The Differentiated Properties of Vascular Smooth Muscle Cells and the Roles of Extracellular Matrix

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Abstract: In the adult, vascular smooth muscle cells (SMC) exist in a highly differentiated, contractile phenotype. During the early developmental stages of atherosclerosis, arterial SMC undergo transition from the contractile to the synthetic phenotype characterized by losing their contractility and instead gaining the ability to proliferate and secrete extracellular matrix (ECM) components. This transition appears to be an early key event in the pathogenesis of atherosclerosis. ECM components may control vascular responses in growth and differentiation and play an important role in determining the phenotypic transition of vascular SMC. Plasma fibronectin and type I collagen bind to cell surface receptors (integrins) and promote the attachment, spread, and transition to the synthetic phenotype, while laminin, type IV collagen, heparin, heparan sulfate, and elastin act in an opposite direction on phenotypic transition. Further studies in this field will help in the understanding of the initial stages in the pathogenesis of atherosclerosis.

Keywords: differentiated properties, vascular smooth muscle cells, extracellular matrix components

INTRODUCTION

The purpose of this article is to review current knowledge of the differentiated properties of vascular smooth muscle cells (SMC) and the roles of extracellular matrix (ECM) components. Vascular SMC can be divided into at least two different states, usually referred to as the contractile and synthetic phenotypes, based on the distribution of myosin filaments and the formation of large amounts of secretory protein apparatus, such as rough endoplasmic reticulum (RER) and Golgi complex. Vascular SMC in the contractile state respond to agents that induce either vasoconstriction or vasodilatation, such as endothelin, angiotensin II, thromboxane A2, prostaglandin E, prostacyclin, leukotrienes, and NO. In contrast, SMC in the synthetic state are capable of expressing genes for a number of growth-regulatory molecules and cytokines, can respond to growth factors by expressing appropriate receptors, and can synthesize ECM components.

Development of Vascular SMC

In the embryo, blood vessels arise from the mesenchyme as a budding network of endothelial-linked channels, which become surrounded by locally derived, irregularly shaped mesenchymal cells. Subsequently, mesenchymal cells accumulate and start to differentiate into SMC. During most of the fetal and early postnatal periods, SMC have a fibroblast-like appearance with extensive RER, a large Golgi complex, and only a few myofilaments. They proliferate in response to platelet-derived growth factor (PDGF) or other mitogens, secrete ECM components, and take part in the formation of the vessel wall. In contrast, SMC in the medial layer of adult aortas are tightly surrounded by ECM components such as elastin fibers and collagen fibrils, and become highly differentiated muscle cells, which contract in response to chemical and mechanical stimuli and take part in the control of blood pressure and flow. These cells show low mitogenic activity. The changes in the structure and function of vascular SMC during development can be referred to as a shift from a synthetic to a contractile phenotype. During the early developmental stages of atherosclerosis, arterial SMC undergo transition from the contractile to the synthetic phenotype characterized by active proliferation and secre-
Fig. 1 Schematic overview of changes in the differentiated properties of arterial smooth muscle cells during normal development and atherogenesis.

Modulation of Phenotype during Atherogenesis

Central to the response-to-injury hypothesis is the proposal that different risk factors somehow lead to endothelial dysfunction, which can elicit a series of cellular interactions that culminate in the lesions of atherosclerosis. However, the initial injurious events do not necessarily lead to endothelial denudation. In fact, with better modes of tissue preservation, it is clear that the early lesions develop at sites of morphologically intact endothelium.

In the early stages of atherosclerosis, arterial SMC migrate from the media to the intima of the arterial wall. Numerous observations suggest that the SMC in lesions have changed from the contractile to the synthetic phenotype, which could have profound effects on the capacity of the lesion to respond to various agonists. SMC in the synthetic state can respond in an autocrine way to PDGF, which they secrete, as well as to other possible growth stimulators, and deposit ECM components, thereby forming a lesion that protrudes into the vessel lumen.

It was recently reported that plasma fibronectin promotes the phenotypic modulation of rat arterial SMC in primary culture without exogenous mitogens. This structural transition...
of the cells in the absence of exogenous mitogens is accompanied by the activation of overall RNA and protein synthesis, but not by a proliferative response. When the cells are exposed to exogenous PDGF or serum, they promptly begin synthesizing DNA and dividing. However, factors derived from platelets and from plasma are not involved in the process of phenotypic modulation. Furthermore, SMC in the contractile state are not stimulated to divide by PDGF alone. Thus, the phenotypic transition to the synthetic state is necessary, but not sufficient in itself, to initiate the growth of SMC.

Taken together, ECM components play an important role in controlling the phenotypic properties of arterial SMC and the control mechanism may be an important key to clarifying the pathogenesis of atherosclerosis.

Identification of the Phenotypic Modulation

The phenotypic transformation of SMC is characterized by a dramatic change in cell morphology with the loss of myofilaments and the formation of extensive RER and a large Golgi complex. The cytoplasm of vascular SMC in the contractile phenotype is dominated by microfilament bundles. A Golgi complex consisting of stacks of short cisternae is found juxtanuclearly, and the RER is poorly developed. In contrast, SMC in the synthetic state are modified structurally with a significant decrease in the content of microfilaments and an increase in the size of the RER and Golgi complex. Some of the cells have started, but not yet completed, the transition from the contractile to the synthetic phenotype. The RER and a stacked Golgi complex have started to form, but much of the cytoplasm is still occupied by microfilament bundles. These cells are categorized as being in an intermediate state. Until recently, this transition has been structurally assessed by electron microscopy, but this method is troublesome and time consuming.

The modulation process also is closely related to changes in the filamentous system. A decrease in the content of smooth muscle α-actin and myosin, an increase in the content of nonmuscle myosin, and the formation of a radiating system of intermediate filaments (composed of vimentin in vascular SMC) have been observed in the synthetic state. However, cells in the intermediate state, in which much of the cytoplasm is still occupied by microfilament bundles, may be indistinguishable by immunocytochemistry from cells in the contractile state.

Arterial SMC could then be identified by the technique of differential cDNA screening. This approach has identified seven genes which are most heavily expressed in differentiated SMC: tropoelastin, smooth muscle α- and γ-actins, phospholamban, SM 22α, calponin, and a membrane channel protein CHIP 28. In situ hybridization studies have recently confirmed high expression of two genes, osteopontin and matrix Gla protein, in atheromatous plaques. These genes have therefore provided new markers which can be used to distinguish differentiated SMC from dedifferentiated SMC in the vessel wall.

We recently examined the phenotypic transition and progression of rabbit arterial SMC from the G0 to the G1 phase by simultaneous flow cytometry, transmission electron microscopy, and immunocytochemistry and found that flow cytometry can rapidly and conveniently monitor the process of phenotypic transition in SMC, thus providing a useful method for the analysis of such transition. According to DNA content, the cells can be divided into those in the G0, G1, S, and G2+M phases. G1 cells can be subdivided into G1A (equalization) and G1B (prereplicative), which are believed to represent different functional states. The G0 phase corresponds to the contractile state, the G1A phase to the intermediate state, and the G1B, S, and G2+M phases to the synthetic state.

Regulation of Phenotypic Modulation

1. Role of ECM Components

ECM components as extracellular adhesive proteins promote the attachment, spread, and migration of vascular SMC and may initiate and control vascular responses in growth, differentiation, and wound repair. ECM components also play an important role in determining the phenotypic transition of vascular SMC.
fibronectin, Arg-Gly-Asp (RGD), mimics intact fibronectin in promoting a change in the differentiated properties of the cells. We recently found that not only basement membrane components such as fibronectin and laminin, but also interstitial components such as type I collagen and elastin, which are involved directly in the attachment and migration of SMC, play an important role in determining the phenotypic transition of cultured rabbit arterial SMC\(^{11}\) (Fig. 2). Type I collagen is as efficient as fibronectin in promoting the transition of cells to the synthetic phenotype without exogenous mitogens, while elastin, a major constituent of the media, acts like laminin to maintain cells in the contractile state\(^{11}\). We also found that types III, IV, and V collagen (bovine placenta), like type I collagen, promote the phenotypic transition to the synthetic state in primary culture, although the efficiency of cell adhesion varied among the collagens\(^{12}\). Furthermore, the major portion of cells in both type I and III collagen gels undergo transition from the contractile to the synthetic phenotype, but this transition is clearly delayed compared with cells grown on collagens (unpublished observations). Hedin et al. indicated that rat SMC attach partially but spread poorly on type 1 V collagen (human placenta) in growth factor-free medium\(^{13}\). In contrast, Stadler et al. (1989) reported that rabbit SMC grown on collagen types I and III undergo phenotype modulation in M199 plus 5% fetal bovine serum (FBS) and 2 mM glutamine, while SMC seeded sparsely onto a layer of type IV collagen (EHS mouse tumor) or in reconstituted type I collagen do not change phenotype. The reason for these discrepancies is not immediately apparent. Perhaps the use of collagens of different species and tissue origins, and different methods may result in different regulation of cell adhesion and phenotypic tran-
sition through interactions with cell surface integrins.

It has been suggested that heparin and heparan sulfate proteoglycans in basement membranes inhibit the phenotypic modulation of SMC from the contractile to the synthetic state in primary culture\(^{13,14}\). However, opinions differ on the effects of heparin\(^1\).

It appears that the thickness of the intima in arteriosclerosis is correlated with the magnitude of substantial defects in the internal elastic lamina and that surface elastases may break down fibrous elastin to provide a tissue gradient of soluble elastin peptides. An elastinolytic enzyme, 68 kDa matrix-degrading metalloproteinase-2 (MMP-2), which degrades insoluble elastin, is produced by cultured human arterial SMC. The 95 and 72 kDa gelatinases (MMP-9 and -2), which are secreted by rabbit arterial SMC, are likely to be involved in the initiation of vascular SMC proliferation. These findings suggest that matrix remodeling can induce phenotypic transition to the synthetic state. However, the roles of MMPs, which have been implicated in tissue remodeling, in atherogenesis, and in other vascular pathologies, have not been widely investigated.

2. Cell-binding Sites and Cell Surface Receptors

Cell surface receptors and signal peptides play a crucial role in cellular functions by allowing SMC to attach and spread over substrates, thus paving the way for cell proliferation and differentiation. Albelda and Buck (1990) suggested that large amounts of \(\alpha_1\beta_1\) and \(\alpha_3\beta_1\) integrins are expressed in situ in vascular smooth muscle. We recently found that with the exception of \(\alpha_4\) integrin (which was undetectable), rabbit SMC express all integrins (\(\alpha_1\), \(\alpha_2\), \(\alpha_3\), \(\alpha_5\), \(\alpha_6\), and \(\beta_1\) integrins) on their surface as detected by flow cytometry\(^{15}\). Belkin et al. (1990) reported that high levels of the \(\alpha_1\) subunit of \(\alpha_1\beta_1\) integrin, which adheres to types I, II, III, and IV collagen, are present in human adult aortic media and muscular-elastic intima, but that the amount of \(\alpha_1\) subunit decreases significantly during primary culture, and subcultured cells do not contain \(\alpha_1\beta_1\) integrin. In contrast, Clyman et al. (1990) reported that \(\alpha_1\beta_1\) integrin is present in subcultured rat aortic SMC and mediates adhesion to laminin and types I and IV collagen. In adult arterial media, \(\alpha_1\beta_1\) and \(\alpha_3\beta_1\) are the major potential laminin-binding integrins, and a variant laminin (possibly A-S-B2) interacting with its receptor (\(\alpha_1\beta_1\) and \(\alpha_3\beta_1\) integrins) may be important for maintaining the differentiated SMC phenotype in vivo (Glukhova et al., 1993).

The interaction between vascular SMC and fibronectin is mediated through a cell surface receptor, \(\alpha_5\beta_1\) integrin, and the minimal cell-binding sequence of fibronectin, RGD\(^{16,13}\). On the other hand, type I collagen promotes the phenotypic transition of rabbit SMC by interacting with a cell surface receptor (of the \(\beta_1\) integrin family) for a cell-binding sequence other than RGD or DGEA (Asp-Gly-Glu-Ala)\(^{14}\). We recently identified cell adhesion receptors and cell-binding sequences in native and denatured type I collagen in rabbit arterial SMC\(^{15}\). Preliminary experiments suggest that rabbit SMC in the contractile state adhere to native type I collagen through \(\alpha_1\beta_1\) and \(\alpha_3\beta_1\) integrins, but that the relative amount of \(\alpha_3\) integrin decreases during primary culture, and the initial adhesion of cells in the synthetic state is mediated only by \(\alpha_1\beta_1\) integrin. In contrast, cell adhesion to heat-denatured type I collagen is mediated only by \(\alpha_1\beta_1\) integrin in the contractile state, and by \(\alpha_1\beta_1\), \(\alpha_2\beta_1\), and \(\alpha_3\beta_1\) integrins in the synthetic state (unpublished observations). In heat-denatured type I collagen, the sequences DGEA and RGD serve as recognition sites for the \(\alpha_2\beta_1\) and \(\alpha_3\beta_1\) integrins\(^{15}\) (Fig. 2). These findings suggest that rabbit SMC can recognize native and denatured type I collagens through interactions with triple helix-binding receptors and \(\alpha\)-chain-binding receptors, and that the expression pattern of integrins changes in conjunction with the phenotypic properties of vascular SMC.

3. Remodulation from the Synthetic to the Contractile Phenotype

Campbell et al. reported that the phenotypic modulation of rabbit and porcine SMC in primary culture with 5% FBS is reversible if the cells are seeded at a high initial density and a confluent monolayer is formed in less than 1 week of proliferation and that heparin and heparan sulfate glycosaminoglycans are responsible\(^{13,14}\). These authors express SMC phenotype as an increase in the volume fraction of myofilaments in the cell cytoplasm and in the expression of smooth muscle \(\alpha\)-actin mRNA. In contrast, in studies on rat aortic SMC cultured...
in the presence of serum, signs of reentry into the contractile phenotype at confluence are scarce\(^4\). However, in subcultured cells detached and reseeded in serum-free medium on substrates of fibronectin or laminin plus type IV collagen, partial remodulation may be possible. On laminin plus type IV collagen, there is an increase in the fraction of cells with smooth muscle α-actin positive stress fibers, and distinct myofilament bundles reappear in central parts of the cytoplasm, although cells on fibronectin have a fine structure characteristic of the synthetic phenotype\(^4\). These findings emphasize the importance of ECM components in the controlling the differentiated properties of vascular SMC.

### Regulation of Cell Proliferation

It is today widely recognized that the proliferation of SMC is a major etiologic event in the formation of atherosclerotic lesions. Following mechanical injury of the endothelium, medial SMC migrate into the intima and proliferate. However, it is difficult to study the regulation of SMC proliferation in vivo, and most of the literature in this field refers to in vitro systems.

#### 1. Phenotypic Modulation and PDGF

Vascular SMC from newborn rats express increased levels of PDGF A- and B-chain transcripts in vitro, secrete high levels of a PDGF-like activity, and proliferate in an autocrine way, whereas SMC from adult rats express only minute amounts of PDGF A-chain and no B-chain RNA is detected. In atherosclerotic lesions, SMC that have changed to the synthetic phenotype express PDGF A-chain mRNA, secrete PDGF-AA, and can proliferate actively, suggesting that an autocrine and/or paracrine proliferative mechanism could be important in atherogenesis. Moreover, it is found that SMC isolated from intimal lesions developing after balloon catheterization produce several-fold larger amounts of PDGF-like activity than cells isolated from the normal media.

Using a primary culture system, it is found that the expression of PDGF A-chain mRNA and PDGF α- and β-receptor mRNA increases in association with phenotypic transition. After modulation to the synthetic phenotype, SMC show high levels of PDGF A-chain mRNA and high PDGF receptor activity, but secrete no detectable amounts of PDGF-like mitogen. After exposure to exogenous PDGF, SMC express PDGF α- and β-receptor mRNA, release a PDGF-like mitogen (presumably PDGF-AA), and can begin synthesizing DNA and dividing in an autocrine way. These cells release a PDGF-like molecule at several-fold higher levels than subcultured cells. The mitogen effect of different isoforms of PDGF may depend on the previous replicative history of the cells. In primary cultures of rat SMC, an equipotent effect on DNA synthesis is obtained with both PDGF-AA and PDGF-BB, while in subcultured cells, PDGF α-receptors are less numerous than β-receptors and the mitogenic response to PDGF-AA is weaker than to PDGF-AB and PDGF-BB\(^4\). However, the simultaneous addition of basic-fibroblast growth factor (FGF) and PDGF modulates the mitogenic activity of PDGF-AA by specific up-regulation of the PDGF α-receptor in bovine vascular SMC. Probably supporting these findings, PDGF A-chain mRNA localizes in human platelets and at sites of acute injury, and SMC isolated from human atheromas express PDGF A-chain mRNA and secrete PDGF-AA-like mitogen. On the other hand, Ross and collaborators suggested the involvement of PDGF-BB derived from macrophages in the development of human atherosclerotic lesions\(^3\). Morisaki et al. reported that the proliferation of SMC in intimal atheromatous lesions is stimulated by autocrine secretion of smooth muscle cell derived growth factor (SDGF).

Taken together, (1) vascular SMC in the contractile state are not stimulated to divide by PDGF alone; (2) when the transition to the synthetic phenotype is promoted by interaction with ECM components, SMC are subjected to initial exposure to platelet PDGF-AA or macrophage PDGF-BB; (3) SMC begin proliferating by autocrine secretion of PDGF-AA or SDGF; (4) SMC can proliferate in response to macrophage PDGF-BB by up-regulation of the PDGF β-receptor. Furthermore, (5) at sites where cell injury and necrosis occur, damaged SMC could release FGF and in so doing could also stimulate neighboring SMC in response to PDGF-AA by up-regulation of PDGF α-receptor, the overlying endothelium, or vascular channels within the lesions.

#### 2. Induction of Cell Proliferation

PDGF is termed a “competence” factor and
has been implicated as a major mitogen for SMC. It is believed to function as a regulator of early events in the cell cycle, allowing cells to traverse from the G0 to the G1 phase. However, PDGF alone is a relatively weak mitogen. To complete DNA synthesis, insulin-like growth factor-I (IGF-I) and epidermal growth factor (EGF) are required as "progression" factors in subcultured porcine and bovine SMC. In contrast, in rat aortic SMC seeded on fibronectin, PDGF alone stimulates DNA synthesis to the same extent as 10% serum in primary culture and in subcultures. On the other hand, our findings indicate that in primary culture of rabbit SMC (G1A phase, intermediate phenotype) on type I collagen, PDGF-BB alone is unable to initiate DNA synthesis and PDGF and IGF-I together are needed for the cells to enter the G1B and S phases (synthetic phenotype) and to divide. To stimulate DNA synthesis and cell proliferation up to the level of 15% FBS, the cells require EGF (Fig. 2).

These differences may be explained by the fact that IGF-I is consistently secreted in rat vascular SMC in culture. In addition, PDGF isoforms (AA and BB) cause increases in IGF-I mRNA levels. IGF-I release, and the number of IGF-I receptors per cell in quiescent rat SMC. A marked increase in IGF-I mRNA levels has been demonstrated in rat aorta after balloon injury. The regulation of IGF-I mRNA expression and IGF-I production in rabbit SMC remains unclear, but it should be noted that in our study of quiescent secondary cultures (G1B phase, synthetic phenotype), we found that PDGF alone is able to initiate DNA synthesis and IGF-I and EGF are required to complete DNA synthesis. This agrees with the findings reported for quiescent subcultured porcine SMC. However, PDGF alone is unable to stimulate DNA synthesis in quiescent primary cultured SMC (G1A phase, intermediate phenotype). These conflicting data may be explained by the fact that these SMC originated from different species and ages of animals. Perhaps more significantly, the phenotypic properties of vascular SMC vary considerably.

Recent studies have demonstrated in rat aortic SMC seeded on fibronectin, PDGF alone stimulates DNA synthesis to the same extent as 10% serum.

CONCLUSION

Phenotypic transition appears to be an early key event in the pathogenesis of atherosclerosis. ECM components promote the attachment, spread, and migration of vascular SMC and may initiate and control vascular responses in growth, differentiation, and wound repair. ECM components also play an important role in determining the phenotypic transition of vascular SMC.

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