E (data not shown).

Cyclin A is essential for cell cycle progression in S phase, and its expression is regulated through the E2F transcription factor, whose activity is controlled by the phosphorylation status of Rb. In quiescent human CASMCs, low levels of cyclin A protein were detected, which increased 3.7±0.4-fold after mitogenic stimulation for 24 hours. Consistent with the inhibitory effects on Rb phosphorylation, each retinoid attenuated the induction of cyclin A (Figure V, please see http://www.atvb.ahajournals.org)

Retinoids Prevent Mitogen-Induced Downregulation of p27kip1 in Human CASMCs

The CDKI p27kip1 inhibits the activities of cyclin E/CDK2 and cyclin D1/CDK4 complexes.21 Downregulation of p27kip1 during G1 in response to mitogens is important for maximal activation of G1 cyclin/CDK holoenzymes.9 We therefore investigated the effect of these compounds on p27kip1 expression after mitogenic stimulation of human CASMCs with P+I. Quiescent G0/G1 CASMCs express substantial levels of p27kip1 protein. Expression of p27kip1 decreased markedly after 24 hours of stimulation with P+I. Each retinoid tested significantly attenuated mitogen-induced downregulation of p27kip1. TTNPB and atRA attenuated mitogen-induced downregulation of p27kip1 at 200 nmol/L to 2 μmol/L (TTNPB: 200 nmol/L, 88±5.3% of quiescent cells; 2 μmol/L, 89±1.2% of quiescent cells, both P<0.01 versus P+I alone; atRA: 200 nmol/L, 91±6.8% of quiescent cells; 2 μmol/L, 92±8.8% of
Retinoids prevent mitogen-induced downregulation of CDK1 p27\textsuperscript{kip1} in human CASMCs. Cells were treated as described in Figure 1. After 24 hours, whole-cell proteins (35 μg) were assayed by Western immunoblotting with anti-p27\textsuperscript{kip1} antibody (top). Each blot is representative of 3 separate experiments. Densitometric analysis of immunoblots is shown as % of quiescent cells (bottom). Results are presented as mean±SEM (n=3), *P<0.05, **P<0.01 vs mitogen-stimulated cells.

To delineate the mechanism by which retinoids increased p27\textsuperscript{kip} levels, we examined its turnover in mitogen-stimulated human CASMCs. After treatment of cells with P+1 plus or minus retinoids for 6 hours to permit a determination of p27\textsuperscript{kip} half-life, cycloheximide (20 μg/mL) was added to inhibit de novo protein synthesis. Selection of this protocol was based on the previous observation by Miano et al\textsuperscript{10} that antiproliferative activity of retinoids was exerted within the first 6 hours after mitogenic stimulation. Steady-state levels of p27\textsuperscript{kip} protein were determined by Western blot at various times after inhibition of de novo protein synthesis (Figure 4A). The half-life of p27\textsuperscript{kip} protein in mitogen-stimulated cells was calculated at 10.7 hours, which is similar to that reported by Servant et al\textsuperscript{22} for PDGF-stimulated rat aortic SMCs. In contrast, pretreatment with retinoids substantially prolonged the half-life of p27\textsuperscript{kip} (44.9 hours for TTNPB, 35.1 hours for atRA, 25.9 hours for AGN4204, and 19.7 hours for 9cRA; Figure 4B). In addition, retinoid-treated VSMCs accumulated higher-molecular-weight, putatively ubiquitinated forms of p27\textsuperscript{kip} (indicated by small arrows in Figure 4A).

Levels of CDK1 p15\textsuperscript{INK4b} or p16\textsuperscript{INK4a} did not change after either mitogenic stimulation or retinoid treatment (data not shown). In marked contrast to p27\textsuperscript{kip}, mitogenic stimulation of human CASMCs increased levels of the CDKI p21\textsuperscript{Cip1}, which was attenuated by retinoids (data not shown). Mitogenic induction of p21\textsuperscript{Cip1} has been observed in a variety of mammalian cell lineages, but its relevance to cell cycle progression is controversial.\textsuperscript{23}

These results suggest that all retinoids block G\textsubscript{1}→S transition of CASMCs and Rb phosphorylation, at least in part, through their effect to antagonize p27\textsuperscript{kip} downregulation after exposure of human CASMCs to mitogens.

**Discussion**

Proliferating mammalian cells pass through several cell cycle checkpoints, mainly G\textsubscript{1}→S and G\textsubscript{2}→M transitions. In this study, we demonstrated that all retinoids block cell cycle progression at the G\textsubscript{1}→S transition, attenuate Rb phosphorylation, and upregulate p27\textsuperscript{kip} levels. In addition, RAR-selective but not RXR-selective retinoids were potent inhibitors of mitogen-induced cyclin D1. Targeting G\textsubscript{1} cell cycle regulators by retinoids supports the prior observation of Miano et al\textsuperscript{10} that the antiproliferative activity of atRA was substantially diminished when its administration to quiescent rat VSMCs was delayed 6 to 12 hours (ie, G\textsubscript{1} phase) after mitogenic stimulation with PDGF.\textsuperscript{10}

Hyperphosphorylation of Rb by G\textsubscript{1} cyclin/CDKs is required for cell to enter S phase.\textsuperscript{7} Rb contains multiple CDK phosphorylation sites that regulate its conformation and ability to bind other cell cycle regulatory protein, such as E2F.\textsuperscript{24} Of the 16 potential CDK phosphorylation sites in Rb, Ser 807/811 has been shown to play a critical role in the growth-suppressive function of Rb.\textsuperscript{20,24} Consistent with their activity to inhibit G\textsubscript{1} exit of human CASMCs, retinoids blocked mitogen-induced phosphorylation of Ser 807/811 in Rb.
To elucidate the mechanism by which retinoids inhibit mitogen-induced Rb phosphorylation, we examined their effect on the expression of G1 cyclins and CDKs, which, as holoenzymes, phosphorylate Rb. Previous studies have shown that a variety of growth factors induce expression of cyclin D, the G1 cyclin that partners with CDK4 or CDK6, which is essential for cell proliferation. The RAR-selective ligands atRA and TTNPB potently inhibited cyclin D1 induction by P+I. In marked contrast, RXR-selective ligands had either a modest effect (AGN4204) or no effect (9cRA) on the mitogenic induction of cyclin D1. These data are consistent with an RAR pathway–dependent mechanism for inhibiting cyclin D1 expression. Selective targeting of cyclin D1 expression by RAR-selective ligands may account for their having a greater antiproliferative activity than RXR-selective ligands in human CASMCs. Transcriptional activation of the cyclin D promoter by growth factors is mediated, at least in part, through the AP-1 binding site at −954, which could be negatively regulated by RAR transrepression of AP-1 activity. Blockade of cell cycle progression by retinoids, however, cannot be fully explained by the effect on cyclin D1 because RXR-selective ligands inhibited human CASMC proliferation at concentrations that had no effect on mitogen-induced upregulation of cyclin D1.

Another logical target for the antiproliferative activity of retinoids is CDKIs, which negatively regulate the activity of multiple cyclin/CDK complexes. The CDKI p27^kip1 plays a pivotal role in the control of cell proliferation by inhibiting both CDK2 and CDK4 activity. Overexpression of p27^kip1 causes cell cycle arrest in G1 phase in both vascular and nonvascular cells. Quiescent VSMCs express high levels of p27^kip1. After mitogenic stimulation, p27^kip1 levels decline precipitously and VSMCs enter S phase to initiate DNA synthesis. As in those studies, we showed that levels of p27^kip1 substantially decreased 24 hours after mitogenic stimulation of human CASMCs. Treatment with either RAR-selective or RXR-selective retinoids attenuated the mitogen-induced downregulation of p27^kip1 and restored levels of this CDKI approaching that observed in quiescent CASMCs. The rank order potency of retinoids for preventing p27^kip1 downregulation closely mirrored their activity to inhibit DNA synthesis with atRA. Treatment with either RAR-selective or RXR-selective retinoids to antagonize p27^kip1 downregulation by mitogens.

Although not explored in the present investigation, retinoids may also affect transcriptional regulation of p27^kip1 as an additional mechanism for elevating levels in human CASMCs. In rat aortic VSMCs, PDGF induces a rapid and transient inhibition of p27^kip1 gene transcription, in addition to activating the proteasome degradation pathway. Activation of the p27^kip1 promoter by atRA or 9cRA has been observed during retinoid-mediated neuronal differentiation of human neuroblastoma tumor cells and mouse embryonal carcinoma cells. Direct induction of p27^kip1 transcription by retinoids through a cognate retinoic acid response element (RARE), however, has not been reported. Further investigations are necessary to define interactions between mitogenic and retinoid receptor signaling pathways that regulate p27^kip1 transcription during G1 phase of the cell cycle.

Recent studies in a variety of animal models have demonstrated a beneficial effect of retinoids on vascular remodeling in response to injury. AtRA suppressed neointimal formation in rat carotid arteries after balloon catheterization and restenosis after angioplasty in atherosclerotic femoral arteries in rabbits. Intimal hyperplasia in rabbit after interposition of jugular vein bypass grafts to the carotid artery was also substantially attenuated atRA. Inhibition of intimal thickening by atRA was hypothesized to result from decreased VSMC proliferation and migration. Reduced BrdU incorporation or lower proliferating cell nuclear antigen labeling indices provided additional evidence for impaired cell cycle progression by SMCs in the injured vessel wall. The present study provides a mechanistic explanation for the observed in vivo antiproliferative activity of RAR-selective retinoids. Tanner et al documented a rapid and sharp decline in p27^kip1 protein levels after mechanical injury of porcine femoral arteries. No significant changes in other CDKIs were observed until much later times postinjury, when substantial neointima had already formed. Elevation of p27^kip1 levels by activating RAR receptors, therefore, may block one of the earliest steps in the injury response prerequisite for VSMC proliferation. Interestingly, the macrolide antibiotic rapamycin, which inhibits VSMC proliferation by blocking the G1→S transition, was recently shown to reduce intimal thickening in porcine coronary arteries after angioplasty by increasing p27^kip1 levels and lowering Rb phosphorylation in vessels.

We observed that 9cRA, which can activate both RAR and RXR, and the RXR-selective agonist AGN4204 are less potent inhibitors of human CASMC proliferation than RAR-selective retinoids. The marked difference in the antiproliferative activity of these 2 major retinoid receptor subtypes may be due to the lack of a substantial effect of RXR-selective compounds on mitogen-induced cyclin D1 expression. By comparison to studies testing RAR-selective retinoids, there is scant evidence that activated RXR confers vascular protection in vivo. Inferential support for RXR vascular activity is provided by our recent findings that activation of peroxisome proliferator–activated receptor-γ (PPAR-γ), which heterodimerizes with RXR-α, inhibits G1→S progression in rat VSMCs. In accordance with our present findings for RXR-selective retinoids, PPAR-γ ligands also attenuated Rb hyperphosphorylation and p27^kip1 downregulation and had no effect on cyclin D1. Moreover, PPAR-γ ligands suppress neointimal formation after injuring the endothelium in rats. Thus, although only atRA has been tested and shown to inhibit intimal hyperplasia in an animal model of vessel wall injury, similar studies are warranted for...
RXR-selective retinoids based on the in vivo activity of PPAR-γ ligands. Significant differences in the vascular activity of RXR-α versus PPAR-γ ligands, however, would not be surprising, because RXR-α is a promiscuous partner for multiple nuclear receptors (vitamin D receptor, thyroid hormone receptor, PPAR-α, etc.).

A wide variety of antiproliferative drugs are currently being investigated for the prevention of restenosis. Adenoviral expression vectors have been used to deliver a cell cycle regulatory gene directly into the arterial wall. Infection of porcine femoral or rat carotid arteries with an adenoviral vector expressing a nonphosphorylatable, constitutively active form of Rb inhibited neointimal formation in animal balloon-injury models. Similarly, adenoviral overexpression of p27Kip1 but not p16INK4a markedly attenuated intimal hyperplasia after balloon injury of porcine femoral arteries. 

In the present study, we demonstrated that the antiproliferative activity of retinoids toward VSMCs resulted from their perturbing the function of the key cell cycle regulators, Rb, cyclin D1, and p27Kip1. By modulating these cell cycle molecules in human coronary arterial cells, natural retinoids or synthetic analogues may provide a novel therapeutic treatment for proliferative vascular diseases.

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References

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