Retinoids and Arterial Smooth Muscle Cells

Pascal Neuville, Marie-Luce Bochaton-Piallat, Giulio Gabbiani

Retinoic acid (RA), the active metabolite of vitamin A, is a crucial signaling molecule during vertebrate development and plays key roles in establishing cell lineages as well as in cell differentiation and proliferation. RA has been shown to influence the expression of many genes (see the Table) through interactions between RA receptors (RARs) and the RA response element (RARE) located in promoter regions. RARs, transducers of the RA signal at the gene expression level, are members of the nuclear receptor superfamily. Essentially 2 subfamilies of nuclear receptor are known to function as RA-dependent transcription factors: the RARs and the retinoid X receptors (RXRs). These receptors, in the form of homodimers or heterodimers, recognize response elements located in the promoter regions of target genes. For each subfamily, 3 different genes (RAR-α, -β, and -γ; RXR-α, -β, and -γ) generate multiple isoforms with specific patterns of expression in both the embryo and adult, suggesting that they perform specific functions in the control of RA target genes. The uptake, transport, and metabolism of all-trans retinol is regulated by the cellular retinol-binding proteins (CRBP I and CRBP II), whereas the cellular RA-binding proteins (CRABP I and CRABP II) exert the same function for RA. Unlike RARs, retinoid-binding proteins modulate the effect of RA by regulating its intracellular level. Several lines of evidence indicate that CRBPs and CRABPs have distinct physiological roles. CRBPs provide the substrate for RA biosynthesis, whereas CRABPs are substrates for RA catabolism. Retinoids have not been extensively studied with regard to smooth muscle cell (SMC) biology. Their role in establishing SMC lineages has long been reported, but their influence on SMC function and their potential therapeutic use for vascular proliferative disorders are only starting to be appreciated. This review summarizes some of these recent advances.

RA and SMC Lineage

RA has a teratogenic effect when applied during critical times of embryonic development. Both the lack of and excess RA may damage embryonic structures, including the cardiovascular apparatus. RA induces cardiac malformations sharing effects with neural crest ablation. The neural crest is a transient, ectodermal structure of the vertebrate embryo that gives rise to a variety of cell types by treatment with different concentrations of RA.

A feature of neural crest–derived SMCs is their ability to be influenced by RA: on one hand, RA modulates differentiation of cardiac neural crest cells and on the other hand, neural crest–derived SMCs express RARs and binding proteins. Recent studies on ductus arteriosus development have shed new light on the action of RA on neural crest–derived SMCs. The ductus arteriosus connects the pulmonary artery and the aorta in the fetal circulation; it arises from the left sixth aortic arch, which is essentially composed of neural crest–derived SMCs. At birth, closure of the ductus arteriosus is caused by the formation of an important intimal thickening followed by vessel constriction. SMCs of the ductus arteriosus exhibit an advanced differentiation of the contractile apparatus compared with those of adjacent large vessels. In particular, the SM myosin heavy-chain isoform found in vessels after birth (SM2) is already expressed in the media of the ductus arteriosus before birth. Colbert et al have generated transgenic mice carrying an RARE-lacZ transgene expressing β-galactosidase in response to endogenous RA. This reporter gene is specifically activated in SMCs of the ductus arteriosus during embryonic and neonatal development and is colocalized with SM2. Taken together, these results suggest that in the developing ductus arteriosus, RA acts as a signal promoting the differentiation of a subset of neural crest–derived SMCs.

Other evidence suggesting interactions between RA and SMC lineage have arisen from studies on differentiation of embryonal carcinoma cells. Embryonal carcinoma cells can be induced to differentiate in vitro into a variety of cell types by treatment with different concentrations of RA. At low
Nonexhaustive List of Genes Regulated by RA and Involved in Different Aspects of Atherogenesis and Restenosis

<table>
<thead>
<tr>
<th>Name</th>
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<th>Cell Type Studied</th>
<th>Location and Relationship to SMCs</th>
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<td>Rat aortic SMCs</td>
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PPAR indicates peroxisome proliferator–activated receptor; IFN, interferon; iNOS, inducible nitric oxide synthase; ICAM, intercellular adhesion molecule; and VCAM, vascular cell adhesion molecule. See text for explanation of other abbreviations.
RA concentrations, they differentiate into fibroblast-like cells \(^{63}\) that express high levels of \(\alpha\)-SM actin and SM myosin heavy chains and thus, resemble vascular SMCs. \(^{64}\) During RA-induced differentiation of P19 embryonal carcinoma cells, \(\alpha\)-SM actin and CRBP are rapidly expressed. \(^{65}\) This CRBP induction occurs at RA concentrations that support embryonal carcinoma cell differentiation into SMCs. Increased levels of CRBP are also observed during F9 embryonal carcinoma cell differentiation induced by RA. \(^{66}\) Another cell line (9E11G), derived from RA-treated P19s, permanently expresses multiple characteristics of differentiated SMCs, including \(\alpha\)-SM actin and SM myosin heavy-chain expression and functional responses to contractile agonists. \(^{67}\) More generally, it appears that induction of RA-responsive genes is a prerequisite for proper embryonal carcinoma cell differentiation, because a mutation affecting the RAR-\(\alpha\) gene of the P19 mutant clone RAC65 blocks the induction of RA-responsive genes as well as RA-induced differentiation. \(^{58,69}\) The fact that a number of genes expressed by differentiated SMCs appear after RA treatment argues in favor of the assumption that RA is a key molecule in SMC differentiation.

### RA and SMC Biological Features

Historically, the first description of distinct SMC phenotypes has been achieved by the identification of contractile and synthetic cells, the first being typical of differentiated arteries and the second, typical of developing and/or pathological arteries. \(^{70,71}\) Moreover, when arterial SMCs from adult animals are placed in culture, they switch their phenotype from contractile to synthetic. It is generally accepted that contractile and synthetic phenotypes can modulate from one to the other. \(^{70,71}\) A further step in the establishment of SMC heterogeneity has been the characterization of spindle and epithelioid phenotypes. \(^{56,72-74}\) These SMC subtypes, studied mostly in the rat, have been isolated from different arterial locations or from the same location of animals at different ages, and they exhibit distinct features: (1) the classic “hill-and-valley” growth pattern for the spindle-shape phenotype that is usually obtained from the normal adult media and (2) growth as a contact-inhibited monolayer for the epithelioid phenotype that has been first and is usually isolated from the intimal thickening induced 15 days after endothelial injury (IT15). \(^{74,76}\) It is accepted that spindle and epithelioid phenotypes permanently maintain their features in culture. \(^{75-77}\) Moreover, when SMCs are cloned from the aortic media or from IT15 cells, both phenotypes are retrieved and again remain stable during culture conditions. \(^{78}\) However, the proportion of clones exhibiting these phenotypes differs according to their origin, with the media yielding a majority of spindle-shape clones and IT15 cells yielding a majority of epithelioid clones. Epithelioid SMCs, cultured as whole or clonal cell populations, show less-differentiated cytoskeletal features (eg, \(\alpha\)-SM actin, SM myosin, and desmin expression), are able to grow in the absence of serum, and exhibit higher migratory activity compared with spindle-shape cells. \(^{77,78}\) Taken together, these findings suggest that intimal thickening develops essentially from a subpopulation of medial SMCs exhibiting epithelioid features in vitro.

In an attempt to identify proteins differentially expressed between different SMC phenotypes, we have observed that CRBP expression is restricted to rat aortic SMCs cultured from IT15 cells displaying an epithelioid phenotype and to epithelioid clones derived from either the normal media or IT15 cells. \(^{2,78}\) Thus, CRBP expression can be used as a marker of epithelioid cells in vitro. Moreover, CRBP is transiently expressed by a subset of SMCs during neointima formation. CRBP-positive cells proliferate at early stages and disappear, allegedly through apoptosis, at later stages of neointimal evolution, \(^{2}\) suggesting that a modulation of the retinoid content may be required for both processes. CRBP upregulation is a direct transcriptional effect of RA, mediated through binding of the RAR-\(\alpha\)-RXR-\(\alpha\) heterodimer to the RARE of the CRBP promoter. \(^{80}\) In SMCs, the pattern of expression of the 3 RAR and RXR genes has been studied. \(^{5}\) Five of the 6 receptors are expressed in rat SMCs in vitro, but in vivo, only RXR-\(\gamma\) is undetectable. The presence of these receptors indicates that SMCs can respond to RA.

RA modulates various SMC features. It has been reported that RA either increases \(^{81}\) or decreases \(2,5,82\) SMC proliferation. This paradoxical effect has recently been clarified by Chen and Gardner. \(^{83}\) RA stimulates quiescent SMCs by increasing expression of cyclin D1, which is involved in cell entry into the G1 phase, whereas serum-stimulated cells are growth inhibited through a decrease in extracellular signal-regulated protein kinase activity. This property seems to be independent of SMC phenotype, because serum-stimulated epithelioid and spindle-shape SMCs are growth inhibited. \(^{28}\) The role of RA in SMC migration in vitro remains unclear. The plasminogen activator (PA) system and the matrix metalloproteinases (MMPs) are both involved in extracellular matrix (ECM) degradation, which is believed to mediate SMC migration. RA has been shown to reduce SMC migration, possibly by decreasing collagenase and stromelysin transcription, 2 MMPs induced by mechanical injury. \(^{84}\) However, we have observed that RA increases migration of both medial and IT15 SMCs, probably by stimulating tissue-type plasminogen activator (tPA) activity. \(^{28}\) tPA is the main PA involved in the proteolytic activities of SMCs in vitro, and the higher proteolytic activity of IT15 cells is mainly due to increased expression of tPA. \(^{29}\) Thus, the opposite effects of RA on SMC migration in vitro are likely due to its differential action on the PA system and MMPs (see the section below on RA and SMC Gene Expression). In addition to SMC proliferation and migration, RA may influence SMC differentiation, because \(\alpha\)-SM actin expression can be enhanced or reduced. \(^{10,28}\) and SMC phenotype, because RA induces the transition from the epithelioid shape to the spindle one. \(^{2}\)

The action of RA in vivo is less controversial. In the rat carotid injury model, RA decreases neointimal cellularity and ECM deposition, leading to a one-third increase in lumen diameter and area. \(^{28,85,86}\) RA also induces favorable remodeling of the injured artery. \(^{85,87}\) Medial area and cell number are unaffected, suggesting, among several possibilities, that in vivo RA specifically influences the SMCs prone to migrate, proliferate, and give rise to the neointima. As discussed above, this RA responsiveness may be correlated with a particular phenotypic and/or embryological origin.

Because different SMC phenotypes are engaged in different genetic programs, it is of interest to investigate possible differential expression of the 5 receptors or of the isoforms corresponding to a given RAR subtype. To identify which...
RAR may have a differential effect on distinct SMC phenotypes, we tested RA agonists specific for each receptor. Among them, only RAR-α agonists were able to inhibit SMC proliferation in vitro and to reduce intimal thickening formation in vivo.28 Thus, RA may specifically influence the SMC phenotype responsible for formation of the neointima through an RAR-α-dependent signaling pathway. It has recently been reported that RAR-γ agonists inhibit in vitro SMC proliferation as well.82 These results suggest that RA may be potentially useful in regulating SMC differentiation, migration, and proliferation in vascular diseases involving neointima formation.

It has been suggested that in some situations, the vascular healing process may involve adventitial fibroblasts in producing arterial remodeling and/or neointima formation.88 These fibroblasts modulate into myofibroblasts expressing α-SM actin and producing ECM components under transforming growth factor-β (TGF-β) stimulation.89,90 We have observed that after induction of an open wound in the back skin of the rat, myofibroblasts start to express CRBP during the proliferation phase; CRBP expression persists until myofibroblasts disappear through apoptosis.91 These observations are compatible with the possibility that CRBP expression and RA modulation are common features of repair processes. Our results suggest that RA may be potentially useful in regulating SMC differentiation, migration, and proliferation in vascular diseases involving neointima formation.

RA and SMC Gene Expression

RA influences the expression of many genes, including those associated with cell growth and differentiation.43 It may activate the transcription of target genes or repress other genes by antagonizing the function of the activator protein-1 (AP-1) transcription factor.92 AP-1 is a heterodimer of c-fos and c-jun that binds to AP-1 consensus sequences and activates genes related to cell proliferation, migration, apoptosis, and ECM production.93 These 4 processes have been shown to play key roles in the progression of atherosclerosis and restenosis.94,95 An impressive number of genes are positively or negatively regulated by RA (see the Table). Most of them are in some way involved in the development of atherosclerotic lesions or in the formation of the arterial neointima. Thus, c-fos and c-jun are rapidly induced after arterial injury, leading to the formation of AP-1 and to the activation of AP-1-regulated genes,33 such as fibroblast growth factor-2, TGF-β1, c-myc, endothelin-1, stromelysin, collagenase, or the precursor of the MMP-1, pro–MMP-1.40,96 RA can inhibit the expression of some of these genes, such as those for collagenase, stromelysin, and pro–MMP-1.32,40,98 The RA-induced inhibition of MMPs is generally accompanied by an RA-mediated activation of genes encoding ECM components, such as fibronectin, laminin, and collagen type IV.99,100 Thus, RA allegedly modifies the SMC genetic program to favor the formation of ECM, which may in turn decrease cell migration and maintain cell differentiation.101,102 In relation to cardiovascular diseases, the action of RA as a negative regulator of AP-1–responsive genes may be considered beneficial. On the other hand, arterial injury results in an increase of platelet-derived growth factor (PDGF), PDGF receptors, and TGF-β194,103 that are responsible for the induction of SMC migration and proliferation and that are also potential target genes for RA.104,105 Moreover, some of the actions of RA are considered to be mediated or potentiated by induction of TGF-β1, and cross-talk between their signal transduction pathways is well established.106,107 Thus, it appears that RA is also capable of enhancing the atherogenic growth factors. These observations underline the complexity of the RA influence on SMCs and support the notion that the action of RA on SMC behavior, and probably on vascular diseases, largely depends on local environmental changes and on the pattern of genes expressed at a given time.

Conclusions and Perspectives

Although it is well accepted that RA plays an important role in vascular development, the multifaceted activity of RA in arterial SMCs of adult animals is presently an emerging, though rapidly developing, topic. It is now well established that RA influences SMC replication and motility, and, through its pleiotropic action, RA may affect other processes involved in the pathogenesis of vascular diseases. Recent work points particularly to the possibility that RA affects SMC participation in such phenomena as restenosis and plaque formation.28,85 The findings that RA can exert distinct actions on epithelial versus spindle SMC phenotypes in culture and can reduce experimental intimal thickening in vivo are encouraging; however, further work is required to elucidate the mechanisms by which RA affects SMC function in vitro and in vivo. Nevertheless, one can confidently anticipate that the field of SMC modulation by RA in biological and pathological situation will develop importantly during the next several years.

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References


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**KEY WORDS:** α-smooth muscle actin ■ myofibroblast ■ atheroma ■ wound healing ■ TGF-β
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