The successful management of vascular occlusive diseases, particularly those due to procedural interventions for preexisting atherosclerosis, will likely require the targeting of numerous cellular processes rather than any 1 gene, protein, or signaling pathway. This fact is underscored by the failure of virtually all clinical trials employing pathway- or factor-specific therapies to limit restenosis after percutaneous vascular interventions. Retinoids are natural and synthetic derivatives of vitamin A that exert myriad effects on such cellular processes as growth, apoptosis, differentiation, and migration. As such, retinoids could be potential therapeutics to test in the context of vascular occlusive disease. Clinically, the prototypic natural retinoid, all-trans retinoic acid (atRA), effects a nearly 100% rate of remission in patients with acute promyelocytic leukemia. Given the similarities in the pathogenesis of neoplasia and vascular occlusive lesions (ie, increased cell growth and loss of cellular differentiated properties), it is surprising that retinoids have only recently been examined with respect to cells of the vascular wall.

Many retinoids exert their pleiotropic effects through the binding and activation of nuclear retinoid receptors. There are 2 families of retinoid receptors, each of which comprises 3 distinct genes. The retinoic acid receptors (RAR α, β, and γ) bind atRA and its 9-cis stereoisomer (9cRA), whereas the more weakly expressed retinoid X receptors (RXR α, β, and γ) bind 9cRA. Numerous retinoids have been synthesized and tested for receptor selectivity as a means of reducing the side effects associated with natural retinoid therapy. Many of these synthetic retinoids have recently found clinical utility for a number of diseases. Ligand-activated retinoid receptors dimerize (preferentially as an RAR-RXR heterodimer), recognize, and bind cis elements (called retinoic acid–response elements, or RAREs) in the genome to activate gene transcription. Identifying retinoid-responsive target genes is critical in defining the molecular actions of these potent, biological response modifiers, although other posttranscriptional processes are likely to play a role as well (see below).

In recent years, retinoids have been examined for their influence on vascular smooth muscle cell (SMC) growth and differentiation, inasmuch as these processes are thought to be of some relevance in the pathogenesis of vascular occlusive disease. Thus, there is a growing body of in vitro data demonstrating that retinoids antagonize growth factor–stimulated SMC hyperplasia while in some cases promoting a more differentiated SMC phenotype. Because cultured SMCs and the aorta express most of the retinoid receptors and display retinoid receptor activity in vitro, it is hypothesized that these observed effects on SMC phenotype are related to retinoid receptor–mediated changes in the SMC transcriptome. Indeed, studies using retinoid receptor–selective agonists with reduced toxicity have shown inhibitory effects on SMC growth.

Collectively, these in vitro data prompted a series of in vivo studies that examined the effects of atRA and other retinoids on the vessel wall’s response to injury. Remarkably, all in vivo studies to date have documented desirable changes in vessel wall geometry with retinoid administration after vascular injury. Such changes in vessel geometry include an attenuation in neointimal mass, an outward remodeling of the vessel wall, and accelerated reendothelialization. The decrease in neointimal mass was shown in most of the aforementioned studies to be associated with reduced SMC DNA synthesis. At this point in time, we have very little insight into the mechanisms through which retinoids exert these desirable effects on SMC growth and neointimal mass.

In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Wakino et al examined the effects of retinoids on PDGF/insulin-stimulated HCASMC growth, a finding that is consistent with another recent study showing 9cRA inhibition of HCA-SMC growth. Flow cytometry experiments were then performed that revealed a significant decrease in the number of cells entering the S phase, which suggested that retinoids might be interfering with the cell cycle machinery. These results are similar to those of a previous study, which suggested that early G₁ events in SMCs might be the targets of retinoids and their activated receptors. Accordingly, Wakino et al examined the effects of retinoids on PDGF-induced phosphorylation of the retinoblastoma (RB) protein, inasmuch as RB phosphorylation represents a pivotal, early cell-cycle progression event. Under normal quiescent conditions, RB is hypophosphorylated, which promotes seques-
tration of the E2F family of transcription factors. On growth stimulation, the RB protein is phosphorylated (on some 16 serine-threonine residues) via several cyclin/cyclin-dependent kinase (cdk) complexes, resulting in the release of E2F. Transcriptionally active E2F stimulates expression of growth-related genes such as c-myc, cyclins A and E, DNA polymerase-α, and thymidine kinase.21 Wakino et al18 showed that each retinoid could dose-dependently inhibit RB phosphorylation after PDGF stimulation, although a corresponding decrease in E2F-dependent transcription (eg, decreased c-myc expression) was not studied. Importantly, there was a strong correlation between each retinoid’s ability to inhibit SMC growth and block RB phosphorylation, suggesting that the inhibition of SMC growth was a result of RB hypophosphorylation.

Several cyclin/cdk complexes actively phosphorylate RB, including cyclin D/cdk4/6, cyclin A/cdk2, and cyclin E/cdk2.21 Wakino et al18 showed that 2 pan-RAR agonists (atRA and TTNBP) decreased protein expression of cyclin D1. An RXR-selective agonist (AGN4204) inhibited cyclin D1 only at the highest concentration, whereas a pan-RAR/RXR agonist (9cRA) had no effect on cyclin D1 levels. These results contrast with those of Chen and Gardner,9 who showed that atRA and TTNBP-induced cyclin D1 at both the mRNA and protein level in aortic SMCs. The reasons for these divergent data probably relate to the cell type (coronary versus aortic SMCs, which represent 2 distinct SMC lineages) as well as species differences. All retinoids tested inhibited cyclin A protein levels.18 Cyclin A is an E2F-dependent gene, so it will be informative to assess its steady-state mRNA expression profile with each retinoid. None of the retinoids tested in the study by Wakino et al18 had any effect on the expression of cyclin E or the cdk’s, which may explain why RB phosphorylation was not completely inhibited with each retinoid. Taken in aggregate, the decrease in growth factor–induced RB phosphorylation with retinoids appears to be a consequence of impaired expression, and thus activity, of cyclin D/cdk4/6, and/or cyclin A/cdk2. The findings of Wakino et al18 are in perfect agreement with numerous reports from the cancer field showing a similar inhibition of RB phosphorylation on retinoid treatment.22–26 What remains to be shown is whether the activities of the retinoid-targeted cyclin/cdk complexes are reduced in SMCs with a substrate such as histone H1 or RB itself.

Whereas cyclin/cdk complexes promote the phosphorylation of RB, there is yet another family of proteins, the cyclin-dependent kinase inhibitors (cdkI’s), that bind to cdk’s, neutralize their activity, and thus minimize the phosphorylation of RB.21 Levels of cdkI’s are high in quiescent cells; however, on mitogenic stimulation or vascular injury, the levels of cdkI’s are rapidly downregulated, which creates a “permissive” condition for RB phosphorylation and cell cycle progression.27 In HCASMCs, Wakino et al18 found that p27kip1 was the only cdkI to decrease with mitogenic stimulation. In contrast, p15INK4b and p16INK4 are the same in HCASMCs and p27kip1 actually increased with mitogens. Growth factor–induced downregulation of the cdkI p27kip1 prevented with each retinoid.18 Interestingly, this apparent stabilization of p27kip1 protein levels appeared to be related to an extended half-life of the protein, because addition of cycloheximide further increased p27kip1 levels in cells treated with each retinoid. A similar posttranscriptional mechanism for retinoid-mediated p27kip1 stabilization was proposed in a B lymphocyte–transformed cell line.28 Wakino et al18 noted higher-molecular-weight forms of p27kip1, a finding that has recently been observed in atRA-treated neuroblastoma cells whose growth and RB phosphorylation are both inhibited with retinoids.29 Thus, the extended half-life of p27kip1 observed in retinoid-treated HCASMCs could be due to a reduction in proteasome-dependent protein degradation, although this specific mechanism awaits formal testing. It will also be important to show that p27kip1 actually binds its corresponding cyclin/cdk complex, leading to diminished activity in HCASMCs treated with retinoids. In summary, in HCASMCs, p27kip1 downregulation coupled with the reduced expression of cyclins A and D appears to be sufficient for the hypophosphorylation of RB. It should be noted that there remains the possibility that retinoids induce or activate a protein phosphatase (PP1?) that could dephosphorylate RB, thereby achieving the same end of reducing cell cycle progression.21 The figure (Figure) illustrates some of the more salient effects that retinoids such as atRA have on vascular SMCs.

As principal “gatekeepers” of RB activity and the cell cycle, cyclin/cdk’s and their inhibitors represent logical targets for therapy of vascular occlusive diseases. The functional importance of RB phosphorylation has already been demonstrated in rat and porcine arterial models of neointimal formation.30 The report by Wakino et al18 in this issue of Arteriosclerosis, Thrombosis, and Vascular Biology provides the first in-depth analysis of the cell cycle and RB phosphorylation in retinoid-treated SMCs. It should be emphasized that therapies directed solely toward cell cycle regulators may not interfere with other complex aspects of human vascular occlusive disease, such as vessel (adventitial) remodeling, cellular migration, inflammation, and thrombosis. It remains to be discovered how retinoids impinge on these and other aspects of the vessel wall’s response to insult and whether
retinoids may be efficacious in the treatment of human vascular disorders.

References

KEY WORDS: retinoid ■ restenosis ■ smooth muscle proliferation and differentiation ■ growth factors/cytokines
Retinoids: New Insight Into Smooth Muscle Cell Growth Inhibition
Joseph M. Miano and Bradford C. Berk

doi: 10.1161/01.ATV.21.5.724

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/21/5/724