Arterial Smooth Muscle Cell Heterogeneity
Implications for Atherosclerosis and Restenosis Development

Hiroyuki Hao, Giulio Gabbiani, Marie-Luce Bochaton-Piallat

Abstract—During atheromatous plaque formation or restenosis after angioplasty, smooth muscle cells (SMCs) migrate from the media toward the intima, where they proliferate and undergo phenotypic changes. The mechanisms that regulate these phenomena and, in particular, the phenotypic modulation of intimal SMCs have been the subject of numerous studies and much debate during recent years. One view is that any SMCs present in the media could undergo phenotypic modulation. Alternatively, the seminal observation of Benditt and Benditt that human atheromatous plaques have the features of a monoclonal or an oligoclonal lesion led to the hypothesis that a predisposed, medial SMC subpopulation could play a crucial role in the production of intimal thickening. The presence of a distinct SMC population in the arterial wall implies that under normal conditions, SMCs are phenotypically heterogeneous. The concept of SMC heterogeneity is gaining wider acceptance, as shown by the increasing number of publications on this subject. In this review, we discuss the in vitro studies that demonstrate the presence of distinct SMC subpopulations in arteries of various species, including humans. Their specific features and their regulation will be highlighted. Finally, the relevance of an atheroma-prone phenotype to intimal thickening formation will be discussed. (Arterioscler Thromb Vasc Biol. 2003;23:●●●●●●●●●.)

Key Words: smooth muscle cell ■ heterogeneity

Smooth muscle cells (SMCs) are important actors in the pathogenesis of atherosclerosis and of restenosis after angioplasty or stent application. In both phenomena, 1 of the characteristic changes is the accumulation of SMCs within the intima. The contemporary paradigm is that the combined action of growth factors, proteolytic agents, and extracellular matrix proteins, produced by a dysfunctional endothelium and/or inflammatory cells, induces migration of SMCs from the media and their proliferation. Moreover, during these processes, SMCs switch from a contractile to a synthetic phenotype. Alternative to the predominant hypothesis that all SMCs of the media can undergo phenotypic modulation is the concept that a predisposed SMC subpopulation is responsible for the production of intimal thickening (IT). This possibility has been raised on the basis of original work by Benditt and Benditt,4 who reported that human atheromatous plaques have the features of a monoclonal lesion, an observation that has been confirmed by several laboratories.5–7 More recently, with use of in situ microdissection techniques, it has been demonstrated that human plaques are at least oligoclonal.8 These findings support the suggestion that SMCs of the arterial wall are biologically heterogeneous, and thus, attempts have been made to isolate distinct SMC phenotypes from arterial vessels. Intimal SMCs have been proposed to originate from diverse sources, including fibroblasts of the adventitia during restenosis,9 endothelial cells,10 and/or circulating bone marrow–derived cells.11 Although the existence of these possibilities is gaining acceptance, the role of each of them is subject to much debate. These possibilities, including the SMC origin of IT, are not mutually exclusive. The ultimate aim of these studies, including those that have investigated SMC heterogeneity, the focus of this review, is to define an atheroma-prone phenotype (APP) involved in atheroma and restenosis formation.

Establishment of Distinct SMC Populations

The concept of SMC heterogeneity has been established by the description of contractile and synthetic phenotypes in vivo and in vitro.2,3 The contractile phenotype is typical of the differentiated artery, and the synthetic 1 is typical of developing and pathologic arteries. A further step was the characterization in vitro of morphologically distinct SMC populations, which has been observed in many species, including humans (Table 1). Until now, the most-studied species has been the rat. The initial description of SMC heterogeneity was made in the rat carotid artery injury model, wherein 2 SMC populations were identified: (1) a spindle-shaped phenotype, with the classic “hill-and-valley” growth pattern, obtained from the normal media (NM), and (2) an epithelioid phenotype, in which cells grow as a monolayer and exhibit a cobblestone morphology at confluence, isolated from the IT 15 days after endothelial injury.12 This has been confirmed by

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TABLE 1. Description of Smooth Muscle Cell Subpopulations

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<tr>
<td>Human</td>
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<td>Nonatherosclerotic artery</td>
<td>Cloning from passage 3</td>
<td>Spindle and broad</td>
<td>30</td>
<td></td>
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<tr>
<td>Pulmonary artery</td>
<td>40–60 y</td>
<td>Nonatherosclerotic artery</td>
<td>Cloning from passage 3</td>
<td>Spindle and polygonal</td>
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<td></td>
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<tr>
<td>Coronary artery</td>
<td>Adult</td>
<td>Nonatherosclerotic and atherosclerotic artery</td>
<td>Cloning from passage 3</td>
<td>Spindle and epithelioid</td>
<td>51</td>
<td></td>
</tr>
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</table>

SHR indicates spontaneously hypertensive rats.

*H-2Kb-tsA58 transgenic mice.
many laboratories (Figure 1; eg, see Orlandi et al,13 Bochaton-Piallat et al,14 and Yan and Hansson15).

The last 2 decades have seen several advances in the understanding of SMC heterogeneity, including the identification of (1) distinct phenotypes in healthy arteries of the rat at different ages16–23; (2) SMC subpopulations from particular compartments within the NM of the rat aorta24; (3) clonal populations from the NM and IT of the rat aorta14,15,25,26; and (4) distinct phenotypes in arteries of various species, such as the dog,27 cattle,28 pig,29 and human.30–32

SMC populations with either spindle-shaped or epithelioid phenotypes were isolated from the healthy rat aorta at different ages.18,19,21,22 In particular, spindle-shaped SMCs were predominant in fetuses at different developmental stages,22 as well as in newborn (4 to 5 days)19,21,33 and adult (6 weeks to 3 months) rats,13,21,34,35 whereas epithelioid SMCs were prevalent in old rats (>18 months).18,21 This suggests that the population of SMCs that exhibit an epithelioid phenotype in vitro increases in rat aortic NM with age. In this respect, several studies have shown that greater IT in response to injury is produced in old rats compared with adult rats.36–38 It is, however, noteworthy that a predominant population of epithelioid SMCs was recovered from the NM of 12-day-old newborn rats,16,17,20,23 an age when sexual maturation occurs. These results suggest that a proportion of SMCs that exhibit an epithelioid phenotype in vitro exists within the media throughout the whole life span and that its size can be influenced by circulating or microenvironmental factors.

Villaschi et al,24 using tissue explantation, isolated epithelioid SMCs from the luminal part of the rat aorta NM. Our group has produced clones from the NM and IT and has demonstrated that spindle-shaped and epithelioid clones can be recovered from both locations. However, the proportion of clones that exhibit these phenotypes differed according to their origin, the NM predominantly yielding spindle-shaped clones and the IT yielding a majority of epithelioid clones.14

Several groups have confirmed the production of SMC clones that exhibit spindle-shaped or epithelioid phenotypes from the NM of rats15,25,26 and mice.39 Taken together, these studies support the possibility that IT develops essentially from a distinct, medial subpopulation that exhibits an epithelioid phenotype when placed in culture. It should be noted that an SMC population that displays an epithelioid phenotype has been obtained from the NM of the spontaneously hypertensive rat aorta,40 extending the role of this subpopulation in arterial diseases other than atherosclerosis and demonstrating that SMCs with a propensity to produce an epithelioid population in culture can increase within the media subjected to pathologic stimuli.

Some experimental data indicate that rodent, including rat, SMCs are not ideal models for human SMCs (vide infra “Mechanisms of SMC Phenotypic Modulation”); hence, attempts have been made to culture SMCs from larger animals. Frid et al28,41 have performed extensive studies of bovine pulmonary artery and aorta. They first characterized the morphologically distinct compartments within the NM41 and then isolated from these compartments, by means of tissue explantation, SMC subpopulations that exhibited spindle-shaped, rhomboid, and epithelioid morphologies, the last 2 being similar to rat epithelioid SMCs.28 However, they did not study arterial lesions. In the canine carotid artery, Holifield et al27 have shown by sequential enzymatic digestion that spherical SMCs, similar to rat epithelioid cells, arise from the abluminal part of the NM and are predominant in the IT produced 14 days after endothelial injury. Our group has recently isolated 2 distinct SMC subpopulations from the porcine coronary artery.29 SMCs isolated by enzymatic digestion from the NM exhibit a spindle-shaped phenotype and grow in a hill-and-valley configuration,29,42 similar to SMCs derived from the porcine aortic NM.43,44 In contrast, SMCs obtained by tissue explantation are either spindle-shaped or rhomboid (flat, but more elongated than epithelioid rat SMCs); the luminal side of the media yields equal proportions of spindle-shaped and rhomboid SMCs, whereas the abluminal side yields a high proportion of rhomboid SMCs (Figure 1).29 With these same techniques, IT induced 15 days after stent implantation gives rise to a high proportion of

![Figure 1. Morphological features of SMC subpopulations. Phase-contrast microphotographs show spindle-shaped (a) and epithelioid (b) phenotypes, respectively, isolated from the NM and IT of rat aorta and spindle-shaped (c) and rhomboid (b) phenotypes isolated from the NM of pig coronary artery. Bar=150 μm.](http://atvb.ahajournals.org/)

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TABLE 2. Biological and Biochemical features of Smooth Muscle Cell Subpopulations

<table>
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<th>Cow</th>
<th>Pig</th>
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<tr>
<td></td>
<td></td>
<td>Spindle</td>
<td>Epithelioid</td>
<td>Rhomboid</td>
<td>Epithelioid</td>
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<tr>
<td>Autonomic growth</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Migratory activity</td>
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<td>High</td>
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**Differentiation features**

<table>
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<tr>
<th>α-SM actin</th>
<th>Desmin</th>
<th>SM-MHC</th>
<th>Smoothelin</th>
<th>SM22α</th>
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<td>++</td>
<td>+/−</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>++</td>
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<td>++</td>
<td>ND</td>
<td>ND</td>
<td>+/−</td>
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</tbody>
</table>

SM-MHC indicates smooth muscle myosin heavy chain.
*Under PDGF-BB stimulation.

rhomboid SMCs. Hence, these cells represent good candidates for the formation of IT in the porcine coronary artery. Identification of particular SMC subpopulations in human arteries represents a difficult challenge for understandable reasons, such as material availability and experiment standardization. Nevertheless, distinct SMC subpopulations have been isolated from various human healthy and pathologic arteries. In the 1980s, Orekhov et al. characterized the morphological polymorphism of SMCs isolated from arterial intima. Among the distinct shapes observed, elongated cells (ie, differentiated SMCs) were predominant in the unaffected intima, whereas stellate cells (ie, undifferentiated SMCs) were predominant in the atherosclerotic intima. Such increase was correlated with collagen deposition and lipid accumulation in the lesion. This cell diversity was partially retained in primary culture. At the same time, Björkerud showed that 2 cell types, distinguishable by their shape and adhesion properties, were present in SMCs cultured from IT. Taken together, these studies suggest the presence of phenotypically distinct SMC populations in human arteries. More recently, it has been shown that SMC subpopulations isolated by tissue explantation or cell cloning from healthy or atherosclerotic arteries display phenotypic features similar to those observed in the rat and pig. In particular, the finding that epithelioid SMCs can be cloned from human arterial media supports the suggestion that expansion of an SMC subset in atherosclerotic lesions is conceivable. However, the relevance of SMC heterogeneity to human disease still remains to be demonstrated.

The distinct phenotypes of arterial SMCs have been mainly identified in vitro, suggesting that specific features of SMC subpopulations arise and are maintained because of the particular environment of cell culture. We have implanted into the rat carotid artery subjected to endothelial injury 2 SMC subpopulations, ie, spindle-shaped, isolated from newborn rats, and epithelioid, isolated from old rats, that exhibit distinct differentiation features (defined in addition to morphology by the expression level of α-SM actin, SM myosin heavy chains [MHCs], and cellular retinol binding protein [CRBP]-1; vide infra “Features of SMC Subpopulations” and “Markers of Epithelioid and Spindle-Shaped Phenotypes”). Once seeded, the 2 SMC populations maintained their distinct features, indicating that the phenotype of SMCs depends more on their intrinsic features rather than their environment, thereby reinforcing the notion of SMC heterogeneity.

**Features of SMC Subpopulations**

Irrespective of the species studied, epithelioid and rhomboid phenotypes, when compared with spindle-shaped SMCs, have in common several features, among which the most relevant are (1) enhanced proliferative activity, including serum-independent growth in some species; (2) enhanced migratory activity; (3) increased proteolytic activity; (4) poor level of differentiation, as defined by cytoskeletal and contractile protein expression (Table 2); and (5) high sensitivity to apoptotic stimuli.

In all species studied, epithelioid and rhomboid SMCs show a higher proliferative activity than do spindle-shaped SMCs; however, contrary to spindle-shaped SMCs, they stop growing at confluence as a result of cell contact inhibition. It is notable that rat epithelioid SMCs are able to grow in the absence of serum. In addition, albeit spindle-shaped, SMCs isolated from rat embryos exhibit serum-independent growth capacity. Epithelioid SMCs produce platelet-derived growth factor (PDGF)-BB, which is a potent SMC mitogen, and fail to respond to the growth-inhibitory effect of transforming growth factor (TGF)-β. However, the factor(s) responsible for serum independence has never been clearly identified. Autonomous growth by epithelioid and rhomboid SMCs has been observed in other species with the exception of the pig, which in this respect is similar to humans.

One of the most striking differences between epithelioid or rhomboid and spindle-shaped SMCs is that the first exhibit a high migratory activity, as has been shown in the rat and pig. With the aim of exploring the mechanisms involved in this activity, we have demonstrated that epithelial SMCs display high tissue plasminogen activator (iPA) activity and that pig rhomboid SMCs display high urokinase-type PA (uPA) activity. Likewise, Lai has shown that rat epithelial SMCs might produce iPA, uPA, and metalloproteinase-2 under particular growth conditions. In this respect, both PAs have been detected in both experimental IT and human atherosclerotic lesions.

The study of cytoskeletal proteins, which are accepted as reliable differentiation markers, has allowed character-
ization of the contractile versus the synthetic phenotype (Table 2). It should be noted that when placed in culture, all SMCs tend to show a dedifferentiated phenotype. With this limitation, the phenotypic variation of cultured SMCs furnishes important information concerning the influence of many factors on their biologic features. α-SM actin is expressed in vascular SMCs, even at early stages of development, and thus represents the most general marker of SMC lineage. Although α-SM actin is permanently expressed in SMCs, it is more abundant in spindle-shaped SMCs than in epithelioid or rhomboid SMCs. Desmin, an intermediate filament protein, and SMMHC are expressed in well-differentiated SMCs and are relatively well studied. In cultured rat SMCs, desmin generally disappears. In larger animals, desmin is maintained at a significant level of expression in spindle-shaped SMCs, whereas it is hardly detectable in epithelioid and rhomboid SMCs. Other cytoskeletal proteins have been less extensively studied. In particular, smoothelin, SM22α, calponin, h-caldesmon, and metavinculin serve as late differentiation markers and are more abundant in spindle-shaped SMCs than in epithelioid and rhomboid SMCs. In general, SMCs isolated from larger animals, including humans, are more differentiated than those isolated from rodents. Porcine spindle-shaped SMCs maintain appreciable expression of α-SM actin, SMMHC, desmin, and smoothelin. Desmin, h-caldesmon, and metavinculin serve as late differentiation markers and are more abundant in bovine spindle-shaped SMCs. SMCs isolated from human arteries behave similarly. An interesting correlation has been demonstrated, albeit occasionally, between dedifferentiated and/or highly proliferating SMC phenotypes and increased LDL uptake or decreased HDL binding sites. The role of LDL and HDL processes in atheromatous plaque formation with respect to SMC heterogeneity should be further investigated. Taken together, the data obtained in different species suggest that the degree of differentiation of SMCs changes with the phenotype; this integrates well into a view that reconciles the heterogeneity of SMCs with the modulation concepts.

An enhanced susceptibility of rat epithelioid SMCs to apoptosis induced by reactive oxygen species, retinoic acid, and antimitic drugs has been recently described. Interestingly, SMCs isolated from healthy human coronary arteries show marked heterogeneity to Fas-induced apoptosis. It is noteworthy that apoptosis is an important phenomenon in the development of experimental IT and has been detected in SMCs of atherosclerotic and restenotic lesions (for a review of different aspects of this problem, see Kockx and Herman, McCarthy and Bennett, and Geng and Libby). Apoptosis could participate in the regulation of cellularity in restenosis and in the stability of the plaque; the role of distinct SMC populations in this context remains to be demonstrated.

Markers of Epithelioid and Spindle-Shaped Phenotypes

Once distinct populations have been defined, the ultimate aim is to identify genes and/or proteins that are differentially expressed and to test whether they are involved in the phenotypic changes that occur in vivo. Using the technique of 2-dimensional polyacrylamide gel electrophoresis, we have identified several proteins that are differentially expressed in rat aortic spindle-shaped versus epithelioid SMCs. Among them, 3 proteins have been sequenced and identified as markers of the rat epithelioid phenotype in vitro: CRBP-1, a protein involved in retinoid metabolism, and cytokeratins 8 and 18, intermediate filament proteins.

In vivo, CRBP-1 is constitutively expressed in rare SMCs of the NM of adult and old rats but not of newborn rats. After endothelial injury, CRBP-1 is rapidly activated in a subset of medial SMCs located toward the lumen and is expressed in the large majority of SMCs present in the IT; however, it disappears when reendothelialization is achieved. Remodeling of IT is associated with SMC apoptosis. CRBP-1, which is present in replicative SMCs during the initial phase of IT formation, is also detected in apoptotic cells of IT. Altogether, these results suggest that a predisposed subset of medial SMCs becomes rapidly CRBP-1–positive after injury, undergoes replication during the early phase of IT development, and then disappears, allegedly through apoptosis, when reendothelialization takes place. Some additional data support this scenario: SMCs cultured from reendothelialized IT (60 days after injury) are exclusively spindle-shaped, suggesting that potentially epithelioid SMCs have disappeared. Moreover, cultured rat epithelioid SMCs are more sensitive to apoptosis than are spindle-shaped SMCs. Taken together, these results indicate that CRBP-1 is a marker of the epithelioid phenotype in vitro and of SMC activation after endothelial injury in vivo.

Unfortunately, when the analysis of CRBP-1 expression was extended to pigs and humans in vitro and in vivo, the role of this protein as a marker was not confirmed (M.-L. Bochaton-Piallat et al, unpublished observations). This further supports the assumption that rodent SMCs do not represent a reliable model for human SMCs.

Cytokeratins 8 and 18, intermediate filament proteins, as well as zonula occludens-2 protein and cingulin, 2 proteins of tight junctions, were thought to be exclusively expressed in epithelial or endothelial cells. They have since been identified as markers of rodent epithelioid SMCs and are expressed in experimental IT or human atheromatous plaque. Studies of these proteins could give further insight into the mechanisms of SMC pathologic modulation.

Several other genes have been discovered, mainly in rodents, as being specific or at least more abundant in 1 SMC population compared with the other. Epithelioid SMCs express osteopontin, troponelastin, PDGF-BB, cytochrome P450, and peroxisome proliferator–activated receptor-γ, whereas spindle-shaped SMCs express procollagen type I and PDGF-α receptor. This pattern of gene expression is broadly observed in cloned SMCs that exhibit phenotypes similar to their parental populations, with the exception of procollagen type I, whose expression appears to be sensitive to cell density and serum level. Although none of these genes has conclusively been proved to be relevant to the pathogenesis of human lesions, the study of osteopontin, an extracellular matrix protein involved in bone mineralization, has shed new light on the mechanisms of IT formation.
Osteopontin is associated with SMC proliferation and migration. In vivo, it is transiently upregulated in experimental IT and accumulates in calcified areas of the atheromatous plaque. Recently, it has been shown that SMCs lose their lineage markers and acquire an osteogenic phenotype in vitro under calcifying conditions and in vivo in transgenic mice whose arteries calcify spontaneously. This suggests that SMCs can play a role in vascular calcification.

Interestingly, the subtractive hybridization approach with RNA isolated from rat embryo SMCs with an autonomous growth property and rat adult SMCs with a nonautonomous growth property allowed identification of several embryonic genes; 1 of them, ie, embryonic growth-associated protein, is involved in the serum-independent growth of embryonic SMCs and is reexpressed in experimentally induced IT.

Recent studies performed in species other than the rat are providing novel insight into the understanding of IT formation. Autonomously growing, rhomboid SMCs isolated from bovine pulmonary artery exhibited constitutively activated extracellular signal–regulated kinase and eicosanoid production. Other studies with microarray techniques or differential-display polymerase chain reaction have been performed with SMCs in various situations, yielding identification of numerous, differentially expressed genes in monkeys and humans. They are either unknown or known to be involved in atherosclerotic processes. A very recent study with suppressive subtractive hybridization that was performed in the pig has compared the coronary artery, predisposed to atherosclerosis, with the mammary artery, which is resistant to atherosclerosis. Genes that are preferentially associated with the mammary artery are involved in cell-cell junction formation, whereas genes that are preferentially expressed in the coronary artery are implicated in lipid metabolism, inflammation, and cell proliferation. However, the presence of distinct SMC populations within the same vessel has not been taken into account in these studies.

Taken together, the studies performed in a variety of species, including humans, provide evidence that arterial SMCs are phenotypically heterogeneous. Moreover, the epithelioid and rhomboid (according to the species) phenotypes are good candidates for representing the APP for several reasons: (1) they always exhibit an enhanced capacity for proliferation and migration that is associated with high proteolytic activity, features essential for the accumulation of SMCs in the IT; (2) they acquire a poorly differentiated phenotype typical of intimal SMCs in vivo; and (3) they express specific proteins crucial for their behavior. In this respect, the most demonstrative example is the identification of CRBP-1 as a specific marker of intimal SMCs in the rat model. The discovery of new genes and/or proteins typical of the APP in other species is still a challenge and, once possible, should provide new insight into the understanding of atherosclerosis and restenosis mechanisms.

**Origin of Epithelioid and Spindle-Shaped Phenotypes**

During vasculogenesis, SMCs have been proved to originate from diverse sources depending on the vessel type: mesoderm, neurectoderm (neural crest), epicardium (for coronary arteries), and, more rarely, endothelium. This has led to the hypothesis that the various SMC phenotypes could arise from distinct lineages. A study performed in the chick embryo aorta has shown that spindle-shaped and epithelioid phenotypes can be isolated from 2 distinct regions of the artery, which differ in their embryologic origin, namely, the mesoderm and neural crest. The 2 populations are quite similar in their cytoskeletal equipment but respond differently to TGF-β. These results suggest that different SMC subpopulations play a role in the formation of the tunica media, at least in the chick embryo.

The morphological similarity of epithelioid SMCs to endothelial cells has led Kohler et al to investigate whether this SMC subtype exhibits an angiogenic capacity. For this purpose, they cultured mouse epithelioid SMCs in a collagen gel and observed that they were capable of forming vessel-like structures, and in coculture, that they induced spindle-shaped SMCs to participate in this process. Moreover, Nicosia and Villaschi have shown that the luminal part of the rat aorta, which contains predominantly epithelioid SMCs (vide infra “Establishment of Distinct SMC Populations”), gives rise to pericytes when placed in coculture with endothelial cells–forming microvessels, whereas SMCs from the deeper part of the aorta (mainly composed of spindle-shaped SMCs) do not. We have shown that the capacity of porcine rhomboid SMCs to invade a collagen gel is remarkably higher than that of spindle-shaped SMCs. These data suggest that epithelioid SMCs could play a major role in angiogenesis and/or arteriogenesis. In addition, they could be considered a potential source of locally derived stem cells, ie, cells present in adult tissues that exhibit pluripotent features.

Recent studies suggest that endothelial cells could acquire SMC features, ie, α-SM actin expression in vitro and in vivo. Moreover, bone marrow–derived cells have been reported to undergo transdifferentiation toward the SMC phenotype in transplant arteriopathy, in IT induced after arterial injury, and in hypercholesterolemia-induced IT. Although considered a minor source of SMCs, it would be of interest to study whether these cells participate in the emergence of those that exhibit the APP.

**Mechanisms of SMC Phenotypic Modulation**

Many attempts have been made to modulate the behavior of distinct SMC subpopulations. The factors tested can be distributed in 4 categories: (1) those described as classic inhibitors of SMC proliferation and/or increasing SMC differentiation, eg, heparin, TGF-β, and retinoic acid; (2) those known to stimulate SMC proliferation and/or decrease SMC differentiation, eg, PDGF-BB, fibroblast growth factor (FGF)-2, and insulin growth factor-I and II; (3) vasoactive substances, such as endothelin-1, angiotensin II, histamine, and norepinephrine; and finally (4) vasodilator factors, such as nitric oxide (NO).

Heparin, which is the most powerful inhibitor of SMC growth in vitro and in vivo, at least in the rat, did not exhibit differences of action on the distinct rat or pig SMC subpopulations. In the bovine pulmonary artery model, heparin exerted dramatic growth inhibition on rhomboid
SMCs, whereas it had almost no effect on spindle-shaped SMCs. These results indicate that the action of heparin on diverse SMC subpopulations depends on the species studied.

In the rat, TGF-β1 as well as retinoic acid changes the morphology of epithelioid SMCs; however, these cells do not achieve a typical spindle-shaped phenotype. In contrast, in pig coronary artery SMCs, TGF-β does not influence SMC morphology, although it does decrease proliferation and increase the differentiation level of both SMC subtypes. In the rat, retinoic acid increases the expression of α-SM actin only in epithelioid but not in spindle-shaped SMCs; however, in both cell types, retinoic acid decreases proliferation and increases migration. All of the effects of retinoic acid are mediated by the nuclear receptor RAR-α. These results indicate that rat epithelioid SMCs, ie, CRBP-1–positive SMCs, are more prone to respond to retinoic acid than are spindle-shaped SMCs, at least so far as their differentiation state is concerned. In vivo, feeding rats with retinoic acid or with an RAR-α agonist inhibits aortic or carotid artery IT formation, thus functionally confirming that CRBP-1 is a marker of the APP in the rat model.

Some articles have shown that spindle-shaped SMCs are more responsive than are epithelioid SMCs to vasoactive factors such as endothelin-1, angiotensin II, histamine, and norepinephrine, either by measuring collagen gel contraction or by evaluating the intracellular calcium concentration. This is in accordance with the contractile feature of spindle-shaped SMCs. Conversely, epithelioid SMCs exhibit increased expression of inducible NO synthase, which is correlated with enhanced nuclear factor-κB expression when compared with spindle-shaped SMCs. Moreover, these cells fail to respond to NO, because of the lack of the β-subunit of soluble guanylyl cyclase. This suggests that despite a large production of NO, epithelioid SMCs are less sensitive than are spindle-shaped SMCs to NO actions. GFG-2 and PDGF-BB similarly increase the proliferation and migration of porcine SMC subpopulations. Human epithelioid SMCs migrate more actively than do spindle-shaped SMCs in response to PDGF-BB. We have shown that FGF-2 and PDGF-BB induce a switch from the spindle-shaped to the rhomboid phenotype in pig SMCs. This is associated with increased proliferation and a decrease in expression of differentiation markers. A similar effect has been obtained for spindle-shaped SMC clones. In both situations, this shape change is reversible when treatment is ceased. These results indicate that the switch depends on phenotypic modulation rather than on selection of a given population. Interestingly, endothelial cells isolated from the porcine coronary artery and placed in coculture with SMCs induce a switch from the spindle-shaped to the rhomboid phenotype. In these experiments, endothelial cells did not exhibit a quiescent state even after confluence, suggesting that they mimic an injured or dysfunctional endothelium. In other species, previous studies with endothelial cell/SMC cocultures have shown that endothelial cells stimulate SMC proliferation and decrease the expression of α-SM actin and SMMHC, particularly when nonquiescent endothelial cells are used. It has been suggested that endothelial cells stimulate the proliferation of SMCs by producing plasmino

Conclusions and Perspectives
The original observation by Benditt and Benditt has stimulated a significant number of experiments, which support the possibility that SMC heterogeneity exists in different species. Epithelioid and/or rhomboid SMCs appear to be the most likely candidate to represent the APP by playing an important role in the formation of IT. Studies on the possibility that an SMC phenotype can evolve into another have shown that the results depend on the species used. This suggests that the mechanisms of IT formation differ according to the model (Figure 2). Thus, in the rat, the most studied model, SMCs show 2 phenotypes that do not appear to be interchangeable, both in vitro and in vivo. Indeed, the specific phenotype of SMC observed in vitro is maintained when they are placed back into an in vivo environment. The evolution of the lesion in vivo appears to depend on the relative replicative activity and/or susceptibility to apoptosis of these phenotypes. Moreover, in the rat, an agent that influences the biologic behavior of epithelioid SMCs, ie, retinoic acid, inhibits IT formation. In the pig, the situation is different, in that spindle-shaped SMCs can modulate into rhomboid SMCs and, if the stimulus ceases, can return to their original phenotype, at least in vitro. This supports the assumption that the evolution of in vivo lesions is more complex in the pig than in the rat. In this respect, porcine SMCs appear to behave similarly to human SMCs.

It should be emphasized that at present, the phenotypic identification of APP SMCs other than in the rat is at a very early stage. Studies at the genetic and/or proteomic levels similar to those performed in the rat are needed to achieve clear identification of the markers of each phenotype in vitro and in vivo. An extension of these studies to vascular development in appropriate species will also furnish useful information on the origin of different SMC phenotypes and on their localization in arterial vessels. In conclusion, the paradigm of SMC heterogeneity has begun to produce experimental information and should be very useful in the future to characterize precisely the biologic features of arterial SMCs and to yield a better understanding of the role of different SMC subpopulations in physiologic and pathologic situations.

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noted that the non–APP-SMCs (ie, spindle-shaped phenotype) and APP-SMCs (epithelioid phenotype) are not interchangeable. In the intima and subsequently acquire CRBP-1 (\(\Phi\)) or first express CRBP-1 in the media before migrating toward the intima (\(\Phi\)). It should be noted that the non–APP-SMCs (ie, spindle-shaped phenotype) and APP-SMCs (epithelioid phenotype) are not interchangeable. In the porcine model, APP-SMCs are located at the abluminal part of the media. The mechanisms (\(\Phi\)) and (\(\Phi\)) described in the rat model are the same for the porcine model. In addition to these 2 possibilities, non–APP-SMCs (ie, spindle-shaped phenotype) can evolve to APP-SMCs (rhomboid phenotype), which then accumulate within the intima (\(\Phi\)).

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Arterial Smooth Muscle Cell Heterogeneity. Implications for Atherosclerosis and Restenosis Development

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