The vascular smooth muscle cell in arterial pathology: a cell that can take on multiple roles

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Abstract

Vascular smooth muscle cells (VSMCs) are the stromal cells of the vascular wall, continually exposed to mechanical signals and biochemical components generated in the blood compartment. They are involved in all the physiological functions and the pathological changes taking place in the vascular wall. Owing to their contractile tonus, VSMCs of resistance vessels participate in the regulation of blood pressure and also in hypertension. VSMCs of conduit arteries respond to hypertension-induced increases in wall stress by an increase in cell protein synthesis (hypertrophy) and extracellular matrix secretion. These responses are mediated by complex signalling pathways, mainly involving RhoA and extracellular signal-regulated kinase1/2. Serum response factor and miRNA expression represent main mechanisms controlling the pattern of gene expression. Ageing also induces VSMC phenotypic modulation that could have influence on cell senescence and loss of plasticity and reprogramming. In the early stages of human atheroma, VSMCs support the lipid overload. Endocytosis/phagocytosis of modified low-density lipoproteins, free cholesterol, microvesicles, and apoptotic cells by VSMCs plays a major role in the progression of atheroma. Migration and proliferation of VSMCs in the intima also participate in plaque progression. The medial VSMC is the organizer of the inwardly directed angiogenic response arising from the adventitia by overexpressing vascular endothelial growth factor in response to lipid-stimulated peroxisome proliferator-activated receptor-γ, and probably also the organizer of the adventitial immune response by secreting chemokines. VSMCs are also involved in the response to proteolytic injury via their ability to activate blood-borne proteases, to secrete antiproteases, and to clear protease/antiprotease complexes.

Keywords

Hypertension • Ageing • Signalling • Atherosclerosis • Protease • Serpin

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1. Introduction

Vascular smooth muscle cells (VSMCs) are the main constitutive stromal cells of the vascular wall, assuming a variety of different structural and physiological functions. VSMCs produce the extracellular matrix (ECM) during development, providing the arterial wall with the capacity of withstanding the high pressure of the circulating blood compartment and are also involved in arterial repair after injury in adult life. VSMCs of arterial resistance vessels maintain a variable contractile tone (peripheral resistance to flow), which is responsible both for the regulation of blood pressure and for the redistribution of blood flow generated by the pumping heart. Owing to the pressure generated, mechanical stress/strain is generated within the arterial wall, and radial hydraulic conductance occurs across the wall, conveying soluble substances from the blood outwards. VSMCs present within the arterial wall are highly sensitive to both the mechanical stress and the radially convected molecules.

Physiologically, VSMCs are the sole cell type of the medial layer of the vascular wall. The media is composed of VSMCs and the ECM synthesized by VSMCs. The medial layer is, for the most part, an avascular tissue, devoid of capillaries and an immune-privileged site compared with the adventitia, as shown in the arterial allogenic transplantation model,1,2 physiologically poorly accessible to leucocytes. The media is accessible to soluble plasma components, which are outwardly convected from the circulating blood through the vascular wall.3 In contrast, the movement of cells inwardly from the media to intima (VSMCs, intimal proliferation), and from the adventitia...
to media (endothelial cells, neo-angiogenesis), depends on the ability of the cells to adhere, to migrate, and to proliferate in relation to transmural gradients of specific growth factors (Figure 1). Compared with the media, the outer adventitia is enriched in capillary vessels where leucocyte extravasation is facilitated. Therefore, the role of VSMCs in human pathologies cannot be interpreted independently of this tissue-specific spatiotemporal structure and the physiology of the arterial wall.

The VSMCs, as stromal cells of the vascular wall, control these physiological environments, but are also the targets of their pathological changes. VSMCs can undertake additional functions in response to the stimuli to which they are subjected. This ability of VSMCs to adapt is related to the high plasticity of these stromal cells to reprogramme their expression pattern in response to (i) acute stimuli, mainly mediated by ligand–receptor interactions, and (ii) chronic stimuli that trigger epigenetic modulations.

Traditionally, proliferative synthetic VSMCs and quiescent contractile VSMCs represent the two ends of a spectrum of VSMCs with intermediate phenotypes. However, it has been suggested that differentiation and proliferation of VSMCs are not mutually exclusive. Cessation of proliferation alone is not sufficient to promote VSMC differentiation. Depending on the signals present in their local environment, contractile VSMCs can acquire distinct phenotypes, such as the ability to migrate and proliferate and to promote ECM production, inflammatory signals, and/or calcification. The phenotypic modulation of VSMCs is determined by the environmental cues/signals, such as mechanical forces, endocytosis of specific molecules, and growth factors that influence expression of a panel of VSMC-specific genes. Notch and Wnt signalling play prominent roles in controlling VSMC differentiation and modulating the phenotypic response following different stimuli via the influence on positive and/or negative transcription factors and cofactors that determine gene expression programme of VSMCs (for reviews, see Joutel et al. and George et al. in this issue). The platelet-derived growth factor-BB (PDGF-BB) and transforming growth factor-β (TGF-β) are the key mediators of VSMC phenotypic switching. PDGF-BB is involved in the active repression of VSMC-specific gene expression, whereas TGF-β promotes the contractile phenotype. In vitro experiments indicate the interaction between these pathways. Notch signalling cooperates with PDGF signalling and TGF-β to regulate VSMC migration and differentiation. Wnt inhibitory factor-1 retarded PDGF-BB-induced VSMC proliferation. It has been established that the regulation of VSMC-specific gene expression depends on unique combinatorial interactions of multiple factors that are either ubiquitously expressed [e.g. serum response factor (SRF)] or selective for VSMCs (e.g. myocardin). Owens et al. have shown that phenotypic modulation of VSMCs in vivo is mediated, at least in part, by transcription repression. The activity of these transcription factors and cofactors is regulated by a wide range of signalling pathways, including extracellular signal-regulated kinase (ERK), c-jun-N-terminal kinase, p38 mitogen-activated protein kinases, Akt, Rho/Rho-kinase, and calcineurin/calmodulin kinases.

2. Changes in contractile stimuli and function of VSMCs lead to hypertension, and hypertension impacts VSMC biology

The arterial VSMC contractile tone, mainly dependent on sympathetic nervous system signalling, has the main circulatory function of generating blood pressure, which allows the redistribution of local flow in relation to organ-specific metabolic demand. The absence of arterial VSMC tone is rapidly incompatible with life, whereas excessive arterial VSMC contractile tone leads to abnormally high blood pressure. Although the pathophysiology of hypertension was originally focused on the determinant role of the kidney in the long-term control of body fluid volumes, compelling evidence now suggests that ‘mechanotransduction disorders’ at the level of the VSMC play a role in its genesis. Hypertension and the excessive strain on the ECM (mainly elastin in physiological conditions) stretches the VSMCs, which transduces intracellular signals of tensile stress (for a review, see 10). The ECM integrin-cytoskeleton interactions play an important role in mechanosensing, which enables VSMCs to detect and respond to changes in intraluminal pressure, allowing hypertrophic inward remodelling of resistance arteries characterized by reduction in lumen diameter and an increased media/lumen ratio. Increases in integrin signalling may in part determine VSMC hypertrophy and hyperplastia, corresponding to an increase in protein synthesis by each cell, observed in hypertensive experimental models. Hypertrophic remodelling contrasts with the hyperplasic remodelling (VSMC proliferation) observed in intimal proliferation. In large arteries, the increase in α1β1 and fibronecin participates in the adaptation to mechanical stress in spontaneously hypertensive rats (SHR) through increased numbers of cell–matrix attachments and phenotypic changes. Integrin α1β1, which is the receptor for collagen IV and I, controls the hypertrophic response of VSMCs during angiotensin II (Ang II)-induced hypertension. VSMC-specific integrin α5β1, the receptor for laminin, regulates the expression of contractile proteins and vascular compliance. The loss of adhesion and, therefore, of tensile stress leads to VSMC apoptosis.

In the early stage of hypertension, VSMCs in conduit arteries may undergo a transition from a contractile phenotype to a more synthetic phenotype, including the production of both contractile and secreted proteins. Activation of the transcription factor network may trigger
changes in the components of the contractile apparatus and cytoskeleton, leading to an increased VSMC stiffness. These structural modifications are largely influenced by haemodynamics and/or the higher level of reactive oxygen species and vasoactive compounds (Ang II, endothelin-1, and aldosterone), leading to the subsequent response of fibrosis.

### 2.1 Intracellular signalling pathways involved in phenotypic modulation

The contractile phenotype can be altered by phenotypic modulation leading to a high rate of proliferation and migration and ECM accumulation while markers of VSMC contractility are down-regulated. Vascular tone is linked to the Ca$^{2+}$-dependent phosphorylation state of the myosin light chain (MLC) and other associated calponsins and caldesmon that allow the interaction of actin and myosin to generate tonic contraction. MLC phosphorylation can be stimulated by a variety of vasoactive hormones and signalling pathways, including the Ca$^{2+}$-dependent ERK pathway. Alteration of either the synthesis or the phosphorylation state of the contractile proteins may influence myogenic tone, and finally blood pressure. During phenotypic modulation, a decrease in ERK1/2-mediated phosphorylation impedes VSMC contraction.

In addition to their role in the cellular hypertrophic/proliferative response to Ang II in hypertension, ERK signalling pathways participate at different levels in the increased VSMC contraction in hypertension, both in the acute phase during the elevation of intracellular Ca$^{2+}$ and during long-lasting contraction.

Under physiological conditions, low activity and stuttering-persistent Ca$^{2+}$ sparklets, as well as integrins, contribute to regulate arterial intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]i) and hence to the development of myogenic tone and the regulation of blood pressure. Increased Ang II signalling activates stuttering persistent Ca$^{2+}$ sparklet signals in the arterial SMC via A-kinase anchoring protein-150/PKCµ signalling. The activation of L-type calcium channels in VSMCs by αβ1 or αβ2 causes vasoconstriction, whereas inhibition by αβ2 causes vasodilatation. More recently, it has been shown that αβ1 is able to potentiate calcium-activated potassium channels to produce sustained vasodilatation following initial vasoconstriction. In addition, Ca$^{2+}$ is negatively modulated by Mg$^{2+}$. VSMCs possess transient receptor potential melastatin-7, which plays an essential role in maintaining intracellular Mg$^{2+}$ levels during the long-term Ang II and aldosterone treatment. Increased intracellular Mg$^{2+}$ concentration ([Mg$^{2+}$]i) causes vasodilatation and attenuates agonist-induced vasoconstriction, whereas reduced [Mg$^{2+}$]i has opposite effects, leading to hypercontractility and impaired vasorelaxation. The Na$^{+}$-K$^{+}$-ATPase works in concert with the Na$^{+}$/Ca$^{2+}$ exchanger to modulate intracellular calcium. A selective Na$^{+}$-K$^{+}$-ATPase inhibitor, ouabain, triggers hypertension, whereas transgenic mice expressing Na$^{+}$-K$^{+}$-ATPase in smooth muscle have a decreased blood pressure.

Other signalling pathways control MLC phosphorylation. Activated G protein-coupled receptors such as Ang II type-1 receptor (AT1R) may increase the phosphorylated form of MLC either via a Ca$^{2+}$-dependent activation of MLC kinase or by inhibition of MLC phosphatase via Rho/Rho-kinase pathways. These two different signalling pathways are connected by the tyrosine kinase, Jak2, activated by a rise in [Ca$^{2+}$]. The basal regulation of blood pressure is dependent on MLCK activation, whereas salt-induced or Ang II-dependent hypertension may result from both pathways.

### 2.2 VSMC-specific transcriptional network regulation

VSMC plasticity, i.e. the switch from contractile to synthetic phenotype, is controlled by many transcriptional regulatory pathways, in particular SRF and its main cofactor, myocardin. Binding of SRF to the CaR-G box sequences in both 5′ promoter and intronic regions activates VSMC-specific contractile genes. The development of hypertension in SHR is linked to an increased SRF binding affinity to the CaR-G box present in the SM-MLC kinase promoter, resulting in higher phosphorylation of MLC.

Function of the SRF/myocardin axis is activated by the Rho/Rho-kinase pathway. It has been previously shown that SKP2 plays a role in VSMC proliferation and more recently the work of Werth et al. suggests that SRF may modulate proliferation by stabilizing the SKP2-containing ubiquitin complex level. Proliferation of VSMCs is enhanced by SRF via the regulation of immediate early genes, and its downstream effectors are impeded by phosphatase and tensin homologue (PTEN), which suppresses pathways involved in SMC proliferation. PTEN has been identified as a downstream effector of SRF since its expression is regulated by SRF through an miR network. In the setting of SRF inactivation, repression of PTEN by miR-21 provides an alternative proliferative pathway. Regulation of the expression level of these transcription factors drives the effects of anti-proliferative molecules such as adiponectin, TGF-β, and peroxisome proliferator-activated receptor gamma (PPAR-γ).

miRs dynamically regulate VSMC differentiation and phenotypic switching, miR-143/145, miR-21, and miR-1 promote the contractile phenotype, whereas miR-221, miR-146a, miR-24, and miR-26a are involved in the switch to the synthetic phenotype and cell proliferation after vascular injury. It has been proposed that miR-143/145 are activated in parallel pathways by Jagged-1/Notch and SRF/myocardin. Consistent with this, smooth muscle miRs are essential for regulating blood pressure levels in resistance vessels, and mice lacking both miR-143 and miR-145 show a significant reduction in blood pressure. In young hypertensive patients, the expression of AT1R is negatively correlated with miR-155, which recognizes only the A allele of the AT1R A1166C polymorphism. It has also been reported that Kruppel-like factor-4/5 (KLF4/5), ETS-like transcription factor-1 (Elk-1), HES-related repressor protein-1 (Herp1), FoxO4, and the p65 subunit of NFκB, all inhibit VSMC-specific gene expression, mediating the effects of PDGF-BB, TGF-β, and Notch signalling on VSMCs, at least in part by disrupting SRF-myocardin interaction, thus promoting the synthetic state of VSMCs.

In summary, although it is now well established that VSMC plasticity plays a role in the genetics of hypertension, a strict association between the degree of VSMC de-differentiation and the severity of hypertension has not been established. The process of phenotypic switching requires a specific pattern of transcriptional factors and miR expression whose mechanisms are now beginning to emerge. miR-145 and miR-143 are direct targets of SRF and myocardin, which themselves target a network of transcription factors such as KLF4 and Elk-1. Although the effects of miR-145 and miR-143 on blood pressure have been reported, further studies are required to establish whether the loss of SRF in VSMCs has any consequences on blood pressure.
3. Impact of ageing on VSMC function

Age-associated arterial changes encompass the activation of the intra-vascular renin–angiotensin–aldosterone system and alterations of functional properties of VSMCs: ECM synthesis, contractility, switch to an inflammatory phenotype, apoptosis, and senescence in response to changes in signalling mechanisms and gene expression patterns. A concurrent impaired regeneration of endothelial cells and enhanced proliferation and migration of VSMCs contribute to vascular remodeling with ageing. Using a proteomic approach, it has been recently shown that a combination of several proteins, Ang II, monocyte chemoattractant protein-1 (MCP-1), and milk fat globule protein epidermal growth factor-8, plays a crucial role in the coordinated process of VSMC proliferation and migration.

3.1 Mechanisms of VSMC proliferation and contractility during ageing

VSMC proliferation is controlled by SRF and its partners, but some mechanisms are more related to the loss of endothelial regeneration capacity, which is a specific feature of ageing. Endothelial expression of a Notch ligand, Jagged-1, controls the expression of Jagged-1 in VSMCs, and the loss of this unidirectional effect is the main mechanism contributing to the growth of the neointima.

The regulation of arterial contractility triggered by the Rho-kinase signalling pathway is markedly altered during the ageing process. Accumulation of fibronectin with ageing has been implicated in the activation of cGMP-dependent protein kinase-β, which mediates relaxation of contractile VSMCs. In Alzheimer’s disease, neurovascular dysfunction and dementia are driven by a hypercontractile phenotype in small cerebral arteries, resulting in reduced cerebral blood flow. This process may be mediated by overexpression of SRF, which down-regulates the low-density lipoprotein receptor-related protein-1 (LRP-1), a key amyloid B peptide clearance receptor in VSMCs. Down-regulation of LRP-1 expression also enhances VSMC proliferation and matrix synthesis by PDGF- and Smad2-dependent pathways, respectively.

3.2 Ageing contributes to the propagation of inflammation via VSMCs

The age-associated pro-inflammatory phenotype is orchestrated by Notch ligand, Jagged-1, controls the expression of Jagged-1 in VSMCs, and the loss of this unidirectional effect is the main mechanism contributing to the growth of the neointima.

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3.3 Ageing is marked by cellular senescence and vascular calcification

Polyplody in VSMCs is a marker of ageing and triggers the senescence process. NADPH oxidase-4 increases during ageing in polyploid cells and promotes the down-regulation of survivin, a mitotic regulator. The senescence phenotype is characterized by a loss of VSMCs from the media and increased markers of premature senescence, including increased β-galactosidase-positive cells, reduced telomere length, and a reduced number of Ki67-positive cells. Hutchinson–Gilford progeria syndrome represents a model of a premature senescence VSMC phenotype associated with advanced atherosclerosis. This syndrome is caused by a mutation in lamin A (LMNA) gene producing a mutant lamin A protein (progerin) which lacks the site of the metalloproteinase FACE1/Zmpste24 required to remove the farnesyl group, leading to protein maturation. This prelamin A is considered to be a new biomarker of VSMC ageing and arteriosclerosis whose accumulation is causal and not a consequence of cell senescence. VSMC loss could lead to the formation of areas of mucoid degeneration involved in chronic aneurysm and acute dissection associated with ageing.

Few reports have demonstrated the association between cellular senescence and VSMC calcification. Vascular calcification, occurring with advancing age, is initiated primarily in matrix vesicles and involves transglutaminase-2-induced collagen crosslinking and osteopontin dimerization. The process of medial calcification is mediated by a switch from a de-differentiated to a pro-calcificatory phenotype of the VSMC via differential expression of specific markers, such as reduced matrix Gla protein and increased bone morphogenetic protein-2.

3.4 Arterial stiffness profiles in ageing

The age-associated pro-inflammatory phenotype is orchestrated by Notch ligand, Jagged-1, controls the expression of Jagged-1 in VSMCs, and the loss of this unidirectional effect is the main mechanism contributing to the growth of the neointima.

The concerted effects of aldosterone and Ang II via mineralocorticoid receptor signalling and aldosterone-mediated ERK1/2 activation, related to increased epidural growth factor receptor expression. Increased expression of adhesion molecules (ICAM-1), matrix metalloproteinase-2 (MMP-2), profibrotic molecules (TGF-β), and chemokines (MCP-1) also participate in the VSMC response. Ang II-induced increased expression and the activity of MMP-2 may be involved in TGF-β1 activation and signalling via TGF-β1 type-II receptor. Within the aged arterial wall, this pathway leads to arterial inflammation, accumulation of fibronectin, collagen, and fibrosis. An age-related shift to a de-differentiated phenotype may contribute to an inflammatory state by increasing the expression of endothelial cell Nfkb, ICAM-1, vascular cell adhesion molecule-1, and E-selectin. MCP-1 and its receptor CCR2 increased the proliferation and migration of VSMCs in response to growth factors within the thickened aortic intima. There is evidence suggesting that cell sex influences phenotypic changes of VSMCs during ageing, mainly related to the role of the oestrogen receptor (ER). This is illustrated by distinct patterns of proliferative rate of male and female VSMCs from ERα or ERβ KO mice following exposure to NO. Indeed, it has been demonstrated that female VSMCs are more resistant to oxidative stress and detachment from a substrate (anokias) than male cells and exhibit a higher propensity to autophagy, which also protects against apoptosis.
the vascular wall function deteriorates. In agreement with this finding, cultured VSMCs require cyclic stretch to interfere with the synthesis and secretion of TFPI. This finding may explain the link between arterial stiffness and coagulation in aged patients.

In summary, an age-related imbalance towards a pro-oxidant state leads to an inflammatory response and thereby to the production of Ang II signalling molecules by VSMCs, mainly TGF-β1, MCP-1, and MMPs. Although the VSMC phenotypic modulation in ageing likely involves activation pathways similar to those in hypertension, their structural and functional consequences are quite different. Whereas essential hypertension maintains arterial function, ageing constantly reduces arterial distensibility, in turn triggering isolated systolic hypertension. The age-associated irreversible cellular senescence process, leading to a progressive decrease in plasticity and reprogramming potential of VSMCs, plays a complementary signalling role contributing to the increase in arterial stiffness.

### 4. Responses of VSMCs to lipid overload and their role in atherothrombotic progression

Atherothrombotic diseases are canonically characterized by an initial lipid overload of the arterial wall, due to the outward radial convection of plasma lipid components from the circulation into the wall and the retention of these lipid fractions in the inner part (intima). Early atheroma evolves from the initial fatty streaks developing on a cellular intimal background to early plaques (fibrolipidic lesion), in which an acellular lipid core becomes encapsulated between a cellular, matrix-rich and lipid poor, focally thickened intima (fibrocellular cap) and a remnant cellular media. Later, human atheroma evolves towards more complicated lesions, involving both neoangiogenesis and leucocyte extravasation, leading to...
repeated intra-plaque haemorrhages, the main determinants of plaque expansion, ultimately leading to plaque rupture and thrombosis. VSMCs, as the vascular stromal cells, are involved in all stages of the disease progression, supporting the lipid overload and orchestrating the response to these micro-environmental stimuli. Radially convected plasma low-density lipoproteins (LDLs) are retained and modified in the intima due to the ability of apolipoprotein B100 to bind to sulphated proteoglycans, fibronectin, and collagens, all secreted by intimal VSMCs. Immobilized LDLs are susceptible to modifications.

The role of VSMCs is directly linked to their ability to take up these lipid components by endocytosis, including pinocytosis of small components in the fluid phase and phagocytosis of nanometric molecular complexes, cell particles, or apoptotic cells. Cationic proteins are modified-LDLs than native LDLs due to the down-regulation of LDLR in response to LDLs. The scavenger receptors SR-A-I, SR-A-II, CD36, and Lecitin-oxidized low-density lipoprotein receptor-1 present on VSMCs are mainly involved in the endocytosis of acetylated or oxidized LDL and many other heterogeneous components, whereas LRP-1 is mainly involved in the endocytosis of aggregated LDLs and accepts more than 30 distinct ligands. Minimally modified LDLs could activate toll-like receptor-4 (TLR4) and participate in lipid uptake and the subsequent response. The endocytosis of LDL content by VSMCs leads to the formation of foam cells containing large lipid droplets within their cytoplasm.

Besides the ability to endocytose soluble molecules such as LDLs, VSMCs also have the ability to phagocytose cell/platelet-derived microparticles, crystals, iron particles, apoptotic cells, etc. In particular, VSMCs are able to phagocytose ageing red blood cells by a phosphatidyl serine-dependent mechanism, and heme-derived iron as do macrophages. Similarly, living VSMCs can engulf and degrade within their phagolysosome-adjacent apoptotic VSMCs, leading to the secretion of both anti-inflammatory TGF-β1 and MCP-1, and cytokine-induced neutrophil chemoattractant-1, without affecting VSMC proliferation or inducing protease secretion.

Of importance, endocytic/phagocytic activities in VSMCs induce overexpression of proteins involved in endosome/phagolysosome activities such as: CD68, a class-D scavenger receptor of the lysosomal-associated membrane protein family; 5100 A9/A10; calprotectin, induced in VSMCs by LPS; CD14, a co-receptor of TLR4 elicited by LPS; Mac-2/galectin-3, and scavenger receptors with a phagocyte-like phenotype. For example, VSMCs stimulated by LDLs overexpress CD68, whereas VSMC antigens such as α-actin, transgelin (SM22a), and myosin can be detected in CD68-positive cells of the intima of arteries with early atheroma. Therefore, we should not consider these antigens as being specific for a cell lineage, but rather as functional markers of the endocytic activity of different cell lineages. These observations imply (i) that the role of VSMCs in early human atheroma has been probably largely underestimated to the benefit of macrophages, (ii) that we critically need alternative, new specific markers of the monocyte and VSMC cell lineages other than the existing functional markers of macrophage phagocytic activities, such as the epigenetic marks that are acquired during the development, and (iii) that we have to revisit former and more recent scientific reports in the light of the phagocytic capacities of VSMCs in human atheroma (Figure 3).

In response to the initial lipid injury, certain medial VSMCs acquire a synthetic and proliferative phenotype and lose several markers of their physiological contractile function. Primary cultures of VSMCs recapitulate these phenotypic modifications in vitro. There is strong evidence that alterations in the differentiated state of VSMCs play an important role in fibrous cap formation and in post-angioplasty restenosis. Medial VSMCs migrate, proliferate, produce proteoglycans with less affinity for LDLs and collagens in the fibrous cap in response to many stimuli, including PDGF, LPS, etc. Foam VSMCs can evolve either towards cell death, promoting intimal proliferation of adjacent VSMCs and plaque calcification, hence participating in plaque progression, or towards more complex and tissue-integrated responses. Apoptosis and cell death are frequent biological events in the initial as well as in more evolved plaques. Lipid LDL contents can accumulate within cells and can be released into the extracellular space as free cholesterol and cholesterol crystals. Phospholipids are metabolized by phospholipases and can generate and secrete soluble products via the arachidonic/cyclooxygenase pathways. The modalities of cell death in response to lipid overload are likely diverse and include the role of free cholesterol and cholesterol crystal formation, injury of cell membranes, inflammus activation, and release of IL-1β in macrophages. The pyroptotic type of cell death, induced by intracellular crystals and involving caspase activation, leads to the release of mixed material, including chromatin filaments (free DNA and histones), cytoplasmic acidophilic proteins and associated lysosomal enzymes, and ECM, which together constitute the ground substance of the acellular initial human plaques. Although the formation and the impact of cholesterol crystals have been studied in macrophages only, the chances are high that similar mechanisms will soon be demonstrated in VSMCs also.

Foci of calcification are probably constituted during the early stages of human atheroma. They are likely linked to early cell death and membrane/annexin-rich particle release, which favour calcium phosphate concentration, whereas extracellular free DNA triggers its precipitation. In this context, VSMCs could acquire an osteoblastic phenotype.

Neo-angiogenesis progressing inwardly from the adventitia towards the plaque is also an event during the development of human atheroma. Neo-angiogenesis takes place in the adventitia and medial layer immediately outside the plaque, suggesting that angiogenic mediators generated by the plaque are conveyed outwards by the orthogonal hydraulic conductance. Neo-angiogenesis is directly responsible for erythrocyte leakage and haemorrhages and participates in leucocyte extravasation into the newly vascularized plaques. In a recent study, Ho-Tin-Noe´ et al. explored the mechanism of this angiogenesis in early stages of human atheroma and designated the medial VSMCs as the main source of vascular endothelial growth factor (VEGF)-A, induced by lipid products generated at the plaque level, and capable of activating PPAR-γ receptors within the medial VSMCs. This indirect effect of lipid overload on the inwardly directed angiogenesis, using the VSMC stromal cell as a relay for VEGF-A synthesis and release, was reproduced in a mouse model.

Owing to the high plasticity of VSMCs and the variety of lipid stimuli, there is probably a considerable diversity of VSMC responses to lipid overload in early human atheroma. A part of these phenotypic modifications could correspond to the reprogramming of the VSMC expression profile by epigenetic regulation. For example, Li et al. successfully cloned two different subpopulations of VSMCs in
primary culture from one human artery, which differently responded to LDL overload, suggesting a differential inherited reprogramming of VSMCs originating from the same vascular wall. One major challenge for the field is to further define the extent and the mechanisms of VSMC plasticity in response to endocytic activities.

5. Intimal proliferation

Intimal proliferation of VSMCs is a hallmark of the wall response to blood-borne injury, including platelet aggregation on the lining of the vascular wall, mechanical injury (ballooning with or without stenting) (for a review, see 96), immune injury in allograft vasculopathy (for a review, see 97), etc. This intimal proliferation is usually induced by the release of platelet growth factors, such as PDGF, but prothrombin activation could also participate, and the neointima is mainly constituted by VSMCs of subjacent medial origin (for a review, see 99). During their migration and intimal proliferation, VSMCs partially loose their contractile phenotype and acquire a synthetic one, involving the appearance of abundant endoplasmic reticulum and the Golgi apparatus and the secretion of proteoglycans and collagen. Intimal sulphate-rich proteoglycans are able to bind apoB100 by ionic interaction with basic amino acids, and therefore to initiate fatty streaks. In contrast to fatty streaks, the fibrocellular cap of the initial plaque and at later stages is usually poor in lipids, as are the atheroma-resistant regions of the arterial wall (for a review, see 102). This duality could be in part due to the ability of VSMCs to reprogramme their expression pattern between sulphate-rich and sulphate-poor proteoglycans.

Allogenic immune injury of the arterial wall in experimental models of aortic transplantation is spatio-temporally characterized by (i) an intimal VSMC proliferative response leading to lumen narrowing, (ii) an associated disappearance of medial VSMCs, in the absence of lymphocyte invasion of the media, and (iii) the formation of tertiary lymphoid organs in the adventitia. This duality between intimal VSMC proliferation and medial VSMC disappearance allowed us to propose a pathophysiological scheme, in which the intimal VSMCs come from the graft recipient, whereas the medial VSMC apoptosis is related to anti-major histocompatibility complex allo-antibodies initially generated in the maturing tertiary lymphoid organs of the subjacent adventitia. Similarly, adventitial lymphoid granuloma have been described in atherosclerotic arteries. Grabner et al. and Lotzer et al. have shown that VSMCs can express a panel of chemokines which are known to be required during lymphoid organogenesis and could be convected outwards towards the adventitia.

6. Responses of VSMCs to proteolytic injury and implications in the development of aneurysms

Aneurysms and dissections are characterized by degradation of the ECM within the media by a proteolytic process, leading to acute

Figure 3 The central role of VSMC endocytosis/phagocytosis capacity in the progression of human atheroma (ATLO, adventitial tertiary lymphoid organ; RBC, red blood cell).
intramural rupture (dissection) or chronic progressive dilatation (aneurysm). This proteolytic injury is directly or indirectly linked to the ability of VSMCs to inhibit and clear proteolytic enzymes and/or to VSMC disappearance. Aneurysms and dissections of the thoracic ascending aorta (TAAD) are primarily diseases of VSMCs, as suggested by monogenic forms of these diseases. Regardless of aetiology—including monogenic forms, association with bicuspid aortic valves, and spontaneous degenerative forms in older patients—TAADs are characterized by the presence of pathological areas of mucoid degeneration (cystic medial necrosis) within the aortic media. These areas, where VSMCs have disappeared, show an accumulation of modified glycosaminoglycans, the presence of vacuoles, the degradation of ECM, the retention of proteases, with the presence of apoptotic cells in the surrounding medial areas. The mechanisms leading to VSMC disappearance could be multiple, depending on the aetiological context. Gomez et al. reported the constitutive overexpression of Smad2 in VSMCs from TAADs independent of their aetiology, and recently related this observation to a VSMC-specific epigenetic phenomenon inducing Smad2 overexpression (see the review by Gomez and Owens in this issue).

In this pathological context, VSMCs can express and release proteases, including MMPs. VSMCs constitutively express and secrete MMP-2, and expression and secretion of MMP-9 are inducible in VSMCs under the control of NF-κB. VSMCs also express MMP-7 (matrilysin) and MMP-3 (stromelysin). Nevertheless, the VSMC secretes these MMPs as inactive proforms, which require activation to become functional. In vitro, cathepsin S rapidly degrades apolipoprotein B-100 at pH 7.4, rendering LDL particles more prone to fusion compared with controls, and breaks down pericellular adhesive proteins such as fibronectin.

In the vascular wall, VSMCs are the main source of tissue inhibitors of metalloproteinases (TIMPs) and of several serpins, such as plasminogen activator inhibitor-1 and protease nexin-1 (PN-1) and probably cysteine inhibitors (cystatin). The expression and secretion of TIMPs and PAIs by VSMCs are mainly under the control of the TGF-β/Smad pathway.

One of the blood-borne proteolytic systems involved in vascular wall pathology is the plasminergic system. The inactive zymogen, plasminogen, is secreted by the liver and circulates at a micromolar level in plasma. Plasminogen mRNA is not detectable in VSMCs in vitro or in vivo. Plasminogen is physiologically convected from the plasma outwards through the vascular wall. VSMCs express t-PA, which, together with plasminogen, binds to free lysine residues present in the annexin A2/S100A10 heterotetramer expressed on the VSMC surface, forming a cell membrane platform for plasmin activation. The genesis of pericellular plasmin induces fibronectin degradation and causes VSMC detachment and apoptosis. In this context, proteoglycan-bound pericellular PN-1 is able to inhibit plasmin and t-PA, forming a complex which can be endocytosed by the scavenger LRP-1 present on VSMC membranes. We can thus propose that VSMCs assume the clearance of blood-borne proteases in physiological conditions and that this function may become overwhelmed in certain pathological conditions (Figure 4). A similar concept was developed with factor VII-activating serine protease.
(FSAP, HABP2, hyaluronic binding protein-2), which is also synthesized by the liver, circulating at 10 μg/mL in plasma as a zymogen, activated by autocatalysis after binding to anionic (sulphated) glycosaminoglycans or nucleic acids, and able to activate, by proteolysis, factor VII, PDGF-BB, and pro-urokinase. In addition to inhibition by other circulating serpins, FSAP is also inhibited by PN-1 in the sinus, factor VII, PDGF-BB, and pro-urokinase.134 In addition to inhibition of glycosaminoglycans or nucleic acids, and able to activate, by proteolytic mechanisms, future research directions include the selection of specific molecular targets to modulate a signalling pathway attributable to one particular function.

7. Conclusions

There is growing evidence for a pivotal role of VSMC plasticity and phenotypic switching in vascular diseases. These phenotypical changes in VSMC biology, in the context of arterial tissue-specific spatio-temporal physiology and structure, are important determinants of the evolution of atherothrombotic and non-atherothrombotic pathologies in the arterial wall.

Although the mechanisms controlling VSMC phenotypic modulation in these diseases have been largely elucidated, the regulatory roles of SRF and miRs still remain to be established. In the same pathological vascular wall, contractible VSMCs co-exist with pools of synthetic, proliferative, or phagocytic VSMCs, which drive the adaptation of vascular function. However, it is difficult to discern primary effects from adaptive effects due to the possibility of intermediate VSMC phenotypes. Future research directions include the selection of specific molecular targets to modulate a signalling pathway attributable to one particular function.

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